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Development of a yeast-free bread using legume and nut flours in a gluten-free flour: Techno-functional characteristics and sensory evaluation

Ayca Tuna,[1] Jordi Ortiz-Solà,[2] Laura López-Mas,[3†] Filiz Baser,[1,4] Zein Kallas,[3] Ingrid Aguiló-Aguayo,[2] Sukru Gulec,[1,4] Banu Ozen[1] & Figen Tokatli[1]*

1 Department of Food Engineering, Izmir Institute of Technology, Izmir, Turkey
2 IRTA, Postharvest Programme, Edifici Fruitcentre, Parc Agrobiotech25003, Lleida, Catalonia, Spain
3 Centre de Recerca en Economia i Desenvolupament Agroalimentaris (CREDA)-UPC-IRTA, Parc Mediterrani de la Tecnologia, Edifici ESAB, Barcelona, Spain
4 Molecular, Nutrition, and Human Physiology Laboratory, Izmir Institute of Technology, Izmir, Turkey

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Summary This study aimed to investigate the effect of combined use of legume and nut flours on physical, nutritional and sensory properties of yeast-free bread by substituting gluten-free flour with hazelnut and white bean flours. Yeast-free bread containing a mixture of 30% hazelnut and white bean flours was found to have the lowest hardness (9.04 N) and the largest specific volume (1.51 mL g^{-1}) compared to the reference gluten-free bread (18 N and 1.43 mL g^{-1}) using a mixture design. Hazelnut and bean flours improved the *in vitro* starch digestion, reducing rapidly digestible starch by 29% and increasing resistant starch compared to the reference bread. Free choice profiling sensory analysis revealed that the developed breads containing nuts and legumes differed from the standard gluten-free formulation and a commercial product available on the market. The combined use of bean and hazelnut flours was demonstrated as functional ingredients for enhancement of nutritional, sensory and textural aspects.

Keywords Free choice profiling, hazelnut flour, *in vitro* starch digestion, white bean flour, yeast-free bread.

Introduction

Research on legumes and their flours has shown an increase since the 1990s, with a substantial growing interest over the last two decades. A global product database provides current market trends and future insights into the potential of variety of foods (MINTEL, 2023). Their data for the legume flour-based products show their growth in the EU food market over the years. The share of legume flour-based products launched was 0.34% in 2003, with a steady increase to 4.8% and 9.8% in 2013 and 2023 respectively. The interest in legumes can be linked to various factors, such as the trends towards healthy, well-balanced diets and gluten-free foods, awareness of sustainable agriculture practices and plant-based diets (Garrido-Galand *et al.*, 2021; Singh *et al.*, 2021). Some statistics were presented on the cultivation of local legumes and nuts in the Mediterranean region, emphasising the urgency to support their production and consumption due to their low demanding agricultural practices and beneficial nutritional aspects (Hernández-López *et al.*, 2022). Another research reported that legumes and nuts are the crops with lower greenhouse gas emissions (Semba *et al.*, 2021).

After soybeans, the main legumes in terms of volume of production are beans, chickpeas, peas, cowpea and lentil (FAO, 2021). Common white bean or white kidney bean (*Phaseolus vulgaris* L.) is one of the most important legumes cultivated in a very wide range of regions. Bean flour has been studied in combination with wheat flour and gluten-free flour in yeast breads. In a study, wheat flour was replaced with different legume flours, including common white bean, in sourdough breads and indicated that 15% replacement produced breads with improved textural, nutritional and sensory qualities (Rizzello *et al.*, 2014). In a recent report, rice flour was mixed with bean flour and concluded that blend of rice and bean flours improved the dough consistency, softness, volume and sensory

*Correspondent: E-mail: figentokatli@iyte.edu.tr
†Previously with Fundació Miquel Agustí (FMA), Carrer d'Esteve Terradas, 08860, Castelldefels, Spain.

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properties (Aguiar *et al.*, 2022). The use of bean flour in some gluten-free couscous and bread was shown as a valuable functional ingredient in terms of nutritional quality (Boukid *et al.*, 2019; Boudouira *et al.*, 2023).

Hazelnut as a major source of healthy fats, fibre and protein with a low carbohydrate content is one of the commonly used tree nuts. According to the report on the global hazelnut market, a growth projection of 8.7% has been given for a forecast period of 2023–2030 in terms of market value of hazelnut products including hazelnut flour, oil and paste (Research and Markets, 2023). Hazelnut flour can be included in food formulations to increase the nutritional and sensory quality. In a recent study investigating gluten-free yeast bread with white bean and hazelnut flours, the rheological characteristics of the flours and the physical properties of breads were reported (Tuna *et al.*, 2023). In one study, researchers observed a significant reduction in postprandial glycaemic response when individuals consumed breads with hazelnuts; therefore, they reported that bread could be a convenient product to include hazelnuts in the daily diet (Devi *et al.*, 2016). Other researchers used defatted hazelnut flour with rice-based gluten-free flour (Tunc & Kahyaoglu, 2016). In another study, wheat flour mixed with hazelnut testa (skin) was used in breads in order to increase the content of dietary fibre (Anil, 2007).

Yeast-free bread is defined in the bread and rolls category as soda bread in the Codex Alimentarius (FAO Codex Alimentarius, 1995), and also as leavened bread or as bread with yeast substitutes in the EU Commission Regulation on food additives (EU Commission Regulation, 2022). Yeast-free bread is a preferred product in cases of baker's yeast allergy or intolerance (Yazar & Tavman, 2012). It also eliminates the fermentation step and shortens the processing time. In the reported studies, the cases of yeast-free bread are very limited. New processing techniques such as supercritical fluid extraction and CO_2 gas hydrates as a leavening agent in baking instead of yeast has been investigated (Hicsasmaz *et al.*, 2003; Srivastava *et al.*, 2022). Knez *et al.* (2014) studied the effect of processing conditions on the final concentrations of arabinoxylans and fructans, which are both dietary fibres and prebiotics, and compared yeast, unleavened, yeast-free breads and concluded that yeast-free breads had higher arabinoxylan and fructan contents than yeast breads.

Legume and nut flours should be incorporated into foods to broaden the range of products with diverse qualities, and supporting sustainable agriculture both locally and globally. The inclusion of legume flours in wheat breads and gluten-free breads has been studied and found to be nutritionally beneficial. Yeast-free breads, on the other hand, are consumed by individuals with yeast allergies and sensitivities, or by those who prefer them for their shorter, practical processing. However, research on yeast-free breads is very limited. Considering the benefits of legume and nut flours and yeast-free bakery products, to the best of our knowledge, there has been no research on the use of these two aspects in gluten-free products. The novelty of this study is that it is the first to develop a yeast-free bread with no gluten using hazelnut and white bean flours. The study initially focused on the physical properties of formulations prepared according to a mixture design in order to evaluate the combined effect of these functional flours. Breads from the optimised formulations were then subjected to *in vitro* starch digestion and sensory evaluation.

Material and methods

Flour samples and other ingredients

Pre-cooked white bean flour (Naturelka, Aydin, Turkey), raw hazelnut flour with skin (testa), rice flour and corn starch (Ingro, Karaman, Turkey), Xanthan gum (Alfasol, Istanbul, Turkey), baking powder and baking soda (Dr. Oetker, Izmir, Turkey), olive oil and apple vinegar (Taris, Izmir, Turkey), white sugar (Irmak, Istanbul, Turkey) and salt (Billur, Izmir, Turkey) were used in bread making. The chemicals utilised in the analyses were analytical grade.

Flour characterisation

Composition

Moisture (M) and crude protein (P) contents were determined according to the official AACC methods (AACC, 2000); crude fat (F) and total ash (A) contents were analysed in accordance with the official AOAC methods (AOAC, 1990); total carbohydrate content of the flours was calculated by the difference method as $C = 100 - (M + P + F + A)$. Crude fibre contents of the samples were determined in two steps: boiling and incineration, following the official AOAC Methods (AOAC, 1990). The samples were prepared for total phenolic content (TPC) by extracting phenolics in accordance with Byanju *et al.* (2021). TPC of the flour samples were reported in terms of gallic acid equivalent (mg GAE g^{-1}). The compositional analyses of flour samples were replicated three times ($n = 3$).

Technological properties

Water retention capacity (WRC) was determined according to the AACC official method (AACC, 2000). Bulk density (BD), emulsion properties (activity [EA] and stability [ES]), foaming properties (capacity [FC] and stability [FS]) were analysed according to the procedures described in Turan *et al.* (2015). Oil

Absorption Capacity (OAC) was determined in accordance with the procedure reported in Falade & Okafor (2013). The colours of the flours were determined using a colorimeter (CR-400, Konica Minolta, Tokyo, Japan) with standard illuminant D65 in terms of L^* (lightness; black to white), a^* (green to red) and b^* (blue to yellow) in the CIELAB space. The technological characteristics of flour samples were replicated three times (n = 3).

Bread formulations

The recipes for gluten- and yeast-free breads were formulated using a mixture design with the proportions varied according to the extreme vertices method (Minitab, demo v, State College, PA, USA) as given in Table 1. Trials were replicated twice (n = 2) and two loaves were produced in each. In a previous study (Tuna *et al.*, 2023), white bean and hazelnut flours were mixed with rice flour–corn starch mixture in the 0%–30% range based on the results obtained in the micro visco-amylograph (MVA), as the pure legume and nut flours did not produce MVA profiles. In this study, the content of these flours in the total mixture was also kept up to 30% with equally mixed rice flour and corn starch (RC) varying between 70% and 100%. The codes for bread samples were: STD (with 100% *R* and *C*), B15 (with 15% bean flour (*B*) replacement), B30 (with 30% *B* replacement), H15 (with 15% hazelnut flour (*H*) replacement), H30 (with 30% *H* replacement), BH15 (with 15% equally mixed *B* and *H* replacement) and BH30 (with 30% equally mixed *B* and *H* replacement). For each recipe, the ingredients per 100 g of flour mixture were: sugar (9.6 g), salt (3 g), baking powder (4 g), baking soda (1 g), olive oil (7.2 g), vinegar (3 g) and xanthan gum (1.8 g). The amount of water was determined according to the water retention capacity of the flour mix in each formulation. After adding water to the ingredients, the dough was kneaded for 15 min at low speed (KitchenAid 5KSM125, USA). The dough was then transferred to a baking tin (Dr. Oetker, Izmir, Turkey), and baked in an industrial oven (Senox, Izmir, Turkey) at 180 °C for 45 min. Breads were analysed after they cooled down to room temperature.

Textural properties of bread dough

Dough samples were analysed using the backward extrusion technique (Encina-Zelada *et al.*, 2018) by a texture analyser (TA-XT2i, Stable Microsystems, UK) equipped with a back extrusion rig (mod.A/BE) and 25-mm cylinder probe (P/25). The probe approached (2 mm s^{-1}), penetrated 20 mm into the sample (3 mm s^{-1}) and returned (10 mm s^{-1}) to its starting position.

Table 1 Bread formulations according to the extreme vertices design for 100 g flour mixture (n = 2)

Ingredients	STD[†]	B15	B30	H15	H30	BH15	BH30
R-rice flour	50	42.5	35	42.5	35	42.5	35
C-corn starch	50	42.5	35	42.5	35	42.5	35
B-bean flour	0	15	30	0	0	7.5	15
H-hazelnut flour	0	0	0	15	30	7.5	15
Water[‡]	92	106	118	88	86	103	108

[†]STD, standard bread formulation with equally mixed *R* (rice flour) and *C* (corn starch). In other bread codes, *B* and *H* indicate the presence of white bean and hazelnut flours respectively. The numbers after the letters stand for the proportion of the respective flour(s) in the formulation.

[‡]Water added was determined with respect to the water retention capacities (WRC) of the flour mixtures.

At the end of each measurement firmness (N), cohesiveness (N), consistency (N.s) and viscosity index (N.s) parameters were determined. The values for firmness and consistency (thickness of the sample) are read as positive, that is, higher values indicate a firm and thick sample. The viscosity index (resistance to flow), and cohesiveness values are read as negative, that is, the more negative the value, the more resistant and cohesive the material. Each dough sample was measured twice in each replication (n = 2).

Bread properties

Baking loss (%) was calculated using the fresh bread weight and dough weight. The specific volume (mL g^{-1}) and moisture content was determined according to AACC method (AACC, 2000). The crumb colours of each loaf were determined using a colorimeter (CR-400 Konica Minolta, Tokyo, Japan) with standard illuminant D65. Texture profile analysis of bread crumbs, which were cut cubically (2.5 cm) was performed at 40% compression applied twice at 1 mm s^{-1} for pre-test, test, and post-test speeds using a texture analyser (TA-XT2, Stable Microsystems, Godalming, UK) equipped with a 5 kg load cell and 75 mm cylinder probe.

Sensory analysis

The free choice profiling (FCP) was chosen as a rapid sensory description method to conduct the sensory analysis. Three sessions were conducted. In the first session, the four samples were presented simultaneously to the trained assessors (n = 8) using a balanced presentation order (Dairou & Sieffermann, 2002; Vit *et al.*, 2011). The gender of the panellists was divided between four women and four men. The age range was between 25 and 55 years old. The sensory analysis was carried out in a specialised tasting room at IRTA Fruitcentre (UNE-EN ISO 8589:2010). Participants were instructed to generate a list of sensory descriptors to differentiate the samples (Dehlholm *et al*., 2012). To simplify the attribute generation process, participants were suggested to list them according to the order of human senses' perception: smell, appearance, flavour and texture (Dairou & Sieffermann, 2002). To assist the evaluators in generating descriptors, they were given related bread lexicons (Heenan *et al*., 2008). Each assessors' list of attributes was used for the second session. Each participant only evaluated their own list of attributes. The second session consisted of scoring each sample according to the intensity of each attribute on a 10 cm unstructured linear scale from 0 (low intensity) to 10 (high intensity) (Guàrdia *et al*., 2010; Tárrega & Tarancón, 2014; Lazo *et al*., 2016). More in detail, for each sample and descriptor, assessors were asked to mark its position in the linear scale and write down its identification code (three random digits). If no difference is perceived, various samples can have the same intensity in the scale (tie). The third session was a repetition of the second session, conducted on a different day, using the same attributes generated in the first session. Ethical approval for the involvement of human subjects in this study was granted by the Centre for Agrofood Economics and Development (CREDA) Research Ethics Committee, dated 03/01/2023. Participants signed a consent form before taking part in the study.

In vitro starch digestion of bread samples

Starch fractions of bread samples were determined based on a method developed earlier (Englyst *et al*., 2000). Minced samples (0.25 g) were treated with 5 mL gastric enzyme solution containing 1 g/100 mL pepsin and 1 g/100 mL guar gum in 0.05 mol L^{-1} HCl (Sigma-Aldrich, Mannheim, Germany), and glass balls were added during incubation at 37 °C by shaking at 150 r.p.m. for 30 min. A sample was taken at the end of the gastric phase. In order to neutralise the pH, 5 mL of 0.25 mol L^{-1} sodium acetate was added (Merck, Darmstadt, Germany). The intestinal fluid was prepared by adding 3 g pancreatin (EC 232–468-9, Sigma-Aldrich, Mannheim, Germany) in 20 mL distilled water, vortexed for 10 min and centrifuged at 4500 r.p.m. for 10 min. Fifteen millilitres of the supernatant was mixed with 0.666 mL amyloglucosidase (EC 3.2.1.3, Sigma-Aldrich, Mannheim, Germany) and 1 mL invertase (10 mg mL^{-1}) (EC 3.2.1.3, Sigma-Aldrich, Mannheim, Germany). A total quantity of 2.5 mL of the intestinal enzyme mix was added to each sample. The intestinal phase was performed at 37 °C by shaking at 150 r.p.m. for 2 h and samples were collected at 20th and 120th min for rapidly and slowly digestible starch fractions (RDS and SDS). Samples were denatured at 95 °C for 5 min and stored at −20 °C for further analysis. Bread samples were analysed for free sugar glucose to determine present available carbohydrate. Minced samples were dispersed with 1 mol L^{-1} sodium acetate and incubated at 90 °C for 30 min. Invertase treatment was applied at 37°C for 30 min after cooling down the samples. Denaturation was performed at 95 °C for 5 min to end the hydrolysis. D-glucose levels in the samples were analysed with glucose essay kit (GAGO20 Sigma-Aldrich, Mannheim, Germany). A microplate reader (MultiskanTM GO, Thermo Fisher, Waltham, MA, USA) was used to collect absorbance at 540 nm at 37 °C. Results were given as the averages of four measurements ($n = 4$).

Data analysis

Analysis of variance (ANOVA) and Tukey's multiple range tests were performed on the data at $P < 0.05$ to determine any significant differences. Mixture Design (extreme vertices) and analysis of experimental data were evaluated in terms of the *P*-values (models and lack-of-fit) and R^2_{adjusted} values (Minitab, demo v., State College, PA, USA). Results from the rapid sensory description method, the FCP, were analysed by means of a Generalised Procrustes Analysis (GPA) (Guàrdia *et al*., 2010; Párraga *et al*., 2022). GPA was performed using the attributes generated by each assessor and its corresponding intensity (from 0 to 10). The data were analysed using XLSTAT software, version 2020.1 (2020) (Addinsoft, Paris, France).

Results and discussion

Flours

The proximate analysis of flours and starch show that *H* can be considered as a source of plant-based protein like *B*, and at the same time, *H* is a source of fat and fibre, in contrast to *R* and *C*, which are generally the basis of gluten-free products on the market (Table 2). The total phenolic content of *H* was found to be significantly higher. In terms of functional properties, water retention capacity is important in determining the amount of water to be added in bakery products. *B* has the highest water retention capacity, which is related to its high carbohydrate and protein content. Water retention capacity of *H* could not be determined, as reported elsewhere (Turan *et al*., 2015). *H* was found to be significantly different from other flours with its lowest L^*, highest a^* and b^* values. The colour characteristics of *B* were observed between those of *R*, *C* and *H*. Statistically, *C*, *B* and *H* did not show any differences in terms of emulsifying and

Table 2 Composition, technological and colour properties of the flours

	R	*C*	*B*	*H*
Chemical properties				
Moisture (g/100 g)	8.76 ± 0.27^{A}	7.66 ± 0.08^{B}	7.89 ± 0.05^{B}	1.84 ± 0.02^{C}
Crude protein (g/100 g)	5.87 ± 0.05^{C}	0.60 ± 0.00^{D}	18.77 ± 0.02^{A}	15.60 ± 0.03^{B}
Crude fat (g/100 g)	1.14 ± 0.06^{B}	0.38 ± 0.01^{B}	2.08 ± 0.33^{B}	66.38 ± 1.60^{A}
Total ash (g/100 g)	0.89 ± 0.01^{C}	0.09 ± 0.02^{D}	3.25 ± 0.21^{A}	2.01 ± 0.07^{B}
Carb. (g/100 g)	83.34	91.27	68.01	14.17
Crude fibre (g/100 g)	0.13 ± 0.01^{C}	0.06 ± 0.00^{C}	3.71 ± 0.05^{B}	13.43 ± 0.07^{A}
TPC (mg GAE g^{-1})	0.18 ± 0.01^{C}	0.11 ± 0.07^{C}	0.34 ± 0.09^{BC}	2.13 ± 0.19A
Technological properties				
WRC (g/100 g)	113.86 ± 5.04B	74.80 ± 3.01C	261.97 ± 2.85A	nd
BD (g mL^{-1})	0.93 ± 0.03A	0.73 ± 0.04C	0.83 ± 0.03B	0.49 ± 0.00D
OAC (g/100 g)	110.38 ± 10.73AB	79.04 ± 13.04BC	114.48 ± 0.88BC	158.50 ± 20.20A
EA (mL/100 mL)	50.00 ± 0.00AB	51.00 ± 1.41B	59.00 ± 1.41A	54.01 ± 4.23AB
ES (mL/100 mL)	97.00 ± 1.41A	97.04 ± 1.47A	83.91 ± 0.81A	87.47 ± 12.06A
FC (mL/100 mL)	10.00 ± 0.00A	nd	8.92 ± 1.53A	12.00 ± 0.00A
FS (mL/100 mL)	1.00 ± 1.41A	nd	1.49 ± 0.72A	2.00 ± 2.83A
Colour				
*L**	99.02 ± 0.50A	100.26 ± 0.07A	93.60 ± 1.28B	71.46 ± 4.37C
*a**	−1.36 ± 0.02C	−2.50 ± 0.06C	0.95 ± 0.05B	3.25 ± 2.49A
*b**	13.82 ± 0.05^{C}	12.53 ± 0.07^{D}	22.13 ± 0.09^{B}	31.14 ± 0.80^{A}

Mean values in the same row with different letters ($^{A–C}$) are significantly different ($P \leq 0.05$). nd, not detected; *R*, rice flour; *C*, corn starch; *B*, white bean flour; *H*, hazelnut flour.

foaming stability and foaming capacity, while for *R*, no foaming capacity was observed. *B* was found to have the highest emulsifying activity, which can be related to its high protein content. Yet, *B* and *H* have higher mean emulsifying activity and mean foaming capacity respectively. These can be considered as beneficial characteristics for volume and textural properties of gluten-free bakery products as presented in a review by Alfaro-Diaz *et al.* (2023). In the same study, the values of emulsifying activity, emulsifying stability and oil absorption capacity of bean flours from different varieties were reported to be in accordance with the characteristics of *B* given in Table 2. Oil absorption capacity has a positive effect on flavour and the sensation in the mouth. The significantly high oil absorption capacity of *H* can be attributed to its low moisture content, along with significantly high protein and crude fibre (Adeloye *et al.*, 2020). This property makes *H* an effective ingredient in bakery products, contributing to the enhancement of both sensory and nutritional qualities.

Bread dough

Dough rheology in gluten-free doughs is important for dough handling practices and physical properties of the product such as the specific volume and firmness of the bread; defined by some factors including the composition of flour (protein, fat, fibre and starch contents), and water content (Ronda *et al.*, 2017). Water significantly affects the viscoelastic properties of the dough and the physicochemical characteristics of the baked product. The amount of water added was adjusted according to the water retention capacity of the flour mixtures used in each recipe (Table 1). The results of the back-extrusion parameters were used to compare the textural characteristics of the dough samples with the ranges of firmness (4.2–5.7 N), cohesiveness (2.5–3.6 N), consistency (16.8–22.6 N.s) and viscosity index (3.7–5.4 N.s) (Table 3). Among these, sample B30 stood out as its mean values for firmness and consistency (5.70 N and 22.60 Ns respectively), and mean values for absolute cohesiveness and viscosity index (3.55 N and 5.38 Ns respectively) indicated a relatively firmer and thicker sample compared to the others. Nevertheless, the addition of water to the B30 formulation was limited to its water retention capacity, as a better final bread product was obtained in terms of the moisture content. In their study, Encina-Zelada *et al.* (2018) found that the less the water added to the dough, the higher the consistency and viscosity index of the dough, resulting in the highest firmness of the bread prepared with less water. Similar to their report, in this study, the high viscosity index and consistency of the B30 dough can be explained by the amount of water added in the formulation. On the other hand, the addition of *H* to the formulation created an opposite effect on the rheology of the dough, as seen in H15 and H30 doughs, reducing the firmness and consistency. The inclusion of *H* in *B* containing

Table 3 Properties of dough and bread samples

	STD†	B15	B30	H15	H30	BH15	BH30
Dough texture							
Firmness (N)	4.39 ± 0.32^{B}	4.86 ± 0.01^{AB}	5.70 ± 0.44^{A}	4.50 ± 0.32^{B}	4.54 ± 0.14^{B}	4.62 ± 0.75^{AB}	4.23 ± 0.27^{B}
Cohesiveness (N)	−2.47 ± 0.29^{A}	−2.99 ± 0.02^{AB}	−3.55 ± 0.30^{B}	−2.88 ± 0.21^{AB}	−2.83 ± 0.20^{A}	−2.60 ± 0.20^{A}	−2.50 ± 0.11^{A}
Consistency (N.s)	16.77 ± 0.83^{B}	18.93 ± 0.20^{AB}	22.63 ± 1.22^{A}	17.75 ± 1.48^{B}	18.11 ± 1.11^{B}	17.65 ± 2.33^{B}	16.85 ± 1.18^{B}
Viscosity Index (N.s)	−3.87 ± 0.50^{A}	−4.48 ± 0.07^{AB}	−5.38 ± 0.45^{B}	−4.13 ± 0.30^{AB}	−3.91 ± 0.40^{A}	−4.08 ± 0.27^{A}	−3.70 ± 0.19^{A}
Physical properties							
Baking loss (g/100 g)	18.03 ± 1.03^{A}	20.14 ± 0.72^{A}	20.66 ± 1.96^{A}	18.49 ± 0.91^{A}	18.38 ± 0.01^{A}	18.67 ± 0.09^{A}	19.78 ± 1.21^{A}
Specific volume (mL g^{-1})	1.43 ± 0.02^{AB}	1.32 ± 0.01^{C}	1.27 ± 0.01^{C}	1.33 ± 0.03^{BC}	1.29 ± 0.04^{C}	1.50 ± 0.04^{A}	1.51 ± 0.02^{A}
Moisture (g/100 g)	33.25 ± 0.34^{AB}	35.49 ± 0.02^{A}	31.06 ± 0.90^{AB}	33.35 ± 0.09^{AB}	32.91 ± 3.28^{AB}	28.41 ± 0.37^{B}	30.49 ± 0.22^{AB}
Crumb texture							
Hardness (N)	17.96 ± 0.59^{A}	17.84 ± 7.46^{A}	22.73 ± 2.52^{A}	16.50 ± 4.38^{A}	16.03 ± 0.96^{A}	14.85 ± 2.07^{A}	9.04 ± 2.06^{A}
Cohesiveness	0.77 ± 0.00^{A}	0.69 ± 0.03^{B}	0.65 ± 0.03^{B}	0.71 ± 0.02^{AB}	0.67 ± 0.02^{B}	0.71 ± 0.00^{AB}	0.71 ± 0.01^{AB}
Springiness	0.94 ± 0.00^{A}	0.91 ± 0.02^{A}	0.87 ± 0.01^{A}	0.89 ± 0.00^{A}	0.86 ± 0.04^{A}	0.93 ± 0.02^{A}	0.91 ± 0.02^{A}
Chewiness (N)	13.03 ± 0.40^{A}	11.06 ± 3.91^{A}	12.94 ± 0.73^{A}	10.38 ± 2.52^{A}	9.21 ± 0.22^{A}	981 ± 1.23^{A}	5.79 ± 1.11^{A}
Crumb colour							
L^*	71.53 ± 0.34^{A}	69.83 ± 0.32^{B}	64.87 ± 0.17^{C}	56.60 ± 0.05^{E}	54.31 ± 0.25^{F}	65.15 ± 0.05^{C}	62.10 ± 0.81^{D}
a^*	−4.14 ± 0.06^{G}	−3.48 ± 0.05^{F}	−2.98 ± 0.00^{E}	1.96 ± 0.04^{B}	2.40 ± 0.04^{A}	−1.84 ± 0.01^{D}	−0.85 ± 0.06^{C}
b^*	24.46 ± 0.11^{A}	24.40 ± 0.12^{AB}	23.49 ± 0.01^{C}	23.83 ± 0.00^{BC}	22.89 ± 0.08^{D}	24.26 ± 0.03^{AB}	22.85 ± 0.35^{D}

Mean values in the same row with different letters ($^{A–C}$) are significantly different ($P \leq 0.05$).
†STD, standard bread formulation with equally mixed *R* (rice flour) and *C* (corn starch). In other bread codes, *B* and *H* indicate the presence of white bean and hazelnut flours respectively. The numbers after the letters stand for the proportion of the respective flour(s) in the formulation.

formulations (e.g. BH30) caused a decrease in the textural parameters compared to those of B30 dough. In the study by Benkadri *et al.* (2020), the amount of water added to the gluten-free bakery product was also adjusted according to the water retention capacity of the flour used in the formulation.

Bread

Moisture was found to be significantly higher in B15 bread than in BH15, which can be explained by the low and high water retention capacities of *H* and *B* respectively (Table 3). The addition of *H* caused a decrease in the moisture levels. In general, bread samples had L^*, a^* and b^* values that reflected colour of the flours used in the formulations. Colour parameters were significantly affected by the addition of *H*, as also noted by others (Anil, 2007; Tunc & Kahyaoglu, 2016). The darkest crumbs belonged to the *H*-containing samples and STD had the highest L^*, whereas colour values of the *B* containing ones were in between *H* containing breads and STD. High a^* values were dominant in hazelnut breads, due to the presence of brown skin. There were no significant differences among the baking losses (%).

The specific volumes of BH30 (1.51 mL g^{-1}) and BH15 (1.50 mL g^{-1}) were significantly larger than other formulations except STD, while those of B15, H30 and B30 were smaller. Inclusion of both *B* and *H* in the recipe would produce larger volume breads. It was also observed that specific volume increased as the dough cohesiveness and viscosity index decreased (in absolute values). The cohesiveness of dough is an indication of elasticity or the work required for cohesion (Ronda *et al.*, 2017). Index of viscosity is the indicative parameter of the resistance to flow. If the cohesive and viscous structure of the gluten-free dough is low, the final volume of the bread would be higher according to the results (Table 3). In a study of breads made from a composite flour containing wheat, soybean and almond flours, it was observed that addition of almond flour to the soy–wheat flour blend caused a significant increase in the specific volume of the bread samples and homogeneous distribution of air cells in loaves, which was explained by a hypothesis about the effect of lipids on the interaction of wheat and soybean protein molecules (Lodi & Vodovotz, 2008). Their findings on the interaction of legume and nut flours on bread quality were considered similar to the effect of the combination of hazelnut flour as the high lipid ingredient and white bean flour on the increased volume of the breads in this study.

The textural characteristics of a gluten-free bread are essential in influencing consumer preferences. The crumbs of the yeast-free gluten-free breads were subjected to a texture profile analysis with double compression to simulate mouth chewing, where hardness (N), cohesiveness (N), springiness and chewiness

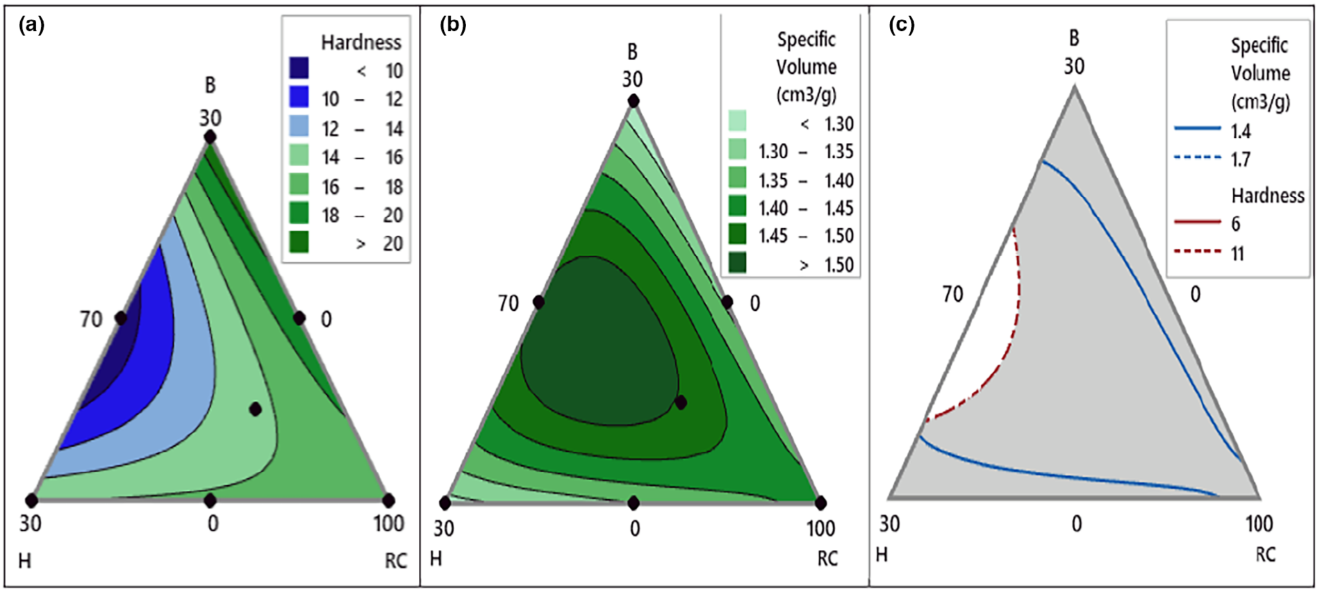

Figure 1 Contour plots for (a) hardness, (b) specific volume and (c) overlay plot for hardness and specific volume. *B*, White bean flour; *H*, Hazelnut flour; RC, rice flour and corn starch mix.

(N.mm) were evaluated. It was generally observed that when flour of a standard formulation was replaced by legume flours, an increase in hardness was observed (Aguiar *et al*., 2022). Statistically, the bread samples had similar hardness values. However, in terms of mean values, the bread with 30% *B* had the highest mean hardness (B30 with 22.7 N), whereas the breads containing *H* in addition to *B* had the lowest mean hardness (BH30 with 9 N). Similar to the findings in this study, another research reported that yeast-leavened breads with the addition of 5%–15% ground hazelnut and walnut had lower hardness than those made with wheat flour alone, which weakened the structure of the dough (Pycia & Juszczak, 2022). Fibres from diverse sources can behave differently in starch matrices such as gluten-free flours. Other researchers observed that the addition of wheat bran increased the crumb hardness, whereas the addition of inulin as a dietary fibre decreased hardness, which was explained by the lower degree of crystallinity (Kiumarsi *et al*., 2019). In a previous study investigating the pasting characteristics of starch-based gluten-free flour and hazelnut flour, it was found that the gelatinisation temperature of gluten-free flour containing hazelnut was significantly higher, and the peak viscosity was lower compared to that of the standard gluten-free flour, and this effect was explained by the high fat and low starch content of the flour mixture (Tuna *et al*., 2023). In the same study, the set back value as an indication of retrogradation was significantly lower in hazelnut flour containing flour mixture, indicating lower consistency of dough samples prepared with hazelnut flour, as also observed in this study. The dough consistency values were found to decrease with the addition of *H* and combination of *H* and *B* (Table 3). This can explain why breads made from the gluten-free flour blend including 30% *H* and *B* (BH30) had the lowest hardness.

Optimised bread formulation

As a result of the mixture design data analysis, significant models ($P < 0.05$) were generated with significant linear terms (*B*, *H* and RC). Some models included significant quadratic and cubic terms (Table S1). The model for hardness ($P < 0.05$, $R^2 = 0.66$ and $R^2_{adjusted} = 0.56$) had a significant interaction term (-BxH) indicating that *B* and *H* alone increased the hardness; however, when used in combination in the formulation, they caused a decrease in the crumb hardness (Fig. 1a). An explanation for this observation can be that *H*, as a high fibre ingredient, could disrupt the starch structure and reduce the degree of crystallinity (Kiumarsi *et al*., 2019). The model for specific volume ($P < 0.01$, $R^2 = 0.94$ and $R^2_{adjusted} = 0.92$) had two significant interaction terms (−BxH and + BxHxRC), and showed a parabolic response. Addition of B or *H* alone decreased specific volume, whereas combined use of *B* and *H* in the formulation caused an increase in the volume (Table 3). The best formulation was determined based on softness and specific volume that are appealing characteristics especially in gluten-free breads. Contour plots for hardness

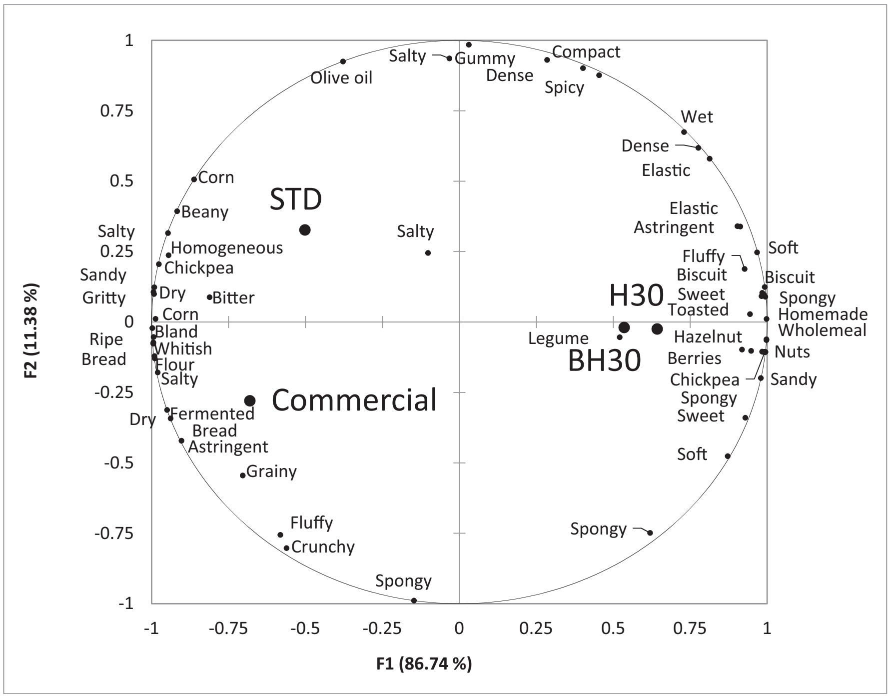

Figure 2 Representation of the first (F1) and second (F2) components of the GPA of data from FCP.

and specific volume (Fig. 1a,b) show that breads with lower hardness and higher specific volumes are breads containing *B* and *H* flours together (15% each). Overlay plot was generated for hardness and specific volume by using their highest and lowest values observed in bread samples (6 and 11 N for hardness, and 1.4 and 1.7 mL g^{-1} for specific volume). It revealed that the best yeast-free bread formulation would be the one with the highest levels of *B* and *H* as shown as the white area in Fig. 1c.

A previous study using a gluten-free flour mixture with 15% hazelnut flour in a yeast-leavened bread reported a specific volume of 3.8 mL g^{-1} and a hardness of 1.6 N (Tuna *et al.*, 2023). In this study, it was observed that the best yeast-free gluten-free breads could be produced with flour mixtures up to 30% bean flour and hazelnut flour. In the latter case, where baking soda and baking powder were preferred to yeast as leavening agents, lower softness and specific volume were observed, however, the enrichment in terms of protein, fibre and healthy fat components can be higher in yeast-free breads.

Sensory analysis

In the final product evaluation, only breads with acceptable texture and appearance were further evaluated. BH30 was selected for the sensory analysis based on the optimisation of the mixture design data due to its larger specific volume and lower hardness values. H30 was also included due to its general appearance, texture and flavour observed during the dough preparation and bread-making stages. The sensory profile obtained with the free choice profiling (FCP) is shown in Fig. 2, which depicts the samples analysed and the sensory descriptors of principal component 1 (F1) and 2 (F2), accounting for 86.74% and 11.38% of the total variance respectively. As can be seen, a pattern with two different groups can be observed: Samples BH30 and H30 are very similar and descriptors such as ' hazelnut flavour' describe them. On the other hand, STD and the commercial samples are also similar for F1 and only differ on F2, although the explained variance is minimal (11.38%). This noticeable trend is mainly due to the flour used in the formulation. In the case of breads made with corn flour (commercial sample) and corn starch and rice flour (STD), the assessors categorised the samples as tasting 'corn' and 'salty'. The commercial sample was most often described as 'bread flavour' and 'fermented flavour'. In terms of appearance, the samples were categorised as 'homogeneous' and 'whitish' compared to the samples H30 and BH30. In terms of texture, the samples are also perceived to be 'gritty' (STD) and 'grainy' (commercial sample), probably due to the absence of gluten in the formulation. An extensive study conducted by Puerta *et al.* (2020), comparing regular bread with gluten-free bread demonstrated the prominent 'sandy' character of bread formulated without gluten, especially the one formulated mainly with rice flour, which is also one of the main components of the gluten-free flour blend used in this study for the STD sample. For the samples with hazelnut flour (H30) and hazelnut and bean flour (BH30), the assessors have described them as having 'sweet taste', 'biscuit flavour' and ' nutty flavour'. These attributes 'sweet taste' or 'biscuit flavour' can be associated to hazelnut flour, as this nut is widely used in the production of confectionary, for example, nougat (López-Mas & Romero del Castillo, 2022). In particular, sample BH30 was described as 'legume flavour', probably due to the predominant taste of the bean flour. Moreover, tasting a bread made with nuts may have influenced assessors to categorise it as a 'wholemeal' product. In terms of texture, the samples were classified as 'spongy' and 'fluffy'. Finally, assessors highlighted its 'homemade' appearance. The aim of comparing commercial products already available on the market with novel food formulations is to ensure that they really have distinctive sensory characteristics (Tóth *et al.*, 2022). Overall, the results of the sensory analysis showed that the novel bread formulations containing nuts and legumes differed from the standard gluten-free (STD) formulation as well as from a commercial product. Therefore, the new bread formulation can offer consumers a product that is different from those already available on the market.

In vitro starch digestion of breads

H30 and BH30 were also analysed for *in vitro* starch digestion and compared with STD. The RDS fraction, reflecting glucose released within 20 min of intestinal digestion, causes a rapid rise in blood glucose levels. The SDS fraction is the result of starch digestion occurring between 20 and 120 min, causing a gradual increase in blood glucose (Englyst *et al.*, 2000). The RDS and SDS fractions (g/100 g dry sample) of H30 and BH30 were found to be significantly lower than those of STD (Table 4). Specifically, the RDS decreased by 22% in H30 and 29% in BH30, compared to standard gluten-free bread. Similarly, the SDS fraction also decreased, being lowest at 3.8 g/100 g in H30. These results can be attributed to the reduced content of the standard gluten-free flour blend (RC) as the main starch ingredient and the low carbohydrate content of *H* in the H30 and BH30 formulations. These factors also explain the absence of an RS fraction in H30 bread, considering that STD based on 100% RC blend was already found to have less than 1 g RS/100 g. Significantly, the highest RS fraction was found in BH30, indicating that *B* was the source of the RS fraction. The combined incorporation of *B* and *H* in gluten-free bread formulations can be nutritionally beneficial in reducing the rapid increase in blood glucose. Approximately 82% of the starch in BH30 bread was found to be digested in the intestine (RDS + SDS), with the remaining 18% as RS. In contrast, for STD, the fraction digested in the intestine was determined to be 99% of the total starch with a very low RS content of 1%.

Table 4 Starch fractions (g/100 g dry sample)

	STD	H30	BH30
RDS	73.8 ± 2.3^{A}	57.0 ± 1.3^{B}	52.4 ± 0.8^{C}
SDS	15.7 ± 0.3^{A}	3.8 ± 0.6^{B}	6.0 ± 2.2^{B}
RS	0.8 ± 1.4^{B}	nd	12.9 ± 2.0^{A}

Mean values in the same row with different letters ($^{A-C}$) are significantly different ($P \leq 0.05$). BH30, bread with 30% equally mixed hazelnut and white bean flours in the formulation; H30, bread with 30% hazelnut flour in the formulation; nd, not detected; RDS, rapidly digestible starch; RS, resistant starch; SDS, slowly digestible starch; STD, standard bread with equally mixed *R* (rice flour) and *C* (corn starch) in the formulation.

In a study of gluten-free yeast-leavened bread samples from the market, RDS, SDS and RS starch fractions (g/100 g) were determined within ranges of 76–93, 2.4–21 and 1–3 respectively (Matos Segura & Rosell, 2011). In another study with gluten-free yeast breads containing rice flour, RDS and SDS contents (%) were reported within ranges of 82–96 and 0.6–11.4 respectively (De La Hera *et al.*, 2014). Similar to the STD bread used in this study, other researchers also compared breads made with an equal mixture rice flour and corn starch to those made with a flour mixture replaced by 30% chickpea flour and acorn flour, and observed lower levels of glucose release and glycaemic index (Gkountenoudi-Eskitzi *et al.*, 2023).

Several factors influence starch fractions, in particular, the type of flour, its microstructure and chemical composition, product texture, preparation and cooking methods. The presence of other compounds, such as water, protein, lipids, fibre and polyphenols affect the bio-accessibility of glucose by limiting gelatinisation, inhibiting digestion enzymes or eliminating the accessibility of enzymes to starch particles (Parada & Aguilera, 2011; Gkountenoudi-Eskitzi *et al.*, 2023). In a research on a pasta formulation with 40% bean flour in a wheat flour base, the reduction in RDS and glycaemic index was explained by the high amylose content of beans and other legumes (Giuberti *et al.*, 2015). The slower digestion of legumes compared to cereals has also been attributed to the partially gelatinised starch granules due to the presence of fibre content (Englyst *et al.*, 2000). In a study, it was reported that incorporating bean flour from different milling stages into gluten-free bread significantly decreased RDS, increased SDS and RS fractions. This result was attributed to the protective action of legume cell walls against the starch digestive enzymes (Boukid *et al*., 2019).

Hazelnut contains high amount of lipids dominated by oleic and linoleic acids (Alasalvar *et al*., 2006). Unsaturated fatty acids tend to form complexes with starch molecules, which in turn, inhibits or slows starch digestion (Parada & Santos, 2016; Shao *et al*., 2022). The lowest moisture content belonged to H30 due to the lowest water additions in the formulation (Table 2). The limited water available for starch gelatinisation in *H* containing breads may lead to a reduction in the RDS fraction (Parada & Santos, 2016). The compact structures in bakery products were also shown to be advantageous in terms of slowly digestible starch content due to the limited access to digestive enzymes (Shao *et al*., 2022). The specific volumes of yeast-free and gluten-free breads were observed lower than those of gluten-free yeast breads (Tuna *et al*., 2023). This property can be considered beneficial for the nutritional value of yeast-free breads.

Starch digestion analyses were carried out on H30 and BH30 breads, and STD as a reference gluten- and yeast-free bread. In order to further discuss the effect of hazelnut and white bean flour content on the starch fractions, it is necessary to analyse the bread samples of other flour formulations in the designed experiments.

Conclusion

A gluten-free flour blend was replaced up to 30% hazelnut and white bean flours to develop a yeast-free bread formulation with no gluten. Optimised levels of both hazelnut and bean flours were determined in terms of lowest hardness and highest specific volume among breads, resulting in 9.04 N and 1.51 mL g^{-1} respectively. The sensory characteristics and starch digestion fractions of the yeast-free breads containing 30% hazelnut flour (H30), and 30% bean and hazelnut flour combination (BH30) were compared with a reference bread (STD) containing only a gluten-free flour base. It was found that the rapidly digestible starch fraction was significantly reduced by 29% and the resistant starch fraction was increased in the breads with hazelnut and bean flours. The resistant starch fraction was 18% of the total starch in BH30, compared to 1% in STD. Based on the results of the nutritional analysis, the combined use of legume and nut flours by partial replacement can reduce the bio-accessibility of glucose. In general, the addition of hazelnut flour together with white bean flour was found to be more beneficial than the addition of either hazelnut or white bean flour alone for the yeast-free bread formulation made with gluten-free flour mixture in terms of technological, textural, nutritional and sensory properties. The inclusion of these functional flours could be an alternative in the gluten-free bread market as their sensory profile differs from the standard formulation and a commercial product.

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Conflict of interest statement

The authors declare no financial and personal conflict of interest.

Author contributions

Ayca Tuna: Investigation; formal analysis; writing – original draft; writing – review and editing. **Jordi Ortiz-Solà:** Investigation; writing – review and editing. **Laura López-Mas:** Formal analysis; writing – review and editing. **Filiz Baser:** Investigation; writing – original draft; writing – review and editing. **Zein Kallas:** Supervision; formal analysis; methodology; writing – review and editing. **Ingrid Aguiló-Aguayo:** Supervision; resources; writing – review and editing. **Sukru Gulec:** Supervision; writing – review and editing. **Banu Ozen:** Supervision; funding acquisition; writing – review and editing; project administration. **Figen Tokatli:** Conceptualization; methodology; supervision; resources; writing – original draft; writing – review and editing.

Data availability statement

Data will be available on request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Models of variables in extreme vertices design.

