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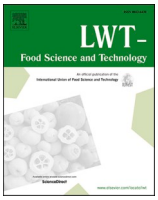

Exploitation of sprouted barley grains and flour through sourdough fermentation

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ABSTRACT

The local barley cultivar Nure was subjected to a controlled sprouting process, obtaining flour and grains that were fermented to produce type II sourdoughs. The germination process led to the reduction of starch and total dietary fibers (−58 and −48% compared to plain barley flour) and to the increase of phenolic compounds and protein bio-accessibility. Fermentation of sprouted barley (SB) with selected lactic acid bacteria determined a further enhancement of its nutritional features, by means of the increased free amino acids (up to 35%) and γ-aminobutyric acid concentrations (up to 57%), and decreased phytic acid content. The potential of SB sourdough to be used as ingredient in bread making was then investigated. Overall, the negative effects (on dough rheology and baking performance) related to the intense enzymatic activities characterizing the sprouted barley flour are strongly mitigated by the fermentation process. Therefore, besides improving bread nutritional and technological attributes, the use of SB sourdoughs, by supplying the native enzymes present in the sprouted grains, but in a less invasive form, could help decrease or substitute the use of commercial enzymes or flour improvers commonly used in the baking industry.

1. Introduction

Nowadays, population growth and lifestyle modifications change the demand for agricultural and food products, pushing food manufacturers towards a deep innovation in the food design. In this context, the use of alternative ingredients to wheat flour, capable to fortify final products in proteins, fibers, bioactive compounds (Amoah et al., 2019) and to diversify the organoleptic profile of conventional staple foods, is strongly increasing. Among these alternative ingredients, the demand for germinated seeds has risen due to the increasingly awareness of their connection with health and nutrition so that in the past decade several European legislations have been implemented to regulate sprouted seeds production (EC no. 208/2013, EC no. 209/2013, EC no. 210/2013).

Germination starts with the uptake of water from the seed and ends with the appearance of the radicle. The complex physical and metabolic events during germination can be grouped in three phases during which the seed becomes fully hydrated, its endogenous metabolism is activated, and a mobilization of reserve material occurs (Lemmens et al., 2019). Once the moisture content reaches the minimum requirement, the seed initiates the synthesis and/or release of plant hormones causing the release of degrading enzymes (amylase, proteases, and lipases). As a result, increases in free amino acids and γ-aminobutyric acid, total phenolic content and consequent antioxidant activity, are observed, as well as decreases of anti-nutritional factors (i.e., phytic acid) with subsequent increased mineral bioavailability (Finnie et al., 2019). These nutrient changes, consequence of the sprouting process, are often associated with health benefits; and although the literature about it is occasionally slender or conflicting, there is supportive data about the direct effect of such changes on *in vivo* health markers. Among cereals, sprouted rice and barley have been largely investigated in clinical studies involving humans or rodents, and compared to their plain flours, germination was found to be correlated to *i*) the reduction of blood serum cholesterol and blood pressure, *ii*) the decrease of blood glucose levels, insulin, and plasma lipid peroxide concentrations, *iii*) the increase of Zn and Fe absorption, *iv*) the reduction of the immunogenicity of gluten, and/or *v*) the increase of short chain fatty acids in the gut (for a review see Lemmens et al., 2019).

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Abbreviations

BF	whole barley flour
CB-B	control barley bread made with non-sprouted in replacement of wheat flour at 10%
CSB-B	control sprouted barley bread, made with sprouted whole barley flours in replacement of wheat flour at 10%
CW-B	control wheat bread made with wheat flour
DPPH	2,2-diphenyl-1-picrylhydrazyl
DY	dough yield
fB100	sourdough made with barley flour
fB100-B	sourdough bread obtained with the addition of 20% of fB100
fSB10	sourdough made with a blend of wheat and sprouted barley flour in the ratio 90:10
fSB100	sourdough made with sprouted barley flour
fSB100-B	sourdough bread obtained with the addition of 20% of fSB100
fSB10-B	sourdough bread obtained with the addition of 20% of fSB10
fSBG10	sourdough made with a blend of wheat flour and sprouted barley grains 90:10
fSBG10-B	sourdough bread obtained with the addition of 20% of fSBG10
GABA	γ-aminobutyric acid
IDF	insoluble dietary fiber
IVPD	*in vitro* protein digestibility
LAB	lactic acid bacteria
PLS-DA	Partial Least-Squares Discriminant Analysis
QF	quotient of fermentation
RH	relative humidity
SBF	sprouted barley flour
SBG	sprouted barley grains
SDF	soluble dietary fiber
TDF	total dietary fiber
TFAA	total free amino acids
WSE	water/salt-soluble extracts

Accordingly, food products containing sprouted grains or flours made thereof, which include baked goods, pasta, breakfast cereals, snacks, and beverages, are perceived by consumers as "natural", "better taste", "more nutritious", and "healthier" (Lemmens et al., 2019). Thanks to their peculiar characteristics, both organoleptic and nutritional, the use of sprouted seeds for wheat bread supplementation is growing (Amoah et al., 2019), nonetheless, the consumption of bread made with sprouted grains actually dates to thousands of years ago. A flourless bread produced from sprouted grains, among which barley, the Ezekiel bread, was mentioned in the Bible (Onyeka & Obeleagu, 2013) and its recipe inspired the many revisitations currently on the market, so that the global Ezekiel bread market was valued hundreds of billions of dollars in 2021 and is expected to grow even more in the forecast period 2022–2028 (Market Research Report, 2022).

It is clear, however, that the indisputably nutritious bread eaten thousands of years ago, does not fit the 21st century concept of bread, mostly due to sensory and technological aspects; indeed, the accumulation of enzymatic activities, if excessive, might hinder dough rheology and baking performance, whereas α-amylase activity can lead to high starch digestibility (Marti et al., 2018).

Therefore, the optimization of recipes for baked goods including alternative ingredients like sprouted grains and derived flours requires the evaluation of all the process parameters that could affect nutritional, sensory and technological aspects of the final product.

In this context, sourdough fermentation has proven to be effective in mitigating the issues of alternative flours while improving the nutritional properties of such matrices and baked goods made thereof (Gobbetti et al., 2019).

Based on the above considerations, in this study, barley grains were subjected to germination and used to prepare type II sourdoughs, obtained by a single-stage fermentation with selected starters (De Vuyst et al., 2021). The effects of germination and fermentation on the main nutritional and functional features were investigated, and experimental breads fortified with sourdoughs made with sprouted grains and flour were also characterized.

2. Material and methods

2.1. Raw materials and microorganisms

Raw materials used in this study included grains of barley (*Hordeum vulgare* var. Nure) provided by Caporalcereali (Gravina di Puglia, Bari, Italy), commercial wheat (*Triticum aestivum)* flour type "0" (Molino Casillo, Corato, BA, Italy) and fresh baker yeast (Lievital, *Lesaffre,* Trecasali, Parma, Italy). Wheat flour was characterized by moisture 12%, protein 13.9% of dry matter; fat 2.3% of dry matter (d.m.), dietary fiber 2.2% of d.m., carbohydrates, 81% of d.m. Lactic acid bacteria (LAB) strains *Lactiplantibacillus plantarum* DSM32248 and *Furfurilactobacillus rossiae* DSM32249, (formerly known as LB1 and LB5 respectively) previously isolated from wheat germ (Rizzello et al., 2010), were selected for their growth and acidification ability and already used as starters for type II sourdough making (Pontonio et al., 2017). LAB strains were singly cultivated in De Man, Rogosa and Sharpe (MRS) at 30 °C until the late exponential phase of growth was reached (ca. 10 h), then cells were harvested by centrifugation (10,000×*g*, 10 min, 4 °C); washed twice in 50 mM phosphate buffer, pH 7.0, and re-suspended in tap water before use.

2.2. Sprouting process and flours production

Barley grains were sprouted according to the protocol previously proposed by Montemurro et al. (2019) with some modifications (Fig. 1) and dried in drying chambers (Binder GmbH, Tuttlingen, Germany) at 50 °C for 25 h. Non-sprouted and dried sprouted grains were milled using a Braun AG (Type 4036, Frankfurt, Germany) laboratory mill to obtain whole barley (BF) and sprouted barley (SBF) flours (particle size <500 μm), respectively. An aliquot of sprouted barley grains (SBG) was used without milling.

2.3. Chemical and biochemical characterization of whole and sprouted barley flours

Protein (total nitrogen × 5.7), fats, ash, starch and moisture were determined according to the AACC approved methods 46-11 A, 30–10.01, 08–01, 76–13.01 and 44-15 A, respectively (AACC, 2010). Carbohydrates were calculated as the difference [100 − (proteins + lipids + ash + total dietary fibres + starch)]. Proteins, lipids, carbohydrates and ash were expressed as % of dry matter. Insoluble (IDF) and soluble (SDF) dietary fibres were determined according to the procedure previously described by Goñi et al. (2009). Water/salt-soluble extracts (WSE) of whole and sprouted barley flours were prepared according to Weiss et al. (1993) and used to determine total free amino acids (TFAA) concentration. TFAA and GABA (γ-aminobutyric acid) were analyzed by a Biochrom 30 series Amino Acid Analyzer (Biochrom Ltd., Cambridge Science Park, England) with a Li-cation-exchange column (4.6 × 200 mm internal diameter), as described by De Pasquale et al. (2021). The

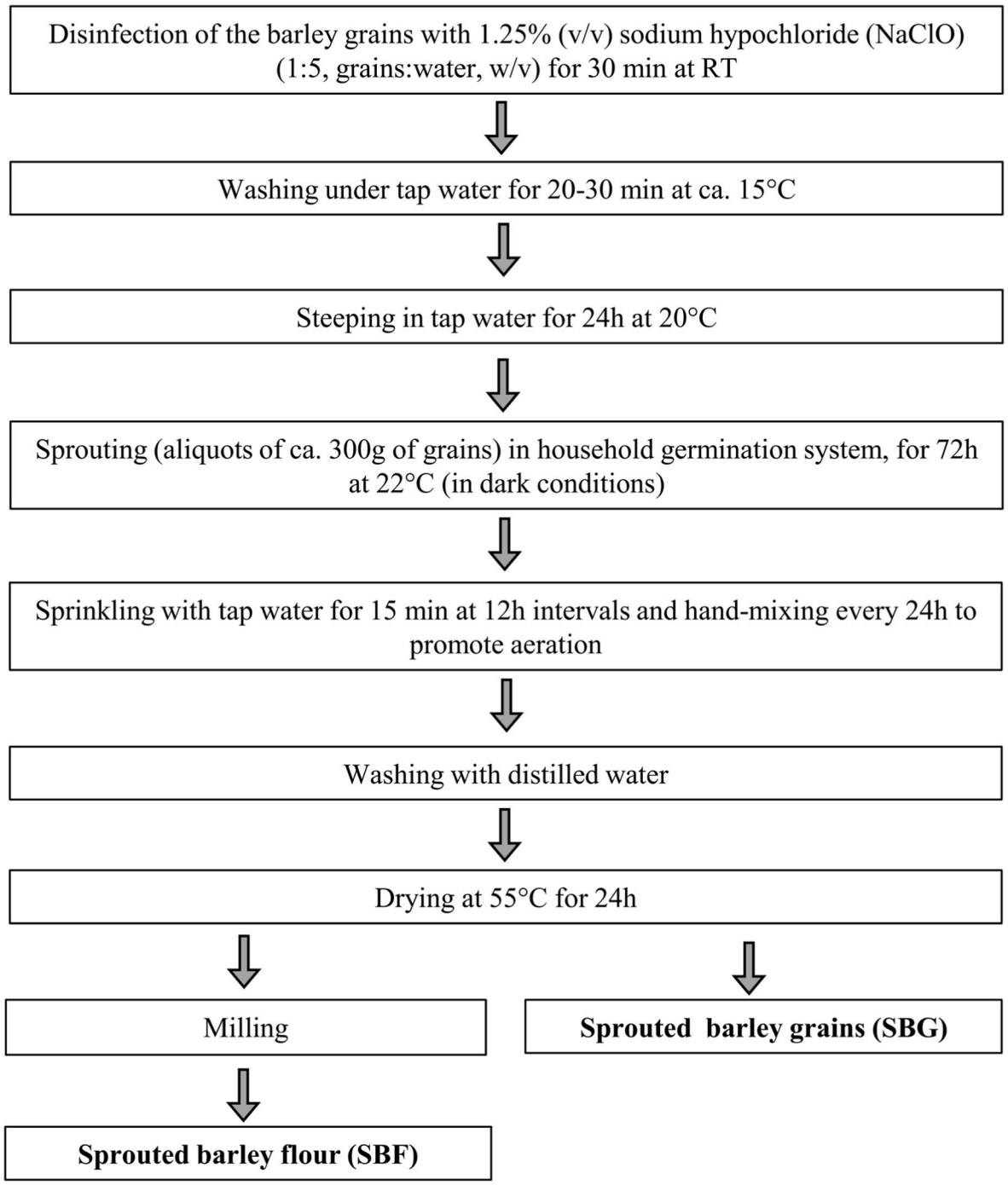

Fig. 1. Flow-chart of the germination process employed for obtaining sprouted barley grains (SBG) and sprouted barley flour (SBF).

methanolic extracts of flours were obtained as reported by Montemurro et al. (2019) to determine the total phenols content and the antioxidant activity. Total phenols concentration was determined as described by Slinkard and Singleton (1977) and expressed as gallic acid equivalent. The radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was used for determining the antioxidant activity as free radical scavenging activity (Rizzello et al., 2010). The synthetic antioxidant butylated hydroxytoluene was included as a reference (75 ppm) in the analysis. The *in vitro* protein digestibility (IVPD) of the flours was determined by the method proposed by Akeson and Stahmann (1964) with some modifications (Rizzello et al., 2010). Samples were subjected to a sequential enzyme treatment mimicking the *in vivo* digestion in the gastrointestinal tract and the IVPD was expressed as the percentage of the total protein which was solubilized after enzymatic hydrolysis. The concentration of protein in digested and non-digested fractions was determined by the Bradford method (Bradford, 1976).

2.4. Microbiological characterization of flours

For microbiological analysis, 20 g of sprouted and non-sprouted barley flour were homogenized with 180 ml of sterile peptone water (1% [w/v] of peptone and 0.9% [w/v] of NaCl) solution. LAB were enumerated using MRS agar (Oxoid, Basingstoke, Hampshire, UK) agar medium, supplemented with cycloheximide (0.1 g/l). Plates were incubated, under anaerobiosis (AnaeroGen and AnaeroJar, Oxoid), at 30 °C for 48 h. Cell densities of yeasts and moulds were respectively

estimated on Saboraud Dextrose Agar (Oxoid) and Yeast Peptone Dextrose Agar medium (Sigma-Merck, Darmstadt, Germany) supplemented with chloramphenicol (0.1 g/l), through pour and spread plate enumeration, respectively, and incubated at 25 °C for 72 h. Total mesophilic aerobic bacteria were determined on Plate Count Agar (Oxoid) at 30 °C for 48 h, and total Enterobacteriaceae were determined on Violet Red Bile Glucose Agar (Oxoid) at 37 °C for 24 h. *Bacillus* spp. cell density was determined on Dextrose Casein Peptone Agar (Sigma-Merck, Darmstadt, Germany) after an incubation at 37 °C for 72 h.

2.5. Sourdough fermentation

Four experimental sourdoughs were produced. All doughs were produced in a 1:1 flour: water ratio corresponding to a dough yield (DY, dough weight × 100/flour weight) of 200 and fermented by *L. plantarum* and *F. rossiae*, each inoculated at the final cell density of 7 Log_{10} cfu/g. Doughs were formulated as follow: fSB100, made with sprouted barley flour; fB100, made with barley flour; fSB10, made with a blend of wheat and sprouted barley flour in the ratio 90:10; fSBG10, made with a blend of wheat flour and sprouted barley grains 90:10. The weight of the sprouted barley grains was considered in the dough yield calculation. All doughs were fermented for 16 h at 30 °C (Fig. S1).

2.6. Sourdough characterization

Before (t0) and after (t16) fermentation, pH and total titratable acidity (TTA) of the experimental sourdoughs were determined. The pH was determined by a pHmeter (Model 507, Crison, Italy) with a food penetration probe. TTA was determined as the amount of 0.1 M NaOH required to adjust the end pH of 10 g dough in sterile water to 8.3. At (t0) and (t16), WSE of the experimental sourdoughs were prepared and used to determine the content of TFAA and GABA, as previously described. The WSE were also used to analyze the organic acids (lactic and acetic) and sugars (glucose and maltose) concentration. Lactic and acetic acids were respectively analyzed with K-DLATE and K-ACET kits (Megazyme International Ireland Limited, Bray, Ireland). The quotient of fermentation (QF) was determined as the molar ratio between lactic and acetic acids. Glucose and maltose were analyzed with K-GLUC and K-MASUG (Megazyme) kits, following the manufacturer's instructions.

2.7. Bread making

Seven experimental breads were manufactured at the pilot plant of the Department of Soil, Plant and Food Sciences of the University of Bari (Bari, Italy). All breads were obtained from doughs with DY 160 corresponding to a flour/water ratio of 62.5/37.5% (w/w). Experimental breads were as follow: CW-B, control wheat bread made using only wheat flour; CB-B and CSB-B, control barley bread and control sprouted barley bread respectively, made using non-sprouted and sprouted whole barley flours in replacement of wheat flour at 10% (w/w) on the dough weight. Four more sourdough breads were produced: fSB100-B, fB100-B, fSB10-B and fSBG10-B, obtained with the addition at 20% (w/w) on the dough weight of the experimental sourdoughs fSB100, fB100, fSB10 and fSBG10 respectively. The amount of the replaced wheat flour with non-sprouted and sprouted barley whole flours was the same for CB-B, CSB-B, fSB100-B, and fB100-B breads (10% (w/w) on the dough basis, corresponding to 16% (w/w) on the flour basis). All doughs were leavened by adding fresh baker yeast at 1.5% on dough weight (w/w) (Table 1). The water content for the bread recipes was that calculated as optimal for the wheat flour based on the Brabender Farinograph determination (Brabender GmbH & Co. KG, Germany). Flours from non-sprouted and sprouted barley and the experimental sourdoughs were mixed with wheat flour, water, and fresh baker yeast in a mixer bowl (Electrolux assistant, EKM4000) for 5 min at low speed and 5 min at fast speed. The doughs were divided into pieces of 200 g, shaped mechanically, and rested in baking trays for 20 min at 25 °C and relative humidity (RH) of 75%, then were leavened in a fermentation cabinet (Zucchelli S. p.a) for 90 min at 25 °C and RH 85%. The loaves were baked in a rotating rack oven (Zucchelli forni S. p.a) at 220 °C for 20 min. After baking, the breads were cooled for 2 h at room temperature. Baking was done on two different days (two independent baking trials) and five loaves were prepared for each type of experimental bread. Each sample was analyzed twice.

Table 1
Recipes for the experimental breads. Control (C-) breads were produced without sourdough addition: CW-B, control wheat bread made with wheat flour; CB-B and CSB-B, control barley bread and control sprouted barley bread, respectively made with non-sprouted and sprouted whole barley flours in replacement of wheat flour at 10% (w/w) of the total dough weight. Sourdough breads fSB100-B, fB100-B, fSB10-B and fSBG10-B were obtained with the addition of 20% (w/w, on the dough weight) experimental sourdoughs fSB100, fB100, fSB10 and fSBG10 respectively. All the sourdoughs had DY 200 and were fermented at 30 °C for 16 h by DSM32248 (*L. plantarum*) and DSM32249 (*F. rossiae*) inoculated at 7 log_{10} cfu/g. All the bread doughs had a final DY of 160 and were leavened by adding 1.5% (w/w) of fresh baker's yeast.

Recipes		CW-B		CB-B		CSB-B		fSB100-B		fB100-B		fSB10-B		fSBG10-B	
		%d.b [a]	f. b.[b]	%d. b.	%f. b.	%d. b.	%f. b.	%d. b.	%f. b.	%d. b.	%f. b.	% d.b.	%f. b.	% d.b.	%f. b.
Type "0" wheat flour		62.5	100	52.5	84	52.5	84	52.5	84	52.5	84	52.5	84	52.5	84
Barley flour		–	–	10	16	–	–	–	–	–	–	–	–	–	–
Sprouted barley flour		–	–	–	–	10	16	–	–	–	–	–	–	–	–
Water		37.5	60	37.5	60	37.5	60	27.5	44	27.5	44	27.5	44	27.5	44
Sourdough (DY 200)		–		–	–	–		20	32	20	32	20	32	20	32
	type "0" wheat flour	-	-	-	-	-	-	-	-	-	-	*9*	*14.4*	*9*	*14.4*
	Sprouted barley flour	-	-	-	-	-	-	*10*	*16*	-	-	*1*	*1.6*	-	-
	barley flour	-	-	-	-	-	-	-	-	*10*	*16*	-	-	-	-
	Sprouted barley grains	-	-	-	-	-	-	-	-	-	-	-	-	*1*	*1.6*
	Water	-	-	-	-	-	-	*10*	*16*	*10*	*16*	*10*	*16*	*10*	*16*
Fresh baker's yeast		1.5	2.4	1.5	2.4	1.5	2.4	1.5	2.4	1.5	2.4	1.5	2.4	1.5	2.4
Total Flour[c]		62.5	100	62.5	100	62.5	100	62.5	100	62.5	100	62.5	100	62.5	100
Total Water[d]		37.5	60	37.5	60	37.5	60	37.5	60	37.5	60	37.5	60	37.5	60

[a] d.b. dough basis.
[b] f.b. flour basis.
[c] Total flour of the recipe was calculated as the sum of flour from sourdough and flour used in baking.
[d] Total water was calculated as the sum of water from sourdough and water used in baking For the control and sourdough breads the total amount of water was the same, 60% of f. b.

2.8. Bread characterization

2.8.1. Doughs biochemical characterization and breads proximal composition

The analysis of pH, TTA, organic acids, QF and TFAA of the dough after proofing process were carried out as reported earlier. Phytic acid was determined by using K-PHYT 05/07 (Megazyme) kit. The proximal composition and energy value of experimental breads were determined following the (AACC, 2010) methods reported above in section 2.3, while the 32–05.01 method was used for the quantification of total dietary fiber.

2.8.2. Nutritional characterization

The IVPD of breads was determined as reported in section 2.3. Starch hydrolysis index of bread (HI) was determined by mimicking the *in vivo* digestion of starch (De Angelis et al., 2009). Aliquots of breads, containing 1 g of starch, were subjected to enzymatic process and the released glucose content was measured with d-D-glucose assay Kit (GOPOD-format, Megazyme) following manufacturer's instructions. The degree of starch digestion was expressed as the percentage of potentially available starch hydrolyzed after 180 min. Control wheat bread (C-WB) was used as the reference to estimate the hydrolysis index (HI = 100). The predicted glycemic index (pGI) was calculated using the equation: pGI = 0.549 × HI+39.71 (Capriles & Arêas, 2013).

2.8.3. Technological characterization

Doughs leavening performance of the different samples, was evaluated determining the volume increase (ΔV, mL) and was expressed as the percentage of volume increase. The specific volume of the breads was calculated as the loaf volume (mL)/loaf weight (g) ratio, after 2–6 h of cooling. Texture profile analysis was performed by using an FRTS-100 N Texture Analyzer (Imada, Toyohashi, Japan) equipped with a 3 cm cylinder probe FR-HA-30 J on boule-shaped loaves (200 g) stored for 2 h at room temperature after baking. The instrument settings were test speed 1 mm/s, 30% deformation of the sample, and two compression cycles, and the parameters evaluated were hardness, cohesiveness, springiness, and chewiness. The chromaticity co-ordinates of the crust and crumb of the bread (obtained by a Minolta CR-10 camera) were reported as color difference, ΔE^*_{ab}, calculated by equation below reported, where ΔL, Δa and Δb are the differences for L, a and b values between sample and reference (a white ceramic plate having L = 93.4, a = − 1.8 and b = 4.4).

$$\Delta E * ab = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

2.8.4. Sensory analysis

Sensory evaluations were carried following the independent method of the "Sensory analysis - Methodology - Flavour Profile" methods (ISO 6564-1985) with some modification. The library of the Department of Soil, Plant and Food Science of the University of Bari (Italy) was used instead of cabinets as previously suggested by Elia (2011). Sensory analysis of breads was carried out by 10 trained panelists (5 males and 5 females, mean age: 30 years, range: 18–54 years). Sensory attributes included: visual and tactual perception (crust and crumb color, elasticity, friability); taste (acidic taste, sweetness, salty, herbaceous taste, bitter flavor); scent perception (acidic odor); chewing (chewiness), using a scale from 0 to 10, with 10 the highest score. Slices (1.5 cm thick) were served in random order and evaluated in two replicates by all panelists. A glass of water was drunk by the panelists after each sample analysis. Final scores for each attribute were calculated as the means of the data collected in three independent evaluations.

2.9. Statistical analysis

All the analysis were carried out in triplicate for each batch of sample. Data were subjected to one-way ANOVA; pair-comparison of treatment means was achieved by Tukey's procedure at $P < 0.05$, using the statistical software Statistica 12.5 (StatSoft Inc., Tulsa, USA). The data obtained from the nutritional and technological characterization of the breads were also analyzed through the Partial Least-Squares Discriminant Analysis (PLS-DA), using the software MetaboAnalyst version 5.0 (metaboanalyst.ca/; accessed online July 14th, 2023).

3. Results

3.1. Sprouted flour

The SBF, obtained by the milling of the dried sprouted grains, was characterized for the main chemical and microbiological features, and compared with a barley flour obtained by non-sprouted barley grains from the same batch. Both the flours, characterized by moisture of 10.5%, were produced without separation of the bran and the germ. Protein, fat, and ash concentrations were similar ($P > 0.05$) between the two flours (Table 2). Protein, in particular, ranged from 13.61 to 14.02% of d.m.

The carbohydrates fraction of the two flours markedly differed: SBF was characterized by a very high concentration of sugars (6-times higher than BF), and a lower concentration in starch (−58%) and total dietary fibers (−42%) compared to BF. An intense degradation of the IDF fraction occurred during the sprouting process (a decrease of the 53% when compared to BF) (Table 2).

Sprouting led to the increase of TFAA content, that resulted more than 5-times higher in SBF compared to BF. The functional amino acid GABA, in particular, was more than 10-times higher, and was found at concentration of 352 mg/kg. IVPD was significantly ($P < 0.05$) higher in SBF (84 vs 54%). As the consequence of the sprouting process, total phenols concentration, determined in the methanolic extract, resulted almost doubled in SBF compared to BF. Accordingly, antioxidant activity, determined as DPPH scavenging activity, was significantly higher in SBF (Table 2).

Sprouting also affected the flour microbiota. In particular, viable mesophilic aerobic bacteria, yeasts, LAB, *Bacillus* spp. and *Enterobacteriacee* were significantly ($P < 0.05$) higher in SBF compared to BF. Aerobic bacteria, LAB, and *Enterobacteriacee* increased of ca 2 logarithmic cycles (Table 3) hereas *Bacillus* spp. density, that increased of ca. 1 log cycle, was lower than 3 $\log_{10}$ cfu/g.

Table 2
Characterization data for sprouted (SBF) and non-sprouted (BF) whole barley flours.

	SBF	BF
Moisture (%)	10.55 ± 0.21[a]	10.47 ± 0.12[a]
Protein (g/100g) d.m.[a]	13.61 ± 0.32[a]	14.02 ± 0.36[a]
Fat (g/100g) d.m.	1.50 ± 0.05[a]	1.41 ± 0.04[a]
Carbohydrates (g/100) d.m.	72.11 ± 2.19[a]	63.35 ± 2.15[b]
Sugars (g/100 g) d.m.	48.61 ± 1.18[a]	7.95 ± 1.02[b]
Starch (g/100 g) d.m	23.5 ± 1.05[b]	55.4 ± 1.12[a]
Total dietary fiber (g/100g)	12.64 ± 0.18[b]	21.52 ± 0.32[a]
Soluble dietary fiber (g/100 g)	3.79 ± 0.11[a]	2.93 ± 0.11[b]
Insoluble dietary fiber (g/100 g)	8.85 ± 0.12[b]	18.59 ± 0.25[a]
Ash (g/100g) d.m	2.12 ± 0.10[a]	2.46 ± 0.16[a]
Total free amino acids (mg/kg)	8119 ± 95[a]	1475 ± 100[b]
GABA (mg/kg)	352 ± 25[a]	32 ± 2[b]
Total phenols (mmol/kg)	5.21 ± 0.50[a]	2.70 ± 0.27[b]
Radical scavenging activity (%)	94 ± 4[a]	81 ± 3[b]
IVPD (In-Vitro Protein Digestibility, %)	83.7 ± 2.4[a]	54 ± 3.1[b]

The data are the means of three independent analysis ± standard deviations (n = 3). [a–b]Values in the same row with different superscript letters differ significantly ($p < 0.05$).

[a] d.m, data are expressed on dry matter basis.

Table 3
Microbiological characterization of sprouted (SBF) and non-sprouted (BF) barley flours.

Parameters	SBF	BF
Mesophilic aerobic bacteria (Log_{10} cfu/g)	6.78 ± 0.14^{a}	4.82 ± 0.11^{b}
Yeasts (Log_{10} cfu/g)	3.44 ± 0.10^{a}	2.03 ± 0.12^{b}
Moulds (Log_{10} cfu/g)	3.03 ± 0.12^{a}	3.27 ± 0.18^{a}
LAB (Log_{10} cfu/g)	3.09 ± 0.09^{a}	1.30 ± 0.11^{b}
***Enterobacteriaceae* (Log_{10} cfu/g)**	5.69 ± 0.18^{a}	2.66 ± 0.05^{b}
***Bacillus* spp. (Log_{10} cfu/g)**	2.53 ± 0.22^{a}	1.52 ± 0.13^{b}

The data are the means of three independent analysis ± standard deviations (n = 3). [a–b]Values in the same row with different superscript letters differ significantly ($p < 0.05$).

3.2. Sourdoughs characterization

Four different type II sourdoughs were produced and characterized. Overall, intense acidification and organic acid production were observed for all, as consequence of an active lactic fermentation (Table 4). In particular, the comparison of the two sourdoughs entirely produced with SBF and BF showed that fSB100 was characterized by an acetic acid concentration ca. 2.5-times higher than fB100. Consequently, QF was significantly ($P < 0.05$) lower in fSB100 compared to fB100 (4.3 *vs.* 13) (Table 4).

The synthesis of lactic and acetic acid was markedly lower (up to 36 and 63%, respectively) when SBF was used in mixture with wheat flour. fSBG, containing the SBG, was characterized by the lowest concentration of both the organic acids among all the sourdoughs (Table 4). Relatively higher amount of maltose was found in SBF-containing sourdoughs compared to BF or SBG (Table 4).

Significant increases of TFAA were found in sourdough after fermentation, compared to t0 (Fig. 2). Clearly, before fermentation, sourdoughs obtained by sprouted barley were characterized by a higher amino acid content (proportional to the addition percentage), compared to sourdough containing non-sprouted barley. Nevertheless, the increments observed after fermentation were markedly higher (more than 3-folds) in fB100 compared to the respective t0; whereas for SB sourdoughs the increments ranged from 23 to 35% (in fSB10 and fSB100, respectively). The trend was similar for all FAA, including the functional amino acid GABA, which almost reached a concentration of 800 mg/kg in fSB100 and 250 mg/kg in fB100.

3.3. Breads characterization

3.3.1. Biochemical and nutritional properties

Four different sourdough breads were produced, characterized, and compared for the main chemical, nutritional, technological, and sensory characteristics to a group of control breads, leavened with baker's yeast and produced without addition of barley LAB pre-fermented ingredients. As expected, pH of sourdough breads was significantly ($P < 0.05$) lower than that of control breads (Table 5). In details, pH of sourdough breads ranged from 4.51 to 4.75 (values corresponding to fSB100-B and fSBG10-B, respectively). Lactic and acetic acid concentration of sourdough breads were in line with concentrations observed in sourdoughs: indeed, although with level ca. 4–6 times lower than the corresponding sourdoughs, fSB100-B and fB100-B were respectively characterized by the highest lactic and acetic acid concentration, respectively (Table 5).

The highest concentration of phytic acid was found in CB-B, while the CSB-B, containing sprouted instead of non-sprouted barley flour, was characterized by a significant ($P < 0.05$) lower amount (Table 5). All the sourdough breads were characterized by the lowest amount of phytic acid, that ranged from 64 (fSBG10-B) to 70 mg/100 g (fB100-B).

The analysis of the proximal composition of the breads did not show significant differences among the breads (Table 5), with the exception of the TDF content, that was the highest for breads containing non-sprouted barley flours (CB-B and fB100 with 3.63 and 3.82%). According to the decrease of TDF occurring during the sprouting process, CSB-B was characterized by a 28% lower TDF concentration compared to CB-B, while sourdough fermentation did not affect TDF content of the breads (Table 5).

IVPD of the CSB-B was significantly higher (+13%) than that of CB-B, and further moderate but significant ($P < 0.05$) increases were found when sourdough fermentation was applied. Indeed, fB100-B had IVPD 7% higher than CB-B, and fSB100-B 4% higher than CSB-B (Table 5).

CW-B was considered as reference for the calculation of the HI. The addition of the BF to the bread caused significant ($P < 0.05$) decrease of the HI (82.66 vs 98.86), while a lower decrease was found when SBF was added to the control baker's yeast bread (90.61 vs 98.86). Sourdough fermentation caused a further decrease of the HI of the bread containing non-sprouted barley flour (fB100-B, 77.63). Sourdough breads fSB100-B, fSB10-B and fSG10-B, that contained SBF or SBG, were characterized by HI values significantly ($P < 0.05$) higher than fB100-B, as the consequence of the higher sugars concentration. Consequentially, pGI was higher for control breads CW-B and CSB-B compared to sourdough breads and CBF-B.

3.3.2. Technological properties

No significant ($P > 0.05$) differences were found in dough volume increase during the proofing step before baking (Table 6), although some differences become evident on loaves only after baking. Indeed, the addition of BF caused a marked (−25%) and significant ($P < 0.05$) decrease of the specific volume of CB-B compared to CW-B, while no differences ($P > 0.05$) were found when SBF was added (CSB-B vs CW-B). The use of the sourdoughs entirely made with BF or SBF caused a decrease of the specific volume up to 11%. When sourdoughs were made with lower amount of SBF (or SBG) no significant ($P < 0.05$) differences of the specific volume were found (fSB10-B and fSBG10-B vs. CW-B, Table 6).

Table 4
Sourdough characterization data. pH, total titratable acidity (TTA), organic acids (lactic and acetic), quotient of fermentation (QF), glucose and maltose concentration were determined before (t0) and after (t16) fermentation. fSB100, made with sprouted barley flour; fB100, made with barley flour; fSB10, made with a blend of wheat and sprouted barley flour in the ratio 90:10; fSBG10, made with a blend of wheat flour and sprouted barley grains 90:10. The weight of the sprouted barley grains was considered in the dough yield calculation. All doughs were inoculated with *L. plantarum* DSM32248 and *F. rossiae* DSM32249 and fermented at 30 °C for 16 h.

	fSB100		fB100		fSB10		fSBG10	
	t0	t16	t0	t16	t0	t16	t0	t16
pH	5.89 ± 0.07^{a}	3.91 ± 0.11^{b}	5.98 ± 0.18^{a}	3.88 ± 0.15^{b}	5.61 ± 0.23^{a}	3.68 ± 0.09^{b}	5.47 ± 0.42^{a}	3.67 ± 0.33^{b}
TTA (ml NaOH)	4.40 ± 0.31^{d}	25.21 ± 1.41^{b}	4.13 ± 0.28^{d}	32 ± 2.12^{a}	1.83 ± 0.33^{e}	13 ± 0.92^{c}	1.43 ± 0.11^{e}	11.4 ± 0.08^{c}
Acetic acid (mmol/kg)	0.12 ± 0.03^{e}	25.45 ± 2.12^{a}	0.08 ± 0.01^{e}	10.9 ± 0.66^{b}	0.43 ± 0.08^{d}	9.39 ± 1.65^{b}	0.11 ± 0.02^{e}	3.21 ± 0.32^{c}
Lactic acid (mmol/kg)	0.78 ± 0.12^{e}	110.14 ± 7.33^{b}	0.67 ± 0.02^{e}	146 ± 9.22^{a}	0.69 ± 0.02^{e}	70.86 ± 4.62^{c}	0.91 ± 0.04^{d}	61.89 ± 3.99^{c}
QF	–	4.3 ± 0.21^{d}	–	13 ± 0.17^{b}	–	7.5 ± 0.12^{c}	–	19.2 ± 0.9^{a}
Glucose (g/100g)	0.49 ± 0.02^{c}	1.23 ± 0.05^{a}	0.11 ± 0.01^{d}	1.18 ± 0.09^{a}	0.14 ± 0.02^{d}	0.35 ± 0.09^{c}	0.05 ± 0.01^{e}	0.13 ± 0.02^{d}
Maltose (g/100g)	0.80 ± 0.03^{c}	1.84 ± 0.07^{a}	0.18 ± 0.02^{d}	n.d.	0.38 ± 0.08^{d}	1.26 ± 0.06^{b}	0.26 ± 0.09^{d}	0.97 ± 0.19^{c}

Data are the means of three independent analysis ± standard deviations (n = 3). [a–d] Values in the same row with different superscript letters differ significantly ($p < 0.05$).

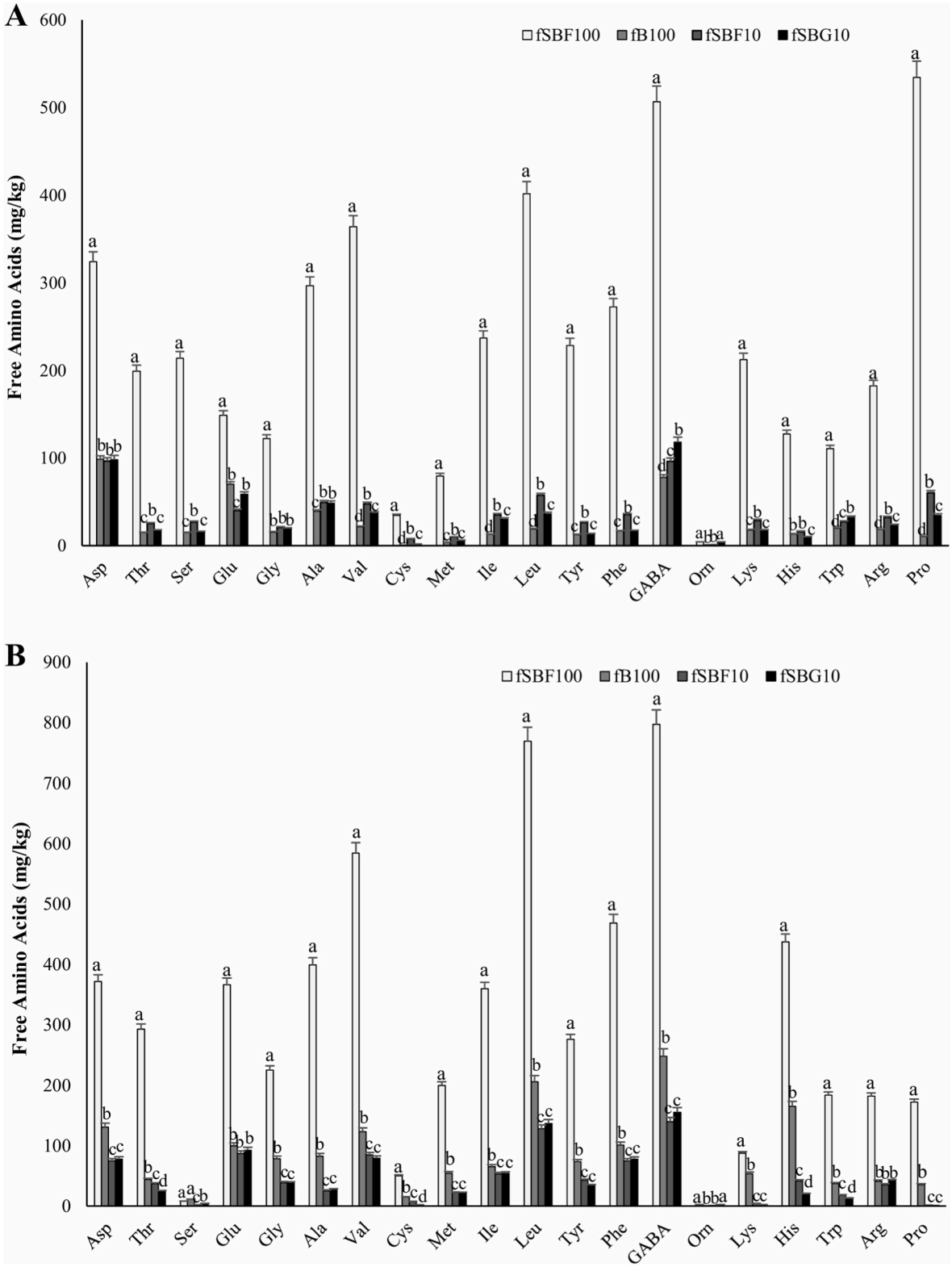

Fig. 2. Concentration of free amino acids and their derivatives (mg/kg) in sourdoughs, before (t0, panel A) and after (t16, panel B) fermentation. fSB100, sourdough made with sprouted barley flour; fB100, sourdough made with barley flour; fSB10, sourdough made with a blend of wheat and sprouted barley flour in the ratio 90:10 (weight based); fSBG10, sourdough made with a blend of wheat flour and sprouted barley grains 90:10 (weight based). All doughs were inoculated with *L. plantarum* DSM32248 and *F. rossiae* DSM32249 (7 $\log_{10}$ cfu/g) and fermented at 30 °C for 16 h. Data ± are the means of three independent analyses. Three-letters amino acid code (IUPAC) was used. $^{a\text{-}d}$ Values with different superscript letters within the same amino acid, differ significantly ($P < 0.05$). Bars of standard deviations are also represented.

Table 5
Bread characterization data. CW-B, control wheat bread made with wheat flour; CB-B and CSB-B, control barley bread and control sprouted barley bread, respectively, made with non-sprouted and sprouted whole barley flours in replacement of wheat flour at 10% (w/w) of the total dough weight. Sourdough breads fSB100-B, fB100-B, fSB10-B, and fSBG10-B were obtained with the addition of 20% (w/w, on dough weight) experimental sourdoughs fSB100, fB100, fSB10, and fSBG10 respectively.

	CW-B	CB-B	CSB-B	fSB100-B	fB100-B	fSB10-B	fSBG10-B
Dough characterization[1]							
pH	5.67 ± 0.23^{a}	5.73 ± 0.31^{a}	5.56 ± 0.38^{a}	4.51 ± 0.17^{b}	4.56 ± 0.19^{b}	4.64 ± 0.24^{b}	4.75 ± 0.33^{b}
TTA (ml)	3.20 ± 0.41^{d}	3.53 ± 0.21^{d}	3.72 ± 0.26^{d}	9.03 ± 1.11^{a}	9.21 ± 0.93^{a}	7.12 ± 0.57^{b}	4.5 ± 0.19^{c}
Lactic acid (mmol/kg)	1.69 ± 0.08^{d}	1.73 ± 0.11^{d}	1.03 ± 0.09^{e}	31.48 ± 3.71^{b}	41.14 ± 4.32^{a}	24.01 ± 1.37^{c}	21.79 ± 2.12^{c}
Acetic acid (mmol/kg)	0.73 ± 0.12^{c}	0.73 ± 0.05^{c}	1.99 ± 0.39^{b}	3.88 ± 0.87^{a}	2.91 ± 0.41^{a}	0.49 ± 0.06^{d}	0.97 ± 0.13^{c}
Phytic acid (mg/100 g)	72 ± 3^{bc}	85 ± 4^{a}	77 ± 1^{b}	66 ± 3^{c}	70 ± 3^{c}	68 ± 1^{c}	64 ± 3^{c}
TFAA (mg/kg)	440.30 ± 11.66^{e}	491.35 ± 7.42^{d}	1175.47 ± 39.14^{b}	1830.36 ± 84.22^{a}	801.26 ± 30.19^{c}	772.63 ± 21.17^{c}	737.50 ± 21.02^{c}
Bread proximal composition and nutritional aspects							
Moisture (g/100 g)	32.26 ± 1.13^{a}	31.38 ± 1.97^{a}	32.21 ± 2.44^{a}	31.12 ± 1.99^{a}	30.88 ± 2.04^{a}	33.36 ± 2.22^{a}	32.36 ± 1.87^{a}
Protein (g/100 g)	9.28 ± 0.34^{a}	10.1 ± 0.62^{a}	9.39 ± 0.44^{a}	9.81 ± 0.32^{a}	9.90 ± 0.52^{a}	9.51 ± 0.65^{a}	9.30 ± 0.27^{a}
Fat (g/100 g)	1.58 ± 0.23^{a}	1.28 ± 0.19^{a}	1.36 ± 0.10^{a}	1.41 ± 0.21^{a}	1.31 ± 0.14^{a}	1.51 ± 0.22^{a}	1.30 ± 0.33^{a}
Carbohydrates (g/100 g)	55.30 ± 1.77^{a}	53.14 ± 1.14^{a}	54.42 ± 1.39^{a}	54.85 ± 0.54^{a}	54.09 ± 0.88^{a}	53.96 ± 1.05^{a}	55.8 ± 1.44^{a}
TDF (g/100 g)	1.58 ± 0.35^{c}	3.63 ± 0.24^{a}	2.62 ± 0.18^{b}	2.81 ± 0.27^{b}	3.82 ± 0.21^{a}	1.66 ± 0.11^{c}	1.33 ± 0.15^{d}
Energy Value (kJ/100 g)	1153 ± 118^{a}	1136 ± 138^{a}	1141 ± 99^{a}	1158 ± 101^{a}	1164 ± 87^{a}	1132 ± 121^{a}	1148 ± 133^{a}
IVPD (%)	68.37 ± 1.79^{c}	66.23 ± 1.61^{c}	74.83 ± 1.11^{b}	81.03 ± 2.92^{a}	70.82 ± 1.14^{bc}	75.13 ± 2.84^{ab}	74.97 ± 3.03^{ab}
HI	98.86 ± 2.84^{a}	82.66 ± 2.48^{b}	90.61 ± 3.07^{ab}	81.39 ± 3.12^{b}	77.63 ± 3.66^{c}	84.95 ± 2.32^{b}	88.23 ± 3.01^{b}
pGI	93.98 ± 2.67^{a}	85.09 ± 2.66^{b}	89.45 ± 2.44^{a}	84.40 ± 2.72^{b}	82.33 ± 1.96^{b}	86.34 ± 1.63^{c}	88.17 ± 2.55^{b}

TDF, Total dietary fiber**; IVPD**, In vitro protein digestibility; **HI**, starch hydrolysis index; **pGI**, predicted glycemic index; Data are the means of three independent analysis ± standard deviations (n = 3). $^{a–e}$Values in the same row with different superscript letters differ significantly ($p < 0.05$).
[1] Data obtained on bread doughs before baking (at the end of leavening at 30 °C for 1.5 h).

Table 6
Technological and structural characterization of the breads. CW-B, control wheat bread made with wheat flour; CB-B and CSB-B, control barley bread and control sprouted barley bread, respectively made with non-sprouted and sprouted whole barley flours in replacement of wheat flour at 10% (w/w) of the total dough weight. Sourdough breads fSB100-B, fB100-B, fSB10-B, and fSBG10-B were obtained with the addition of 20% (w/w, on the dough weight) experimental sourdoughs fSB100, fB100, fSB10 and fSBG10 respectively.

	CW-B	CB-B	CSB-B	fSB100-B	fB100-B	fSB10-B	fSBG10-B
Volume increase (%)	36.47 ± 2.78^{a}	38.46 ± 2.17^{a}	36.32 ± 2.55^{a}	41.18 ± 2.97^{a}	40.00 ± 2.40^{a}	41.18 ± 3.11^{a}	38.82 ± 2.25^{a}
Specific volume (g/cm^3)	2.48 ± 0.21^{a}	1.88 ± 0.08^{b}	2.21 ± 0.19^{a}	1.96 ± 0.11^{ab}	1.85 ± 0.09^{b}	2.22 ± 0.14^{a}	2.26 ± 0.17^{a}
Hardness (N)	49.34 ± 2.94^{a}	51.20 ± 3.01^{a}	16.09 ± 1.27^{c}	38.64 ± 1.66^{b}	55.73 ± 3.19^{a}	37.28 ± 2.33^{b}	35.78 ± 1.95^{b}
Cohesiveness	0.646 ± 0.15^{a}	0.620 ± 0.16^{a}	0.269 ± 0.21^{c}	0.529 ± 0.22^{b}	0.621 ± 0.17^{a}	0.663 ± 0.29^{a}	0.625 ± 0.21^{a}
Springiness	0.90 ± 0.07^{a}	0.92 ± 0.04^{a}	0.90 ± 0.09^{a}	0.88 ± 0.08^{a}	0.92 ± 0.07^{a}	0.93 ± 0.10^{a}	0.92 ± 0.06^{a}
Chewiness (N)	28.51 ± 2.26^{a}	29.15 ± 2.38^{a}	5.11 ± 0.66^{d}	17.99 ± 1.77^{c}	31.72 ± 2.24^{a}	22.92 ± 0.97^{b}	20.46 ± 1.55^{b}
Crust color							
L	59.36 ± 1.66^{a}	58.11 ± 2.02^{a}	41.63 ± 1.45^{c}	43.73 ± 2.23^{c}	62.77 ± 2.73^{a}	48.66 ± 1.86^{b}	53.55 ± 2.17^{b}
a	5.66 ± 0.41^{b}	4.86 ± 0.87^{b}	8.48 ± 1.12^{a}	10.04 ± 1.37^{a}	4.31 ± 1.01^{b}	8.81 ± 1.45^{a}	8.02 ± 0.88^{a}
b	20.32 ± 1.40^{a}	18.80 ± 1.22^{ab}	16.10 ± 1.13^{b}	17.01 ± 2.24^{ab}	18.34 ± 1.88^{a}	19.11 ± 0.78^{a}	21.12 ± 1.95^{a}
dE	38.26 ± 2.24^{c}	38.68 ± 2.07^{c}	53.93 ± 3.33^{a}	52.43 ± 3.09^{a}	34.15 ± 2.75^{c}	48.16 ± 2.51^{ab}	44.21 ± 2.77^{b}
Crumb color							
L	64.52 ± 2.22^{a}	57.42 ± 4.85^{a}	53.90 ± 1.89^{b}	60.58 ± 1.66^{a}	60.54 ± 2.94^{a}	60.67 ± 2.33^{a}	64.17 ± 4.00^{a}
a	−0.35 ± 0.07^{e}	0.09 ± 0.02^{c}	−0.14 ± 0.01^{d}	0.38 ± 0.08^{b}	0.99 ± 0.11^{a}	−0.84 ± 0.14^{f}	−0.82 ± 0.17^{f}
b	11.63 ± 1.77^{a}	12.76 ± 1.04^{a}	12.74 ± 1.55^{a}	12.72 ± 0.98^{a}	12.51 ± 0.67^{a}	11.04 ± 1.44^{a}	11.43 ± 1.02^{a}
dE	29.93 ± 2.50^{c}	37.07 ± 2.08^{ab}	40.52 ± 3.55^{a}	34.01 ± 2.15^{b}	34.01 ± 1.97^{b}	33.53 ± 3.09^{bc}	30.21 ± 3.05^{c}

The data are the means of three independent experiments ± standard deviations (n = 3).
$^{a–f}$Values in the same row with different superscript letters differ significantly ($p < 0.05$).

The instrumental analysis of the texture revealed that the addition of the SBF caused a significant ($P < 0.05$) decrease of hardness, cohesiveness and chewiness of the CSB-B compared to CW-B and CB-B. Moreover, the textural parameters showed significant differences between breads containing sourdough fermented BF or SBF compared to those produced with the non-fermented one. CSB-B, in particular, was characterized by markedly lower values of hardness and cohesiveness compared to those observed for fSB100-B, fB100-B, fSB10-B, and fSBG10-B. Compared to fSB100-B, produced with a 100% SBF sourdough, fSB10-B and fSBG-10 were both characterized by similar hardness and slightly but significant ($P < 0.05$) higher chewiness.

From the colorimetric analysis emerged that crust color was mostly influenced by the addition of SBF, both fermented or not. Indeed, compared to CW-B, lower lightness (up to 30%) and higher red/green (a) index (up to 78%) were found for CSB-B, fSB100-B, and fSBG10, and fSBG10-B, proportionally to the addition. On the contrary, the addition of non-sprouted barley flour did not impact chromaticity indexes (Table 6).

The data collected from the nutritional and technological characterization were analyzed through a PLS-DA, that resulted in a high accuracy for principal components 1 and 3 (Fig. 3). The VIP scores also reported in Fig. 3, which can be considered as indexes of the importance of the variables in the PLS-DA model, demonstrated that the ones that mainly contributing to the stratification were lactic and phytic acid concentration, hydrolysis index, and springiness.

3.3.3. Sensory profile

The sensory analysis revealed that the addition of fB100 and fSB100, as consequence of higher barley supplementation rates and its fermentation, had the highest impact on bread taste and flavor, emphasizing acidity attributes, herbaceous and bitter taste (Fig. 4). Sourdoughs made with a blend of wheat flour and sprouted barley (fSB10 and fSBG10), provided a more neutral flavor to the breads, similar to that of CW-B and CB-B. It is worth of notice that CSB-B was characterized by the highest sweetness. The scores for crumb color, instead, were influenced by the percentage of barley addition being significantly higher in CB-B, CSB-B,

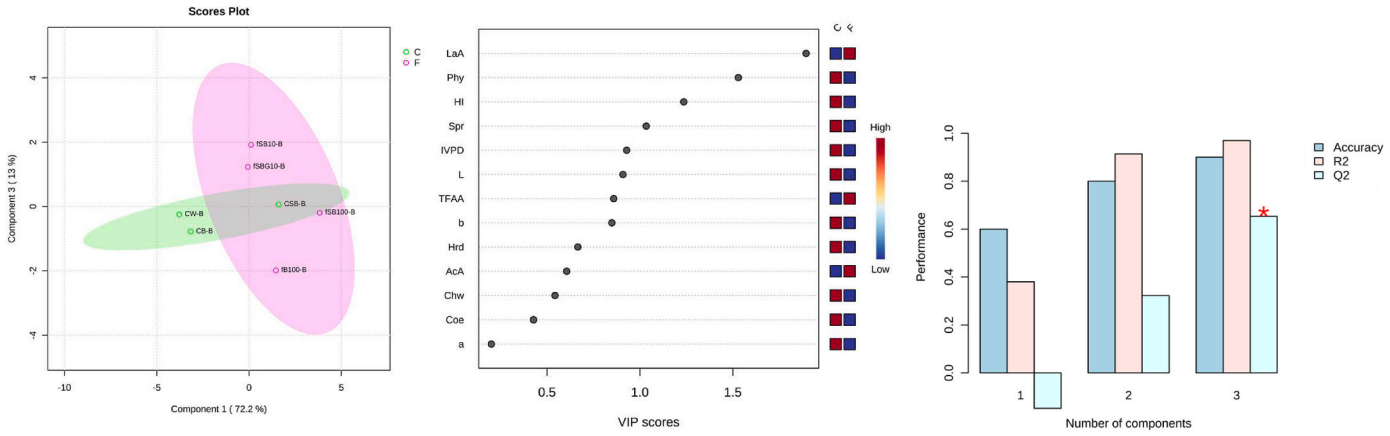

Fig. 3. Partial Least-Squares Discriminant Analysis (PLS-DA) of breads nutritional and technological features. Score plot, VIP scores, and cross validated Q2/R2 coefficients produced as a result of the PLS-DA analysis of control (C, green) and sourdough (F, pink) breads. CW-B, control wheat bread made with wheat flour; CB-B and CSB-B, control barley bread and control sprouted barley bread, respectively, made with non-sprouted and sprouted whole barley flours in replacement of wheat flour at 10% (w/w) of the total dough weight. Sourdough breads fSB100-B, fB100-B, fSB10-B, and fSBG10-B were obtained with the addition of 20% (w/w, on dough weight) experimental sourdoughs fSB100, fB100, fSB10, and fSBG10 respectively.

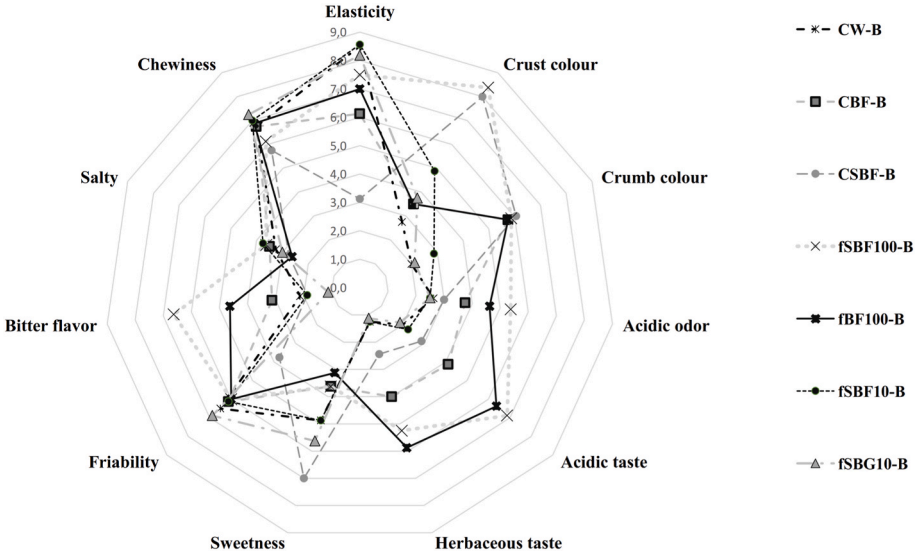

Fig. 4. Spider web chart of the results obtained in the sensory analysis of the experimental breads. CW-B, control wheat bread made with wheat flour; CB-B and CSB-B, control barley bread and control sprouted barley bread, respectively made with non-sprouted and sprouted whole barley flours in replacement of wheat flour at 10% (w/w) of the total dough weight. Sourdough breads fSB100-B, fB100-B, fSB10-B and fSBG10-B were obtained with the addition of 20% (w/w, on the dough weight) experimental sourdoughs fSB100, fB100, fSB10 and fSBG10 respectively.

fB100-B, and fSB100-B compared to CW-B, fSB10-B, and fSBG10-B. Overall, descriptors for structure were similar in all breads except for the control bread containing only SBF, which was the most affected by the supplementation, that led to the lowest elasticity, friability and chewiness.

4. Discussion

Promoting the utilization of barley, one of the oldest cultivated crops in the world, might benefit the sustainability of the agrifood system mainly because of its good level of adaptability to unfavorable environments like cold, drought, or poor soils (Gürel et al., 2016). This concept is particularly amplified if addressed to local and adapted varieties, pivotal for resilient agroecosystems, especially in the current global change (Ficiciyan et al, 2018). Furthermore, for many years, the consumption of whole-grain barley and its components has been linked to a decreased risk for several chronic diseases, including cardiovascular diseases, metabolic syndrome, and some forms of cancer (Zhang et al., 2021). Still, despite its agronomic and health-promoting potential, most of the barley produced is used for animal feed or malting, whereas only 2% is used directly for human consumption (Sharma et al., 2021) and the food industry is faced with the challenge of producing novel barley-based foods that are both healthy and tasty.

In this framework, the local barley cultivar Nure, known to be more resistant to abiotic stress compared to other landraces (Landi et al., 2019), was subjected to a controlled sprouting process, obtaining whole flour and grains that were fermented to produce type II sourdoughs. The potential of sprouted barley grains to be used as ingredient in bread making was then investigated.

According to the literature, when SBF composition was analyzed and compared to that of non-germinated barley flour, a clear reduction of starch and fibers was observed. Indeed, the sprouting process initiates the *de novo* synthesis of starch degrading enzymes such as α-amylase and α-glucosidase leading to the partial hydrolysis of starch into glucose,

maltose, and maltotriose, and a wide range of dextrins (Lemmens et al., 2019). A decrease of insoluble fibers, often followed by an increase in soluble fiber as in the case of this study, was already observed in the first 48 h of germination of wheat (Arora et al., 2010; Koehler et al., 2007) and barley (Montemurro et al., 2019), reaching up to 50% of the initial content when 96 h of germination are exceeded (Koehler et al., 2007). Fiber breakdown could also be ascribed to β-galactosidases, which act on galactomannan yielding galactose (Arora et al., 2010). Another crucial event occurring during germination is the production and secretion of endopeptidase from the aleurone layer and scutellum, resulting in the degradation of seed storage proteins (Lemmens et al., 2019) and consequent higher protein bio-accessibility compared to the non-germinated flour. Indeed, as already reported by Montemurro et al. (2019), SBF in our study showed an *in vitro* protein digestibility 30% higher than BF. Similarly, increases of the phenolic compounds and antioxidant activity of sprouted cereals and pseudocereals were reported elsewhere (Alvarez-Jubete et al., 2010; Ha et al., 2016) and ascribed to the role of polyphenols which act as defense components against environmental stress. However, such increment was reported to be the highest after 48 h of barley germination since, after that, the process of lignification initiates, resulting in the conversion of phenolic compounds to lignans or lignin (Ha et al., 2016).

Nevertheless, if on one side, the degradation of macronutrients by these enzymes provides an energy source for the developing embryo, and consequently improves the nutritional properties of sprouted grains, it can have a significant impact on its performances as ingredient. Indeed, *i*) the high amylase activity results in a decrease of the starch pasting peak viscosity, impacting the starch properties of the ingredient but also its digestibility; *ii*) the increase in proteases may lead to the breakdown of gluten-forming proteins, reducing the overall stability of the dough; whereas *iii*) the high lipase activity can result in the degradation of lipids and their potential autooxidation, generating off-flavors in the finished product (Finnie et al., 2019; Marti et al., 2018). Sprouted grains cannot be therefore used as such, yet a pre-treatment is necessary to bring out the positive nutritional features of germination while compensating the negative once; and a few studies have demonstrated that the synergistic application of germination and fermentation can be a suitable tool to reach this goal (Arora et al., 2010; Montemurro et al., 2019; Perri, Coda, et al., 2021; Perri, Rizzello, et al., 2021).

Herein, sprouted barley was used to manufacture type II sourdoughs using *Lactiplantibacillus plantarum* DSM32248 and *Furfurilactobacillus rossiae* DSM32249, selected for their growth and acidification ability and already used as starters for the fermentation of several matrices (Pontonio et al., 2017; Rizzello et al., 2010). SBF was used as sole ingredient for the sourdough production or at 10% wheat flour replacement, as flour or whole grains. The two percentages of barley (100 and 10%) employed for sourdough making were chosen as representative of two extremely different usage conditions, whose investigation can provide the basis for future applications at intermediate concentrations. Sourdoughs biochemical features were in line with the percentage used and further increases of FAA concentrations were observed in accordance with those already reported (Montemurro et al., 2019).

The sourdoughs fSB100, fSB10, and fSBG10 were then used to produce fortified breads which were compared to common wheat bread, a bread made with SBF and breads made with non-sprouted barley flour and its sourdough. Sprouted flours are often used as ingredient, resulting in a stealth effect, still studies show that grains and particulates thereof, which maintain piece identity in the dough, may be used to enhance texture for consumers desiring visually distinct whole grains or grain particulates (Finnie et al., 2019). To the best of our knowledge, the use of whole germinated grains in sourdough production was never exploited but it draws inspiration from a soaking technique used in bakeries, originally coming from the need to produce bread with whole kernels. Indeed, baking bread with whole grains is a laborious process which requires soaking prior to dough kneading, yet the soaking process can lead to a contamination of the kernels with consequent undesired fermentation and off-flavors. For this reason, companies leaders in the bakery-ingredients sector have developed sourdoughs containing whole kernels and paved the way in to the market for similar products with sprouts (Brandt, 2007). The experimental breads in our study reflected the composition of the sourdoughs used in terms of organic acids and TFAA. The higher protein bio-accessibility deriving from the germination process increased the IVPD which was further enhanced by the fermentation process as already reported (Montemurro et al., 2019). As for starch digestibility, a combination of factors should be considered to explain the differences in breads HI. Compared to CW-B, the addition of whole barley flour, due to the presence of fibers, reduced HI in CB-B, yet further improved after fermentation in fBF100-B, as reported elsewhere (Gobbetti et al., 2019). On the contrary, the supplementation of SBF provided a lower amount of fibers, more soluble sugars and partially digested starch, which led to a HI in CSB-B, slightly but not significantly lower than CW-B. Still the combination of germination and fermentation, due to the presence of organic acids and possibly a higher amount of resistant starch, determined a significant decrease of fSB100-B, fSB10-B, and fSBG10-B hydrolysis index.

Regarding the rheological features of products containing sprouted grains, collapses of the dough structure during leavening were previously reported for bread containing sprouted wheat (Marti et al., 2018) and ascribed to excessive enzymatic activities. Indeed, as predicted, CSB-B presented clear texture problems, being the one characterized by the lowest hardness and cohesiveness values, appearing almost sticky, most likely due to the excessive α-amylase activity of the sprouted flours, which led to the release of maltodextrins also responsible for the highest sweet taste perceived in the sensory analysis and the darkest crust color as consequence of the Maillard reaction. This aspect, hindering the structure of bread, did not affect fSB100-B, fSB10-B, and fSBG10-B which, on the contrary, were characterized by a significantly lower hardness, being considered softener and more elastic than CW-B, CB-B and CSB-B. It is indeed possible that the acidic pH reached during sourdough fermentation limited α-amylase activity, which generally has an optimum around neutral pH. It was indeed found that acidic pH (below 4) can lead to the irreversible inactivation of cereals amylases (Muralikrishna & Nirmala, 2005). Structural improvements of sourdough bread were previously proposed through the *in-situ* synthesis of dextran in a mixture of sprouted lentil and barley fermented with *Leuconostoc pseudomesenteroides* DSM20193 (Perri, Rizzello, et al., 2021). However, due to the low percentage of barley addition (less than 3% on dough weight) compared to our experimental bread (fSB100-B), and to a limited metabolic activity of the strain at the low fermentation temperatures necessary to synthesize dextran, the nutritional advantage imparted by the sprouted barley sourdough, was moderate.

Therefore, our results provided a valid option to promote the use of sprouted barley as food ingredient, especially if the increasing health consciousness of consumers, which is currently fueling the demand for sprouted grains, is taken into account. Indeed, sprouted grains are preferred over the normal counterpart due to their enhanced nutritional values, thus feeding their constant market growth.

In our study, fSB10-B and fSBG10-B, although containing sprouted barley were the once providing the best compromise between nutritional and technological features of the bread, being perceived more like the control breads in terms of sensory and structural properties while providing a more balanced nutritional profile. Though, it remains clear that higher percentages of sprouted barley flours/grains could be exploited by the food industry as part of the recipe optimization process. Moreover, besides improving bread nutritional and technological attributes, the use of germinated and fermented barley as proposed in this study, by supplying the native enzymes present in the sprouted grains, but in a less invasive form, could help decrease or substitute the use of commercial enzymes or flour improvers commonly used in the baking industry.

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CRediT authorship contribution statement

Giuseppe Perri: Investigation, Data curation, Visualization. **Andrea Minisci:** Conceptualization. **Marco Montemurro:** Methodology. **Erica Pontonio:** Resources, Supervision. **Michela Verni:** Writing – review & editing, Visualization. **Carlo G. Rizzello:** Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115326.

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