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Defatted durum wheat germ to produce type-II and III sourdoughs: Characterization and use as bread ingredient

Giuseppe Perri [a], Marcello Greco Miani [b], Gianfranco Amendolagine [b], Erica Pontonio [a], Carlo Giuseppe Rizzello [c,*]

[a] Department of Soil, Plant and Food Science, University of Bari Aldo Moro, Via Amendola 165/a, 70126, Bari, Italy
[b] Casillo Next Gen Food s.r.l, Via Sant'Elia, SNC, 70033, Corato, Bari, Italy
[c] Department of Environmental Biology, "Sapienza" University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy

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ABSTRACT

A fermentation protocol including selected lactic acid bacteria has been applied to defatted durum wheat germ, resulting from the oil extraction, to produce a nutritionally valuable ingredient for bread production. An integrated approach was used to evaluate the microbiological, nutritional, technological, and sensory properties of the fermented ingredient and the corresponding fortified bread. The fermentation led to a significant increase of the concentration of free amino acids (3-times) and decrease of the phytic acid (50%) and raffinose (93%) contents. The bread fortified with the sourdough-fermented defatted wheat germ could be labelled as source of fiber (3.3 g/100 g of bread) and source of protein (15.4% of the energy value was provided by proteins), according to the Regulation EC No. 1924/2006. When the fermented ingredient was used, the free amino acids concentration was 80% higher and the glycemic index lower (84 *vs* 95) than the control bread. Although final volume, hardness and chewiness of bread fortified with the fermented ingredient were similar to those of the control bread, an easier fracturability was found probably due to the high content of dietary fibers and acidity. Sensory analysis showed that fermented defatted wheat germ conferred perceptible acidic odor and taste to the bread.

1. Introduction

The actual trend of the global population growth, will lead to reach 9 billion people by 2050, thus requiring a notable increase in food production (Foresight, 2011) together with a deep rearrangement of the supply food chains under the long-term sustainability approach. Although very difficult to achieve, it has been estimated that a 70% increase in food production will be necessary (Hodges, Buzby, & Bennett, 2011). One of the main issues related to the global requirement is associated to the remarkable amount of wasted edible food, corresponding to a third of the total weight of production (FAO, 2014). In a modern vision of the global resources, by-products discarded during food processing could be considered as a useful biomass, still containing valuable components that can be further used to produce new products (Chandrasekaran, 2012). The by-products valorization should be associated with the growing interest in food products with added healthy and functional benefits (Petrovic'et al., 2017).

Together with bran, wheat germ (WG), corresponding to the 2–4% of the whole kernel weight, is considered as the most important by-product of the milling industry: the world amount of WG produced is estimated to be, annually, *circa* 25,000,000 tons (Rizzello, Nionelli, Coda, De Angelis, & Gobbetti, 2010). WG is removed during milling to prolong the shelf-life of flour and to avoid the development of oxidative processes and related rancid off-flavors (Geng, Harnly, & Chen, 2015; Rizzello, Nionelli, Coda, De Angelis, & Gobbetti, 2010). Rancidity is due to the activity of endogenous oxidative and hydrolytic enzymes on the unsaturated fats (Xu et al., 2013). Hence, the stability of WG separated through milling process is limited to a few days, thus limiting its commercial and industrial applications (Sjövall, Virtalaine, Lapveteläinen, & Kallio, 2000; Srivastava, K., Sudha, Baskaran, & Leelavathi, 2007).

Despite its instability, it was previously reported that WG possess several functional properties (e.g., antioxidant, antihyperlipidemia, hypocolesterolemic, antimicrobial, and anticancer effects) (Ghafoor et al., 2018; Kumar et al., 2011; Mueller & Voigt, 2011; Rizzello, Cassone, Coda, & Gobbetti, 2011; Rizzello et al., 2013). WG is moreover

* Corresponding author.
E-mail addresses: giuseppe.perri483@virgilio.it (G. Perri), marcello.miani@casillogroup.it (M. Greco Miani), gianfranco.amendolagine@casillogroup.it (G. Amendolagine), erica.pontonio@uniba.it (E. Pontonio), carlogiuseppe.rizzello@uniroma1.it (C.G. Rizzello).

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Abbreviations

WG	wheat germ
DWG	defatted wheat germ
fDWG	fermented defatted wheat germ
VRBGA	violet red bile glucose agar
PCA	plate count agar
WSE	Water/salt-soluble extracts
C-WB	control wheat bread
DWG-B	defatted wheat germ bread
fDWG-B	fermented defatted wheat germ bread
DY	dough yield; FQ fermentation quotient
pGI	predicted glycemic index
GI	glycemic index
HI	starch hydrolysis index
LAB	lactic acid bacteria
MRS	De Man, Rogosa and Sharpe
RH	relative humidity
TPA	texture profile analysis
TDF	total dietary fibers
TFAA	total free amino acids
TTA	total titratable acidity

considered as an excellent source of nutrients such as proteins, essential amino acids, dietary fibers, unsaturated fatty acids, vitamins E and B, minerals, flavonoids and sterols (Niu, Jiang, Pan, & Pang, 2013; Sun, Zhang, Hu, Xing, & Zhuo, 2015; Zhu, Wang, & Guo, 2015). Nowadays, WG is mainly used as a feed, while applications in human nutrition are limited and probably not fully explored (Boukid, Folloni, Ranieri, & Vittadini, 2018; Rizzello et al., 2011).

An emerging application for WG valorization includes the extraction of WG oil (Boukid et al., 2018). WG lipid fraction corresponds to 9.0–17 g/100 g (Boukid et al., 2018) and it is characterized by abundance of unsaturated fatty acids such as oleic, linoleic and α-linoleic acids (Rizzello, Nionelli, Coda, De Angelis, & Gobbetti, 2010), suggesting WG oil can be marketed as dietary supplement or food ingredient (Giménez et al., 2013). Moreover, WG oil has been already proposed as ingredient for pharmaceuticals and cosmetic formulations (Gorusupudi & Baskaran, 2013; Niu et al., 2013).

Nevertheless, also the extraction of wheat germ oil leads to the obtainment of another by-product, the Defatted Wheat Germ (DWG) (Niu et al., 2013), that still deserves to be further exploited as food ingredient or supplement. Indeed, DWG contains dietary fibers (more than 30%) and about 10–30% proteins (Sun et al., 2015) having quality comparable to the reference protein defined by FAO/WHO (Arshad, Anjum, & Zahoor, 2007; Boukid et al., 2018) and to egg and milk proteins (Ge, Ni, Yan, Chen, & Cai, 2002). Hence, DWG potentially represents a valuable ingredient with high functional and nutritional interest (Boukid et al., 2018).

Recently, technological, nutritional, functional, and shelf-life advantages of the fermentation of WG using selected lactic acid bacteria (LAB) have been highlighted (Verni, Rizzello, & Coda, 2019). Such sourdough-inspired processes have been identified as suitable for the exploitation of WG as valuable ingredient for the fortification of staple foods such as baked goods (Rizzello, Nionelli, Coda, Di Cagno, & Gobbetti, 2010) and pasta (Pontonio, Lorusso, Gobbetti, & Rizzello, 2017; Schettino, Pontonio, & Rizzello, 2019).

In this work, fermentation with selected LAB strains was applied to DWG resulting from the oil extraction. The microbiological, nutritional, technological, and sensory characterization of the fermented DWG (fDWG) and of experimental bread fortified with fDWG was carried out following an integrated approach.

2. Materials and methods

2.1. Defatted wheat germ

Defatted wheat germ (DWG), certified for mycotoxins levels (aflatoxins, zearalenone, deoxynivalenol, ochratoxin A, and fumonisin) under the thresholds defined by Reg. UE 1881/2006, Reg. UE 1126/2007, and Reg. UE 165/2010 was kindly provided by Molino Casillo, Corato (BA) Italy. DWG resulted from the wheat germ oil extraction by a solvent extraction technique, based on the use of *n*-hexane (BP. 68 °C). After oil extraction and solvent removing, DWG was crushed and sifted (200-mesh sieve, 74 μm) to obtain a powder (here called DWG flour), which was used for experiments. DWG yield corresponded to *circa* 82% of the raw wheat germ subjected to the oil extraction process.

The *n*-hexane content in DWG flour was under 5 ppm, as required by Directive 2009/32/EC on extraction solvents used in the production of foodstuffs and food ingredients from defatted cereal germs. Proteins (total nitrogen × 5.7), lipids, moisture, total dietary fiber, and ash of DWG flour were determined according to Approved Methods of the American Association of Cereal Chemists 46–11.02, 30–10.01, 44–01.01, 32–05.01, and 08–01.01 (AACC, 2010). Available carbohydrates were calculated as follows: [100 – (proteins + lipids + ash + total dietary fiber)]. Results were reported as % (wt/wt).

DWG flour was also subjected to microbiological analyses. Ten grams of sample were suspended in 90 ml of sterile sodium chloride (0.9%, wt/vol) solution and homogenized in a Stomacher blender (2 min at room temperature). Total mesophilic bacteria cell density was determined on Plate Count Agar (PCA, Oxoid), at 30 °C for 48 h.Yeasts were enumerated on Sabouraud Dextrose Agar (SDA, Oxoid), added with 0.1 g/L chloramphenicol, at 25 °C for 48 h. Mesophilic presumptive LAB were enumerated on modified MRS medium (mMRS), obtained by adding 1% [wt/vol] maltose, 5% [vol/vol] fresh yeast extract to MRS (Oxoid, Basingstoke, Hampshire, United Kingdom), at 30 °C for 48–72 h, under anaerobiosis. mMRS was adjusted at pH 5.6. Molds were determined on Potato Dextrose Agar (PDA, Oxoid) at 25 °C for 48 h. Total Enterobacteria were enumerated on Violet Red Bile Glucose Agar (VRBGA, Oxoid) at 37 °C for 24 h.

2.2. Fermentation

Starters for DWG fermentation were chosen among 20 strains belonging to *Lactiplantibacillus plantarum* and *Fructilactobacillus sanfranciscensis* species already employed as starters for fermentation of their own isolation matrices. In detail, a preliminary trial, aimed at the strain selection, was carried out on DWG doughs having dough yield (DY, dough weight x 100/flour weight) of 200. Strains were singly inoculated at cell density of approx. log10 7.0 cfu/g and fermentation was carried out at 30 °C for 24 h. *L. plantarum* T6B10 (Rizzello, Lorusso, Montemurro, & Gobbetti, 2016) and *F. sanfranciscensis* A2S5, previously isolated from quinoa flour and wheat sourdough, respectively, were selected according to the acidification and proteolytic activities (data not shown) and used as starter to produce a DWG-based Type-II sourdough.

The selected LAB strains (belonging to the Culture Collection of the Department of Soil, Plant, and Food Science of the University of Bari, Italy) were routinely propagated at 30 °C in MRS broth (Oxoid) and, for the inoculum, cultivated until the late exponential phase of growth was reached (approx. 12 h). Cells were recovered by centrifugation (10,000×*g*, 10 min, 4 °C) and washed twice in 50 mmol/L sterile potassium phosphate buffer (pH 7.0). LAB cells were then suspended in the water used for dough preparation and inoculated at an initial cell density of approx. log10 7.0 cfu/g of dough. The cell density of the suspension was determined by measuring the optical density (OD) at 620 nm (OD = 2.5 corresponded to log10 9.0 cfu/ml). Fermented defatted wheat germ (fDWG) was prepared by mixing DWG flour and tap water at

the DYof 200, corresponding to the ratio DWG flour:water of 1:1 (weight). Fermentation was carried out at 30 °C for 24 h. LAB cell density, pH, and total titratable acidity (TTA) were determined before (0 h) and after (24 h) the fermentation process. The pH was determined by a pHmeter (Model 507, Crison, Italy) equipped with a food penetration probe. TTA was determined as the volume (ml) of 0.1 M NaOH required to bring the pH of a suspension of 10 g dough in 90 ml sterile water, to 8.3 (Rizzello, Calasso, Campanella, De Angelis, & Gobbetti, 2014). LAB cell density in DWG doughs was determined through plate count on mMRS (30 °C for 48–72 h, under anaerobiosis).

To be used as ingredient for breadmaking, fDWG was dried at 50 °C for approx. 10 h in ventilated stove (Binder, Germany) until a moisture content of 6.5% was achieved. Then fDWG was milled by a laboratory mill Braun AG (Type 4036, Frankfurt, Germany) to obtain a fine powder. During fermentation process with selected LAB strains, the pH for the kinetics of acidification was determined on-line as reported above. Acidification data were modelled according to the Gompertz equation, as modified by Zwietering, Jongenburger, Rombouts, and Van't Riet (1990). Although originally proposed for the description of microbial growth curve, this model is also largely applied to the study of the acidification kinetic (Di Cagno et al., 2003; Vermeiren, Devlieghere, De Graef, & Debevere, 2005) by using the following equation:

$$y = k + A\exp - \{-\exp[(\mathrm{Vmaxe}/\mathrm{A})(\lambda - \mathrm{t}) + 1]\},$$

where y is log (dpH dt^{-1}, units of pH min^{-1}); k is the initial level of the dependent variable to be modelled; A (ΔpH) is the difference in pH (units) between the initial value (pH_0) and the value reached in the stationary phase (pH_t) of the dough fermentation; Vmax is the maximum acidification rate (ΔpH 10 min^{-1}), λ is the length of the latency phase of acidification expressed in minutes, and t is time expressed in min. The stationary phase of the kinetics of acidification was identified in correspondence of dpH 10 min^{-1} value lower than 0.02. The experimental data were elaborated through the nonlinear regression procedure using the Statistica 12.5 software (Statsoft, Tulsa, Oklahoma, USA).

2.3. Characterization of the fermented defatted wheat germ

For the analyses of organic acids and total free amino acids (TFAA), water/salt-soluble extracts (WSE) of the fDWG before and after the fermentation, and after drying, were prepared. WSE were obtained through the procedure proposed by Osborne (1907) and modified by Weiss, Vogelmeier, and Görg (1993). K-DLATE and K-ACET (Megazyme International Ireland Limited, Bray, Ireland) kits were used for the quantification of lactic acid and acetic acids. The quotient of fermentation (QF) was determined as the molar ratio between lactic and acetic acids. Megazyme kit Raffinose/D-Galactose Assay Kit K-RAFGA (Megazyme) and Megazyme test kit K-PHYT 05/07 (Megazyme) were respectively employed for the analysis of raffinose and phytic acid. A Biochrom 30+ series Amino Acid Analyzer (Biochrom Ltd., Cambridge Science Park, England) with a Li-cation-exchange column (20 by 0.46 cm inner diameter) was used for TFAA analysis, as previously described by Rizzello, Nionelli, Coda, Di Cagno, and Gobbetti (2010).

2.4. Breadmaking

Three types of breads were prepared: control wheat bread (C-WB) manufactured only using wheat flour (*Triticum aestivum*, commercial wheat flour type "0", Molino Casillo, moisture 12%, protein 13.9% of dry matter, d.m; fat 2.3% of d.m, dietary fiber 2.2% of d.m, carbohydrates, 81% of d.m); defatted wheat germ bread (DWG-B); fermented defatted wheat germ bread (fDWG-B). DWG and dried fDWG were used at 6% (wt/wt), in replacement of wheat flour. Recipes are reported in Table S1. The optimal water content for the breads was based on wheat flour as determined with a Brabender Farinograph (Brabender GmbH & Co. KG, Germany). Thus, the amount of flour and water was same in all breads (DY 160). which were manufactured at pilot plant scale, and leavened with baker's yeast (2% wt/wt). DWG and dried fDWG flours were mixed with wheat flour, water, and fresh baker's 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 pans for 20 min at 25 °C and relative humidity (RH) of 75%. Doughs were leavened for 90 min at 25 °C and RH 85% in a fermentation cabinet (Zucchelli S.p.a). The leavening performances of the doughs, by means of volume increase (ΔV, mL), were determined, and expressed as the percentage of volume increase (Minervini, Pinto, Di Cagno, De Angelis, & Gobbetti, 2011). pH, TTA, organic acids and TFAA of the dough after proofing process were determined as reported above. The breads were baked at 220 °C for 20 min in a rotating rack oven (Zucchelli S.p.a). Then, the breads were cooled for 2 h at room temperature and weighed. Two independent baking trials were carried out, producing five breads for each type. Each bread was analyzed twice.

2.5. Bread characterization

2.5.1. Nutritional properties

Proximate composition of experimental breads was calculated by the methods (AACC 2010) reported in section 2.1. For the *in vitro* protein digestibility (IVPD), breads were subjected to a sequential enzyme treatment mimicking the *in vivo* digestion as originally proposed by Akeson and Stahmann (1964) and modified by Rizzello et al. (2016). The IVPD was expressed as the percentage of the total protein which was solubilized after enzyme hydrolysis. The Bradford method (Bradford, 1976) was used to determine the concentration of the protein in digested and non-digested fractions.. Starch hydrolysis index of bread (HI) was determined by mimicking the *in vivo* digestion of starch (De Angelis et al., 2009). Bread portions, containing 1 g of starch, were subjected to the enzymatic treatment and the released glucose concentration was determined with the 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 equation: pGI = 0.549 × HI+39.71 proposed by Capriles and Arêas (2013) was used to calculate the predicted glycemic index (pGI).

2.5.2. Technological properties

The baking loss (%) of the breads was evaluated as follow: (dough weight−bread weight) * 100/dough weight. Bread volume was determined by rapeseed displacement method 10–05.01 (AACC, 2010). The specific volume of the bread was calculated as the loaf volume (mL)/loaf weight (g) ratio, after 2–6 h of cooling. Texture profile analysis was performed on boule-shaped loaves (200 g), stored for 2 h at room temperature after baking, by using an FRTS-100N Texture Analyzer (Imada, Toyohashi, Japan) equipped with a cylinder probe FR-HA-30J. The instrument was set as follows: test speed 1 mm/s, 30% deformation of the sample, and two compression cycles. The parameters evaluated were hardness, fracturability, cohesiveness, springiness, and chewiness. The chromaticity co-ordinates of the crust and crumb of the breads (measured by a Minolta CR-10 camera) were reported as color difference, ΔE^*_{ab}, calculated by the following equation:

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

where ΔL, Δa and Δb are the differences between sample and reference L, a and b values. Reference corresponded to a white ceramic plate having L = 93.4, a = − 1.8 and b = 4.4.

2.5.3. Sensory analysis

Ten trained panelists (5 males and 5 females, mean age: 30 years, range: 18–54 years) were recruited for the sensory analysis carried out as previously described by Rizzello et al. (2014). Sensory attributes

included: visual and tactual perceptions (color of crust and crumb, elasticity, friability); taste (acidic taste, sweetness, salty, herbaceous taste, bitter flavor); smell perception (acidic odor); chewing (chewiness), using a scale from 0 to 10, with 10 being the highest score. Samples were served in random order and evaluated by all panelists in two replicates. Before the sensory evaluation, the loaves were thawed at room temperature for 5–6 h, then cut into slices 1.5 cm thick. Slices were cut into 4 pieces and each panelist received 2 pieces per sample. Final scores for each attribute were calculated as the means of the data collected in three independent evaluations.

2.6. Statistical analyses

All the microbiological, chemical, textural and sensory 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).

3. Results

3.1. Defatted wheat germ

DWG was characterized by a moisture of *circa* 7% (Table 1). About the 35% in weight was represented by dietary fibers, while proteins were *circa* 25% (wt/wt) (Table 1). The residual fat was lower than 1% (wt/wt) (Table 1). As expected, a high level of ash (almost the 5%) characterized the raw material. The oil extraction process, including the desolventizing and drying steps led to a very low density of viable microorganisms. All the microbiological groups considered in the analyses (mesophilic LAB, yeasts, Enterobacteria, total mesophilic bacteria) were found at cell densities lower than 2 log10 cfu/g.

3.2. DWG type-II/III sourdough

The DWG dough had a pH of *circa* 6.22 before fermentation, and the 24 h-fermentation with the binary selected starter caused a decrease of *circa* 2.5 units. Accordingly, a relevant increase of the TTA was observed (Table 2). LAB cell density increased of 2 log cycles, and relevant concentrations of lactic and acetic acids were found in fDWG (both the acids were in traces before fermentation) (Table 2), resulting in a FQ of *circa* 11.

The modelling of the kinetic of acidification showed the following parameters: lag phase (λ), 5.47 ± 1.11 h, maximum acidification rate (V_{max}), 0.48 ± 0.04 ΔpH/h, A (ΔpH) of 2.45 ± 0.66.

The TFAA concentration, as expected, was higher than a common wheat flour (more than 1.3 g/kg) in unfermented DWG and increased more than 3-times during the fermentation (Table 2).

In details, arginine (Arg), glutamate (Glu), lysine (Lys), and leucine (Leu) were the FAA found at highest concentration in fDWG (all at concentration higher than 350 mg/kg) (Fig. 1). Compared to the unfermented DFW, only cysteine (Cys) and ornithine (Orn) did not show a significant ($P > 0.05$) increase (Fig. 1).

The antinutritional compounds content significantly ($P < 0.05$) decreased during fermentation. Indeed, final concentration of phytic acid and raffinose were respectively half and a tenth of the initial ones (Table 2).

Aiming at the inclusion in bread formulation, the DWG-based Type-II sourdough was dried, following the procedure industrial employed for obtaining the Type-III sourdough (De Vuyst, Comasio, & Kerrebroeck, 2021), and characterized. As expected, the thermal treatment led to a decrease of the viable LAB to 3.51 log10 cfu/g. Lactic and acetic acids were respectively found at concentration of 335.4 ± 11.0 and 13.8 ± 2.0 mmol/kg. Due to the volatility of acetic acid, a relevant amount was lost during drying, thus leading to a FQ of the dried formulation of 24.3. TFAA in dried fDWG were 8891 ± 57 mg/kg, with Arg (1129 ± 18 mg/kg), Glu (875 ± 14 mg/kg), Lys (800 ± 12 mg/kg), proline (Pro) (743 ± 12 mg/kg), and Leu (740 ± 11 mg/kg) found at the highest concentrations. γ-Aminobutyric acid (GABA) was found at 547 ± 8 mg/kg.

Table 1
Proximate composition of the defatted wheat germ (DWG).

Chemical composition (g/100 g)	DWG
Moisture	7.00 ± 0.28
Protein (d.m.)[a]	25.20 ± 0.77
Fat (d.m.)	0.51 ± 0.20
Carbohydrates (d.m.)	28.19 ± 1.30
Total dietary fibers (d.m.)	35.44 ± 3.13
Salt (d.m.)	0.02 ± 0.00
Ash (d.m.)	5.05 ± 0.55

The data are the means of three independent experiments ± standard deviations (n = 3).
[a] d.m.: on dry matter basis.

Table 2
Cell density of lactic acid bacteria (log10 cfu/g), pH, concentration of lactic and acetic acids, fermentation quotient (FQ), total titratable acidity (TTA), phytic acid, raffinose and total free amino acids (TFAA) content, of the fermented defatted wheat germ (fDWG) before (0 h) and after (24 h) fermentation at 30 °C with *L. plantarum* T6B10 and *F. sanfranciscensis* A2S5. Data refer to wet samples (DY 200).

	fDWG	
	0 h	24 h
Lactic acid bacteria (log10 cfu/g)	7.43 ± 0.48[b]	9.76 ± 0.20[a]
pH	6.22 ± 0.15[a]	3.74 ± 0.31[b]
TTA (ml NaOH)	2.70 ± 0.11[b]	44.14 ± 2.25[a]
Lactic acid (mmol/kg)	0.27 ± 0.02[b]	167.7 ± 9.57[a]
Acetic acid (mmol/kg)	1.04 ± 0.09[b]	15.01 ± 1.15[a]
FQ	0.25 ± 0.02[b]	11.17 ± 2.50[a]
TFAA (mg/kg)	1307.61 ± 118[b]	4268.5 ± 301[a]
Phytic acid (g/100g)	1.43 ± 0.24[a]	0.77 ± 0.15[b]
Raffinose (g/100g)	0.66 ± 0.18[a]	0.06 ± 0.02[b]

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

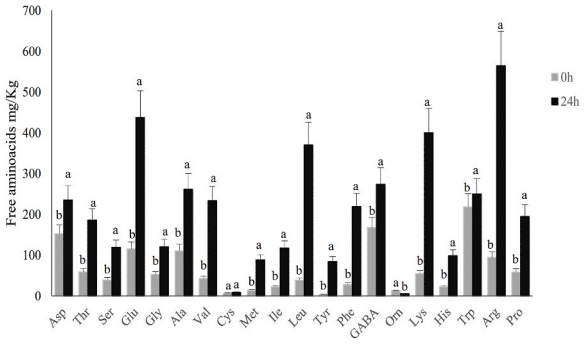

Fig. 1. Concentration of free amino acids and their derivatives (mg/Kg) of the defatted wheat germ (DWG), before (0 h) and after (24 h, fDWG) fermentation at 30 °C with *L. plantarum* T6B10 and *F. sanfranciscensis* A2S5. Data refer to wet samples, dough yield (DY) was 200. Data ± are the means of three independent analyses. Three-letters amino acid code suggested by IUPAC (International Union of Pure and Applied Chemistry) is used. [a-b] Values with different superscript letters within the same amino acid, differ significantly ($P < 0.05$). Bars of standard deviations are also represented.

3.3. Fortified bread

3.3.1. Biochemical and nutritional characteristics

The fDWG was used as ingredient for breadmaking. Two control breads were also prepared: one containing the same amount of unfermented DWG, and one without supplementation. The presence of DWG positively affected the volume increase after proofing (Table 3), which was significantly ($P < 0.05$) higher than those found for C-WB and fDWG-B. The presence of fDWG lead to a significant ($P < 0.05$) decrease of the pH, due to the presence of relevant concentration of organic acids (mainly lactic) in the fermented ingredient. TFAA concentration of both the fortified breads, were significantly ($P < 0.05$) higher than C-WB, with the highest value observed for fDWG bread (Table 3).

The fortification significantly ($P < 0.05$) affected also the proximal composition of the breads (Table 4). Indeed, increases of protein (up to 13.5%) and total dietary fibers (up to 2.0 times higher) contents were observed in DWG- and fDWG-B compared to C-WB. Overall, energy values did not show significant differences (Table 4).

Nevertheless, the nutritional indexes markedly varied among the three breads (Table 4). C-WB was characterized by the lowest IVPD, while DWG-B and fDWG-B had significantly ($P < 0.05$) higher values (respectively 13 and 19% higher than control).

The pGI, calculated based on the starch HI, was the highest for the C-WB (Table 4). Significantly ($P < 0.05$) lower pGI values were found for DWG-B (−9%) and fDWG-B (−11%).

3.3.2. Technological properties and sensory profile

Similar ($P > 0.05$) baking losses characterized the experimental breads during baking (Table 5). The specific volume of the fDWG-B did not differ ($P < 0.05$) from that of the control. Nevertheless, DWG-B showed a higher (+30%) value.

The instrumental analysis of the texture revealed similar hardness and springiness for the three breads, while fracturability was the highest for the C-WB and significantly lower for the fortified breads, especially for that containing the fDWG (Table 5). Moreover, fDWG-B showed chewiness values similar ($P < 0.05$) to the control, while it was markedly and significantly ($P < 0.05$) higher in DWG-B.

The crust colorimetric coordinates of both the fortified breads, expressed through the color difference index (ΔE), resulted similar ($P > 0.05$) (Table 5). Compared to C-WB, significantly ($P < 0.05$) lower values for L (lightness) characterized the crust and the crumb of DWG-B and fDWG-B.

Breads were subjected to a sensory analysis by trained panelists. The fortified bread containing fDWG was characterized by higher scores for acidic odor and taste, and herbaceous taste compared to the others (Fig. 2). Nevertheless, the scores for these attributes were lower than 5.5 in a scale of perceived intensity from 0 to 10. Also, the elasticity and crumb color intensity were scored higher compared to both the other breads. Moreover, friability and bitter flavor differentiated the sensory profiles of both the fortified breads from that of C-WB (Fig. 2).

Table 3
Volume increase (%), pH, total titratable acidity (TTA), concentration of lactic and acetic acids, fermentation quotient (FQ), and total free aminoacids (TFAA) of the doughs (after proofing for 1.5 h at 25 °C) containing defatted wheat germ (DWG-B) or fermented and dried DWG (fDWG-B) (6% wt/wt in replacement of wheat flour). A wheat flour dough (C-WB) was used as control. Doughs for bread making had DY 160 and data refer to wet samples.

	C-WB	DWG-B	fDWG-B
Volume increase (%)	31.82 ± 2.11^{b}	38.64 ± 2.98^{a}	34.09 ± 2.22^{b}
pH	5.33 ± 0.34^{a}	5.45 ± 0.41^{a}	4.73 ± 0.16^{b}
TTA	4.04 ± 0.21^{b}	7.40 ± 0.54^{a}	8.60 ± 0.44^{a}
Lactic acid (mmol/kg)	0.33 ± 0.01^{c}	0.87 ± 0.04^{b}	18.07 ± 1.31^{a}
Acetic acid (mmol/kg)	n.d	0.35 ± 0.07^{b}	1.64 ± 0.09^{a}
FQ	–	–	11 ± 1.2^{a}
TFAA (mg/kg)	580 ± 4^{c}	683 ± 10^{b}	1043 ± 6^{a}

n.d., not detected.
The data are the means of three independent experiments ± standard deviations (n = 3).
$^{a–c}$Values in the same row with different superscript letters differ significantly ($p < 0.05$).

Table 4
Proximate composition and nutritional indexes of breads containing defatted wheat germ raw (DWG-B) or fermented and dried (fDWG-B) (6% wt/wt in replacement of wheat flour). A wheat flour bread (C-WB) was included as control. Data refer to wet samples.

	C-WB	DWG-B	fDWG-B
Chemical composition			
Moisture (g/100 g)	31.26 ± 1.81^{a}	28.13 ± 2.02^{a}	29.75 ± 1.99^{a}
Protein (g/100 g)	9.28 ± 0.11^{b}	10.39 ± 0.28^{a}	10.45 ± 0.31^{a}
Carbohydrates (g/100 g)	56.30 ± 0.27^{a}	55.85 ± 0.62^{a}	54.01 ± 1.44^{a}
Fat (g/100 g)	1.58 ± 0.22^{a}	1.56 ± 0.41^{a}	1.53 ± 0.54^{a}
TDF (g/100 g)	1.58 ± 0.14^{b}	3.24 ± 0.21^{a}	3.36 ± 0.17^{a}
Salt (g/100 g)	0.002 ± 0.000^{a}	0.003 ± 0.001^{a}	0.003 ± 0.002^{a}
Energy Value (KJ/100 g)	1157 ± 113^{a}	1167 ± 138^{a}	1136 ± 123^{a}
Nutritional indexes			
IVPD (%)	64.5 ± 1.12^{c}	73.2 ± 0.98^{b}	77.1 ± 1.04^{a}
HI	100 ± 0.82^{a}	86.65 ± 0.77^{b}	81.07 ± 0.35^{c}
pGI	94.61 ± 1.01^{a}	86.72 ± 0.86^{b}	84.21 ± 0.48^{c}

TDF, Total dietary fiber**; IVPD**, In vitro protein digestibility; **HI**, starch hydrolysis index; **pGI**, predicted glycemic index.
The data are the means of three independent experiments ± standard deviations (n = 3).
$^{a–c}$ Values in the same row with different superscript letters differ significantly ($p < 0.05$).

Table 5
Technological characteristics of breads containing (6% wt/wt in replacement of wheat flour) defatted wheat germ raw (DWG-B) or fermented at 30 °C for 24 h with *L. plantarum* T6B10 and *F. sanfranciscensis* A2S5 and dried (fDWG-B). Wheat flour bread (C-WB) was used as control. Dough for bread making had DY 160.

	C-WB	DWG-B	fDWG-B
Baking loss (%)	13.01 ± 1.31^{a}	14.47 ± 1.44^{a}	13.69 ± 1.36^{a}
Specific volume (g/cm^{3})	2.24 ± 0.28^{a}	2.92 ± 0.41^{a}	2.28 ± 0.23^{a}
Hardness (N)	40.7 ± 2.98^{a}	40.5 ± 3.77^{a}	45.1 ± 3.23^{a}
Fracturability (N)	10.81 ± 2.97^{a}	5.73 ± 0.97^{b}	2.20 ± 0.78^{c}
Cohesivness	0.619 ± 0.14^{a}	0.503 ± 0.13^{b}	0.606 ± 0.27^{a}
Springness	0.914 ± 0.068^{a}	0.968 ± 0.048^{a}	0.941 ± 0.029^{a}
Chewiness (N)	23.6 ± 2.07^{b}	53.9 ± 4.11^{a}	25.6 ± 1.29^{b}
Crust color			
L	73.5 ± 1.95^{a}	67.3 ± 3.04^{b}	66.9 ± 1.47^{b}
a	- 0.90 ± 0.67^{b}	3.4 ± 1.53^{a}	3.2 ± 0.92^{a}
b	29.1 ± 1.22^{b}	34.4 ± 2.23^{a}	34.5 ± 1.29^{a}
ΔE	29.5 ± 1.77^{b}	38.3 ± 4.89^{a}	38.6 ± 2.02^{a}
Crumb color			
L	69.8 ± 3.42^{a}	66.4 ± 2.02^{b}	60.8 ± 1.66^{c}
a	−3.3 ± 0.50^{c}	−3 ± 0.07^{b}	−2 ± 0.02^{a}
b	16.8 ± 1.52^{b}	20.6 ± 1.02^{a}	20.4 ± 0.83^{a}
ΔE	39.4 ± 6.48^{a}	30.7 ± 2.52^{b}	36.1 ± 3.38^{a}

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

4. Discussion

In cereals processing, the two major by-products obtained during the traditional milling procedures are bran and germ, that are separated from the endosperm, the main constituent of the refined flour. Despite the high content of vitamins, minerals, and especially dietary fiber, germ and bran adversely affect the technological properties of flours and are therefore discarded (Patel, 2012; Poutanen, Sozer, & Della Valle, 2014). The milling by-products were mainly employed as feed or intended for compost production. However, to alleviate the environmental and

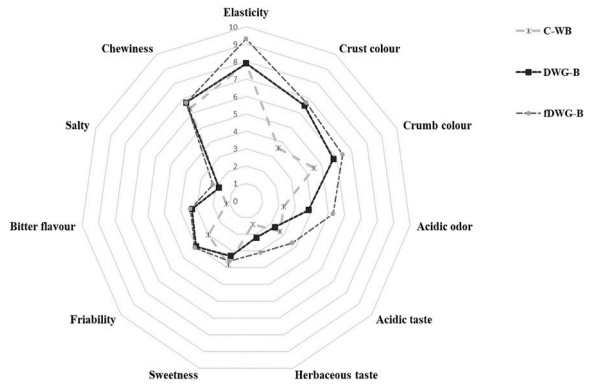

Fig. 2. Spider web chart of the sensory analysis of breads containing defatted (DWG-B) or fermented defatted wheat germ (fDWG-B). A wheat flour bread (C-WB) was included in the analysis.

economic burden of such losses, different approaches have been explored (Ravindran & Jaiswal, 2016). In biorefinery industry, milling by-products can be used to produce biofuels (Vanholme et al., 2013), lactic (Yun, Wee, Kim, & Ryu, 2004) or phytic acid (Kalscheur, Garcia, Schingoethe, Royón, & Hippen, 2012). Insoluble dietary fibers, fructans, antioxidants, and other bioactive compounds can also be extracted from cereal by-products and used in food manufacturing (Ravindran & Jaiswal, 2016). The oil obtained from WG is further processed for vitamin extraction, or used by the cosmetic industry as well as in food, feed, and as biological insect control agent, while the DWG can be potentially employed as food ingredient (Brandolini & Hidalgo, 2012).

DWG was obtained at the pilot plant level from an industrial mill of the South Italy, in which very large amount of durum wheat are daily processed, generating side streams having a tremendous impact on the global food chain: besides the economic loss (both are actually used as feed or disposed as waste), their high organic load represents a critical environmental issue (Verni et al., 2019).

Durum wheat (*Triticum turgidum* subsp. *durum*) is cultivated worldwide over almost 17 million ha, with a global production of 38.1 million tonnes in 2019 (Xynias et al., 2020). The largest producer is the European Union (EU) with cultivation areas concentrated in the Mediterranean. Among EU Countries, Italy is considered the leader of durum wheat production, with an average production of 4.26 million tonnes in the last decade (Xynias et al., 2020). Solvent extraction is the most common process used to extract oil today. The process includes the unit operations of oil extraction, meal desolventizing, meal drying and cooling, miscella distillation, and solvent recovery (Kemper, 2005). Similarly to other defatted meal intended for food and feed purposes, such as those deriving from oleaginous seeds, residual solvent used for oil extraction in DWG is below the food threshold (Directive 2009/32/EC).

Besides enzymes production or bioplastics synthesis, microbial fermentation is a very efficient way to enhance the added-value of cereal by-products, moreover allowing their potential reuse in food production (Verni et al., 2019). Fermentation by LAB, in particular, offers a variety of tools to modify nutritional, functional, and technological features of the cereal matrices. During fermentation, both endogenous and microbial enzymes can modify the grain constituents affecting the structure, bioactivity, and nutrient bioavailability (Coda, Katina, & Rizzello, 2015; Hole et al., 2012). Since LAB fermentation biotechnologies inspired to sourdough processes, both natural or guided through the use of selected starters, were extensively employed to produce cereal-based foods with enhanced health properties (Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003; Capozzi et al., 2012), its application to by-products could, to a wider extent, improve the overall eco-sustainability of the food system, providing a suitable alternative to reduce malnutrition and hunger (Torres-León et al., 2018).

Sourdough fermentation was already applied to not-defatted WG aiming at its stabilization. Two autochthonous LAB were used as starters for fermentation (Rizzello, Nionelli, Coda, De Angelis, & Gobbetti, 2010): compared to the native germ, after 40 days of storage at room temperature, the fermented one presented very low amount of compounds occurring in lipid oxidation and responsible of the rancidity perception.

In this work, LAB fermentation was applied to DWG to improve its nutritional, sensory and technological properties, aiming at obtaining a high-fiber and high-protein fermented ingredient to be used similarly to liquid or dried (Type-II and III) wheat flour sourdoughs. The durum DWG used in this study was characterized by 35% TDF and 25% proteins. Microbial contamination of the DWG was extremely low, as the consequence of the oil extraction process, in which beyond the hexane treatment, temperature reached 68 °C. DWG resulted a suitable substrate for the LAB growth. Growth and technological performances of the selected starters in DWG was comparable to those obtained in similar conditions for other selected starters (Pontonio et al., 2017; Rizzello, Nionelli, Coda, De Angelis, & Gobbetti, 2010), while the lag phase (kinetic of acidification) was longer than that commonly observed for wheat-derived substrates (Pontonio et al., 2017; Rizzello, Nionelli, Coda, De Angelis, & Gobbetti, 2010). It can be hypothesized that the low activity of the endogenous enzymes, denatured during the oil extraction process, such as the removal of soluble fraction from the matrix, could be responsible for a scarce presence of nutrients in the early step of the fermentation process thus requiring a prolonged adaptation phase for LAB.

Nevertheless, at the end of 24 h of incubation, the pH of the fermented DWG was lower than 4.00, and LAB increased of *circa* 2 log10 cycles. An abundant production of lactic acid and a moderate synthesis of acetic acid were observed during fermentation, thus resulting in a QF of 11. Although a low QF is often considered as preferable compared to higher values, in this case it can considered useful. Indeed, the fortification of baked goods aiming at increase total dietary fiber concentration could be carried out with consistent amount of fDWG without worrying about the increase of acetic acid concentration, whose high level are undesirable (Katina, Heiniö, Autio, & Poutanen, 2006).

It was previously demonstrated that wheat germ fermentation by LAB allows the decrease of antinutritional factors, like phytic acid and raffinose (Pontonio, Dingeo, Gobbetti, & Rizzello, 2019; Pontonio et al., 2020; Verni et al., 2019), both present at high levels in the embryo of the kernel. Phytases (endogenous or microbial) and α-galactosidase are involved in the degradative mechanisms. Under the study conditions, the final concentration of phytic acid and raffinose was respectively 50 and 93% lower than the unfermented DWG.

As previously reported, LAB are able to release peptides and FAA from the matrix proteins, during fermentation, thanks to their efficient proteolytic system, including several peptidases with high specificity for wheat polypeptides (Rollán, Gerez, Dallagnol, Torino, & Font, 2010). The fDWG contained more than 4.5 g/kg (on wet weight) or 8.9 g/kg (dry weight) of FAA. The role of FAA is of pivotal importance in the definition of the sensory profile of a baked goods, thanks to the direct effect on taste of different amino acids (like aspartic acid and glutamate) and as precursors of volatiles, especially during baking (Rollán et al., 2010; Thiele, Gänzle, & Vogel, 2002). Moreover, it was recently demonstrated that essential FAA are more bioavailable than those encrypted in native protein sequences, thus resulting in higher nutritional and protein quality indexes of the derived baked good formulations (Rizzello et al., 2019). The concentration of the FAA, together with the degree of proteolysis, is considered one of the main factors affecting the protein digestibility of the bread (Rizzello et al., 2019). Among the most abundant FAA found in fDWG, Lys, that is the limiting amino acid in wheat flour and Arg, involved, through the arginine deiminase (ADI) pathway and the thermal effect of baking in the generation of the aroma compound 2-acetyl-1-pyrroline (Thiele et al., 2002), were found at very high level. Moreover, 273 mg/kg (wet weight) or 547 mg/kg (dry

weight) of the functional non proteic amino acid GABA (Diana, Quílez, & Rafecas, 2014) were also found in fDWG.

Aiming at investigating the application of the fDWG as food ingredient, it was dried at low temperature and used to produce a fortified bread. Besides the very high concentration of TFAA, the dried fDWG was characterized by high concentration of lactic acid (335 mmol/kg).

The dried fDWG was included in bread formulation as wheat flour replacer (6% of the wheat flour). pH of the fortified bread was 4.7, a value comparable to many common sourdough breads (Arora et al., 2021). This level of fortification allowed the labeling of the final product as source of fiber (3.3 g/100 g of bread) and proteins (15.4% of the energy value was provided by proteins), according to the Regulation EC No. 1924/2006.

Beyond the proximate composition, the use of fermented DWG also affected other nutritional characteristics. FAA content was 80% higher than control bread, while the IVPD, a nutritional index that in wheat bread appears to be directly correlated to the degree of proteolysis occurring during fermentation, was *circa* 20% higher than that of the unfortified bread. The supplementation with unfermented DWG improved the IVPD to a lesser extent. It can be hypothesized that the increase of protein digestibility in fortified breads is correlated to the supplementation of albumins and globulins, that characterize the WG composition (Boukid et al., 2018), that are easily hydrolyzed by digestive enzymes as well as subjected to the proteolytic activity of LAB during long time fermentation (Rizzello, Nionelli, Coda, De Angelis, & Gobbetti, 2010).

Glycemic index, quantified using a predictive *in vitro* index based on a multi-step enzymatic treatment mimicking the digestion in gastrointestinal tract (De Angelis et al., 2009), resulted lower than that of the control (84 *vs* 95). The decrease of the glycemic index, as largely confirmed by *in vitro* and *in vivo* studies, depends on the high fiber concentration, but also to the LAB-related acidification, that leads to the increase of the resistant starch ratio (Rizzello et al., 2019).

The presence of the DWG affected the technological characteristics of the bread: in particular, when added in not-processed form, it improves the specific volume, probably thanks to the abundance of FAA that stimulates baker's yeast metabolisms. The addition of the fDWG did not lead to an improvement of loaf volume, probably due to the side-effect of the acidification on bread leavening (Su et al., 2019) although final volume, hardness and chewiness were similar to those of the control wheat flour bread. As expected, the abundance of fibers (in DWG-B and fDWG-B) and the effect of acidification (in fDWG-B) caused easier fracturability (less force needing) compared to control bread, as effect of the weakening of the gluten network (Liu, Ma, Li, & Wang, 2019). The high concentration of FAA, involved in the Maillard reaction at the high temperature of the baking, conferred a more intense and dark color to the crust of the bread. Sensory analysis showed that fermented DWG conferred perceptible acidic odor and taste to the baked good. These are peculiar features of a conventional sourdough bread.

According to the results, DWG can be considered as a suitable substrate for LAB fermentation, in a sourdough-derived bioprocess effective in the conversion of an underutilized by-products in a high-added value ingredient with enhanced nutritional and sensory features.

Declaration of competing interest

The authors declared that there is no conflict of interest.

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CRediT authorship contribution statement

Giuseppe Perri: Methodology, Investigation, Formal analysis, Writing – original draft. **Marcello Greco Miani:** Methodology, Resources, Validation. **Gianfranco Amendolagine:** Methodology, Investigation, Validation. **Erica Pontonio:** Conceptualization, Methodology, Writing – review & editing. **Carlo Giuseppe Rizzello:** Funding acquisition, Data curation, Conceptualization, Methodology, Supervision, Project administration, Writing – original draft, Writing – review & editing.

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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.2022.113566.

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