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Effect of sourdough fermentation on stabilisation, and chemical and nutritional characteristics of wheat germ

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ABSTRACT

Lactic acid bacteria strains were identified from wheat germ by 16S rRNA partial sequencing, subjected to RAPD-PCR typing and screened. *Lactobacillus plantarum* LB1 and *Lactobacillus rossiae* LB5 were used as starters to produce sourdough fermented wheat germ (SFWG). The chemical and nutritional characteristics of SFWG were compared to those of the raw wheat germ (RWG). Lipase activity in SFWG was ca. 2.6-fold lower than that found in RWG. As shown by SPME/GC/MS analysis, most of the volatile compounds derived from lipid oxidation during storage (40 days) were at markedly lower levels in SFWG compared to RWG. Fermentation of wheat germ increased of ca. 50% the concentration of free amino acids. Glu markedly decreased in SFWG, due to its conversion in GABA. The concentration of the anti-nutritional factor raffinose also decreased in SFWG. The *in vitro* protein digestibility, the concentration of total phenols, phytase and antioxidant activities were increased by fermentation.

1. 1.Introduction

Wheat germ is the component of wheat kernel with the highest nutritional value (Zhu, Zhou, & Qian, 2006). In spite of this, wheat germ is one of the main by-products from milling industries. It is known that wheat germ may adversely affect the keeping and reprocessing quality of the flour. According to bakeries demand, milling industries aim at extracting the maximum of the kernel endosperm with the lowest contamination by germ. Therefore, the world annual deposit of wheat germ is estimated to be ca. 25,000,000 tons (FAOSTAT, 2008; Ge, Sun, Ni, & Cai, 2000). The human consumption of wheat germ is very limited, since the major part of it is used for animal feeding and other purposes (Zhu et al., 2006).

Wheat germ is a rich source of α-tocopherol, vitamins of group B, proteins, dietary fibre and minerals (Amadò & Arrigoni, 1992). Most of the essential amino acids from wheat germ proteins are present at concentrations higher than in the reference egg protein pattern (FAO, 1995; Ge, Sun, Ni, & Cai, 2001). Since the rapid increase of the global demand for protein consumption, wheat germ may represent one of the most attractive and alternative source of proteins from cheap vegetable sources (Ge et al., 2000; Zhu et al., 2006). Wheat germ is also rich in unsaturated fatty acids, mainly oleic, linoleic and α-linoleic acids (Sjovall, Virtalaine, Lapvetelainen, & Kallio, 2000), and in functional phytochemicals, mainly flavonoids, sterols, octacosanols and glutathione (Zhu et al., 2006). Wheat germ containing as much as 10% oil, is used in medical and cosmetic industries (Kahlon, 1989). Recently, it was shown the potential of processed wheat germ in the prevention and therapy of carcinogenesis (Reddy et al., 2000; Zalatnai et al., 2001).

The human consumption of wheat germ is mainly limited by the presence of some anti-nutritional factors: (i) raffinose (Dubois, Geddes, & Smith, 1960), not digested by pancreatic enzymes and metabolised by gas-producing bacteria of the large intestine (Connes et al., 2004); (ii) phytic acid, which markedly decreases the mineral and amino acid bioavailability (Febles, Arias, Hardisson, Rodríguez-Alvarez, & Sierra, 2002); and (iii) wheat germ agglutinin (WGA), responsible for the hyperplastic and hypertrophic growth of the small bowel and pancreas (Matucci et al., 2003). Nevertheless, baking and even milder thermal treatments almost completely eliminated the WGA activity from wheat germ (Matucci et al., 2003).

High lipase and lipoxygenase activities are also present in wheat germ. These activities favour sensitivity to oxidation, and the consequent destruction of essential fatty acids and vitamins (Sjovall et al., 2000). Besides, lipase and lipoxygenase activities determine the poor and unstable sensory properties of baked goods made of wheat flour containing the germ, especially during storage (Paradiso, Summo, Trani, & Caponio, 2008). Nowadays, increasing efforts are pursued to stabilise wheat germ towards oxidation (Kapranchikov, Zherebtsov, & Popova, 2004). Oxidation may be prevented by: (i) inactivating enzymes under heat, microwave and extrusion cooking treatments; (ii) removing the oil fraction

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from wheat germ (Matucci et al., 2003); and (iii) using antioxidants (Paradiso et al., 2008). Despite their proved effectiveness, the above treatments are in some cases too expensive, not completely resolving, causing the decrease of the nutritional value of wheat germ, and, more in general, the use of synthetic antioxidants are looked at with suspicion since their potential risks for consumer health (Paradiso et al., 2008). High nutritional and sensory quality, preservative-free, safe but mildly processed foods with extended shelf-life are certainly preferred by consumers (Coda et al., 2008).

This study aimed at exploiting the potential of sourdough fermentation to stabilise wheat germ. The technological and nutritional properties were determined to demonstrate the advantageous use of fermented wheat germ as food ingredient.

2. Materials and methods

2.1. Wheat germ

The mill Tandoi Pellegrino (Corato, BA, Italy) supplied six samples of wheat germ. Samples were produced from different batches. During milling of wheat (*Triticum aestivum* Appulo cv.), the germ was separated from refined flour by using a degerminator and a set of rollermills. Samples of wheat germ were characterised for moisture, ash and fat according to the Approved Methods 44-16, 08-01 and 30-10 of the American Association of Cereal Chemists, respectively (AACC, 2003). Proteins were determined by Kjeldahl method. Total titratable acidity (TTA) was determined on 10 g of wheat germ homogenised with 90 ml of distilled water and expressed as the amount (ml) of 0.1 M NaOH to get pH of 8.3.

Water/salt-soluble extracts from raw or sourdough wheat germ were prepared according to the method originally described by Osborne (1907) and modified by Weiss, Vogelmeier, and Gorg (1993). Fifteen grams of sample were suspended in 60 ml of 50 mM Tris–HCl (pH 8.8), held at 4 °C for 1 h, vortexing at 15 min intervals, and centrifuged at 20,000 *g* for 20 min. The supernatant was used for analyses.

The concentration of proteins in the water/salt-soluble extracts were determined by the Bradford method (Bradford, 1976). Organic acids contained in the water/salt-soluble extract were determined by HPLC (High Performance Liquid Chromatography), using an ÄKTA Purifier system (GE Healthcare) equipped with an Aminex HPX-87H column (ion exclusion, Biorad) and a UV detector operating at 210 nm. Elution was at 60 °C, with a flow rate of 0.6 ml/min, using H_2SO_4 10 mM as mobile phase (Zeppa, Conterno, & Gerbi, 2001). Total and individual free amino acids of the water/salt-soluble extract were analysed by a Biochrom 30 series Amino Acid Analyser (Biochrom Ltd., Cambridge Science Park, England) with a Na-cation-exchange column (20 by 0.46 cm inner diameter) as described by Rizzello, Cassone, Di Cagno, and Gobbetti (2008). Sugars (arabinose, fructose, glucose, sucrose, maltose and raffinose) were determined by HPLC using an ÄKTA Purifier system equipped with a Spherisorb column (Waters, Millford, USA) and a Perkin Elmer 200a refractive index detector operating at 32 °C. Elution was at 32 °C, with a flow rate of 1 ml/min, using acetonitrile 80% as mobile phase. Organic acids, amino acids, and sugars used as standards were purchased from Sigma Chemical Co. (Milan, Italy).

2.2. Plate count, isolation and identification of lactic acid bacteria

Twenty grams of raw wheat germ were suspended in 180 ml of sterile sodium chloride (0.9%, w/v) solution and homogenised with a Classic Blender (PBI International Milan, Italy) for 2 min at room temperature. Mesophilic lactic acid bacteria were determined on modified MRS (maltose and fresh yeast extract were added at 1% and 5%, respectively, and the final pH was 5.6), at 30 °C for 48–72 h, under anaerobiosis. Yeasts were plated on Yeast extract–Peptone–Dextrose agar (YPD, Oxoid, Basingstoke, Hampshire, United Kingdom), and 150 ppm chloramphenicol was added, at 30 °C for 72 h. Total enterobacteria were determined on Violet Red Bile Glucose Agar (VRBGA, Oxoid) at 37 °C for 24 h.

Colonies of presumptive mesophilic lactic acid bacteria, possibly with different morphology, were isolated from modified MRS plates of the highest dilutions. Gram-positive, catalase-negative, non-motile isolates were cultivated in modified MRS at 30 °C for 24 h, and re-streaked into the agar medium. Genomic DNA was extracted as described by De Los Reyes-Gavilán, Limsowtin, Tailliez, Séchaud, and Accolas (1992). Two primer pairs (Invitrogen), LacbF/LacbR and LpCoF/LpCoR (De Angelis et al., 2006), were used to amplify 16S rRNA gene fragment of lactic acid bacteria. The expected amplicons of about 1400 and 1000 bp were eluted from the gel and purified by the GFX™ PCR DNA Gel Band Purification Kit (GE Healthcare, Buckinghmshire, UK). Taxonomic strain identification was carried out by comparing the sequences of each isolate with those reported in the Basic BLAST database (Altschul et al., 1997) (http://www.ncbi.nlm.nih.gov). Identification of *Lactobacillus plantarum* and *Weissella confusa* was further confirmed by partial sequencing of the *recA* and *rpoB* genes, respectively (De Angelis et al., 2007).

Three primers (Invitrogen Life Technologies, Milan, Italy), with arbitrarily chosen sequences (M13, 5′-GAGGGTGGCGGTTCT-3′, P4 5′ CCGCAGCGTT 3′ and P7 5′ AGCAGCGTGG 3′) (Rossetti & Giraffa, 2005), were used singly in three series of amplification for RAPD-PCR analysis. The molecular weight of the amplified DNA fragments was estimated by comparison with 1 Kb Plus DNA Ladder (Invitrogen). The presence or absence of fragments was recorded as 1 or 0, respectively. Only reproducible well-marked amplified fragments were scored, with faint bands being ignored. The RAPD-PCR profiles were evaluated and combined to obtain a unique dendrogram, calculating an index of genetic similarity by the Simple Matching coefficient (Sokal & Michener, 1958).

2.3. Kinetics of acidification

Pediococcus pentosaceus G1 and G17, *W. confusa* G6, G8, G15, and G11, *Lactobacillus rossiae* LB5 and *L. plantarum* LB1 were cultivated in mMRS broth at 30 °C for 24 h. Cells were harvested by centrifugation (10,000 *g*, 10 min, 4 °C), washed twice in 50 mM sterile potassium phosphate buffer (pH 7.0) and re-suspended in tap water before making wheat germ dough. For all fermentation assays, wheat germ from the six samples were pooled and used as a mixture. Sixty-two grams of wheat germ and 37.5 ml of tap water, containing the cell suspension of each lactic acid bacterium (cell density in the dough of ca. 10^8 cfu/g), were used to prepare 100 g of dough (dough yield of 160). Mixing was carried out manually for 5 min. Wheat germ sourdoughs were fermented at 30 °C for 8 h. The kinetic of acidification was determined on-line by a pHmeter (Model 507, Crison, Milan, Italy) with a food penetration probe. Acidification data were modelled according to the Gompertz equation (Zwietering, Jongeberger, Roumbouts, & van't Riet, 1990): $y = k + A \exp\{-\exp[(V_{max}\, e/A)(\lambda - t) + 1]\}$; where y is is the acidification extent expressed as dpH/dt (units of pH/h) at the time t; 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; V_{max} is the maximum acidification rate dpH/h, λ is the length of the latency phase of acidification expressed in hours, and t is the time. Fermentations were carried out in triplicate. The experimental data were modelled through the nonlinear regression procedure of the statistic package Statistica per Windows (Statsoft).

2.4. Sourdough fermentation of wheat germ

L. plantarum LB1 and *L. rossiae* LB5 were cultivated in mMRS until the late exponential phase of growth was reached (ca. 10 h), washed twice in 50 mM phosphate buffer, pH 7.0, and re-suspended in tap water. Two hundred grams of wheat germ, 115 ml of tap water and 5 ml of the cell suspension, containing each lactic acid bacterium (final cell density in the dough, ca. 10^8 cfu/g), were used to produce 320 g dough (dough yield of 160) with a continuous high-speed mixer (60 g; dough mixing time, 5 min). Sourdough fermentation was carried out at 30 °C for 24 h. After fermentation, wheat germ sourdough was freeze dried and used for analyses.

2.5. Lipase activity

Tributyrin was used as the substrate to determine the lipase activity of the wheat germ extract by agar diffusion assay (Lawrence, Fryer, & Reiter, 1967). Agar plates contained 1% (wt/vol) of triglyceride, 0.02% (wt/vol) sodium azide, and 50 mM phosphate buffer, pH 8.0. As reported by Kapranchikov et al. (2004), this value of pH was the optimum for wheat germ endogenous lipase activity. Activity was expressed as the minimum dilution of the enzyme preparation that failed to give a detectable zone of hydrolysis after 24 h of incubation at 30 °C.

2.6. Headspace analysis

Raw and freeze dried sourdough fermented wheat germ samples were stored for 40 days at room temperature and volatile compounds were extracted by solid-phase micro-extraction (SPME). Non stored raw wheat germ was used as the control. Samples were weighed (10 ± 0.05 g) in 50 ml vials, closed by butylic rubber septa and aluminum seal. Before extraction, the stabilisation of the headspace was get by equilibration at 40 °C for 30 min. Extraction was carried out using a divinylbenzene/carboxen/polydimethylsiloxilane (DVB/CAR/PDMS) 50/30 μm SPME fibre assembly (Supelco, Bellefonte, PA, USA) at 40 °C for 30 min. Fibre was desorbed for 6 min in the injection port of the gas chromatograph, operating in splitless mode. For SPME analyses a Fisons gas chromatograph GC800, equipped with a mass spectrometer MD800 was used. Compounds were resolved on a Supelco capillary column SPB-624 (30 m × 0.23 mm × 1.4 μm), under the following conditions: injection port temperature, 250 °C; helium pressure, 30 kPa; oven temperatures, 40 °C for 2 min then 5 °C/min to 230 °C and final isothermal for 10 min. Mass detector was set at the following conditions: detector voltage, 500 V; interface temperature, 250 °C; ionisation energy, 70 eV; emission, 200 Å, scan range 30–270 amu. Peak identification was carried out by comparing retention times with those of standards (Sigma Chemical Co.). Computer matching with the reference mass spectra of NIST and Wiley libraries was carried out. The identification of some compounds was carried out only by matching with the reference mass spectra.

2.7. Total phenols and antioxidant activity

To determine the total phenols and antioxidant activity, extracts were prepared by weighing 5 g of samples and mixing with 50 ml of 80% methanol. The mixture was purged with nitrogen stream, mixed for 30 min and centrifuged at 6000 rpm for 20 min. Extracts were transferred into culture tubes, purged with nitrogen stream and stored at ca. 4 °C before analysis.

Analysis of total phenols was according to the method of Slinkard and Singleton (1997). Gallic acid was the standard. The reaction mixture contained 20 μl of wheat germ extract, 100 μl of Folin–Ciocalteu reagent (Sigma Chemical Co.) and 1.60 ml of distilled water. Three-hundred microliters of saturated sodium carbonate solution were added after few min. The mixture was incubated at 20 °C for 2 h and the absorbance at 765 nm was determined. The concentration of total phenols was calculated as gallic acid equivalent.

The free radical scavenging capacity of wheat germ extracts was determined using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) as reported by Yu et al. (2002), Yu, Perret, Harris, Wilson, and Haley (2003). The antioxidant reaction was started by transferring 1 ml of wheat germ extract into a test tube, containing 4 ml of 80% methanol and 1 ml of freshly prepared DPPH solution. The final concentration of DPPH in the reaction mixture was 100 μmole. The reaction was monitored by reading the absorbance at 517 nm every 2 min for 30 min. A blank reagent was used to study stability of DPPH over the test time. The absorbance measured after 10 min was used for the calculation of the μmoles DPPH scavenged by wheat germ extract. The kinetic of the antioxidant reaction in the presence of wheat germ extract was also determined over 30 min and compared with butylated hydroxytoluene (BHT) as the antioxidant reference.

The radical cation (2,2′-azino-di-[3-ethylbenzthiazoline sulphonate]) ($ABTS^+$) scavenging capacity was measured using the Antioxidant Assay Kit CS0790 (Sigma Chemical Co.), following the manufacturer's instruction. Trolox (6-hydroxy 2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the antioxidant standard. The scavenging activity of wheat germ extract was expressed as trolox equivalent.

2.8. Phytase activity and free minerals

Phytase activity of the water/salt-soluble extracts was measured in terms of inorganic orthophosphate released from the phytic acid by phytase (Shimizu, 1992). The reaction mixture, containing 150 μl of extract and 600 μl of substrate (3 mM Na-phytate in 0.2 M Na-acetate, pH 4.0), was incubated at 45 °C. The reaction was stopped by adding 750 μl of 5% trichloroacetic acid. The released inorganic phosphate was measured by adding 750 μl of colour reagent, prepared daily by mixing four volumes of 1.5% (wt/vol) ammonium molybdate in 5.5% (vol/vol) sulphuric acid solution and one volume of a 2.7% (wt/vol) ferrous sulphate solution. The absorbance was measured at 700 nm. One unit (U) of phytase activity was defined as the amount of enzyme required to liberate 1 nmol of phosphate per min under the assay conditions.

The concentration of free Ca^{++}, Fe^{++}, K^+, Mg^{++}, Mn^{++}, and Zn^{++} contained in the water/salt-soluble extracts was determined at the laboratory Redox SNC, Monza, Italy. The determination was according to method of the Inductively Coupled Plasma (I.C.P.) by using atomic absorption spectrophotometric (IRIS Intrepid, Thermo Elementhal, Thermo Fisher Scientific, Waltham, MA) analysis and air/acetylene flame.

2.9. In vitro protein digestibility

The *in vitro* protein digestibility was determined according to the methods of Hsu, Vavak, Satterlee, and Miller (1977) and Dahlin and Lorenz (1993), with some modifications. Fifty milliliters of wheat germ suspension, containing 6.25 mg of crude protein/ml, were allowed to rehydrate at 5 °C for 60 min. After rehydration, sample was placed in a water bath at 37 °C and the pH was set to 8.0, using 0.1 N NaOH and/or 0.1 N HCl. Lyophilised, crystallised trypsin (Sigma Chemical Co.), at a concentration of 1.6 mg/ml, was maintained in an ice bath and the pH was adjusted to 8.00 with 0.1 N NaOH and/or 0.1 N HCl. Five milliliters of enzyme solution were then added to the protein suspension under stirring at 37 °C. The activity of trypsin was 13,000 BAEE units/mg protein.

A rapid decline in pH occurred immediately. The pH drop was recorded 15 s after enzyme addition and at 1 min intervals for 10 min. The enzyme solution was freshly prepared before each test. The percent protein digestibility (Y) was calculated according to the following equation (Dahlin & Lorenz, 1993): $Y = 210.4–18.1x$, where x is the change in pH after 10 min. Wheat flour was included as the reference.

Table 1
Chemical and nutritional properties of raw (RWG) and sourdough fermented (SFWG) wheat germ.

	RWG	SFWG
pH	6.4 ± 0.08[a]	4.15 ± 0.05[b]
Total titratable acidity (TTA)	18.1 ± 0.23[a]	25.5 ± 0.11[b]
Total free amino acids (g/kg)	15.711 ± 0.13[a]	23.489 ± 0.09[b]
Acetic acid (%)	0.03 ± 0.01[a]	0.24 ± 0.01[b]
Lactic acid (%)	–	0.96 ± 0.03[a]
Lipase activity[A]	56.7 ± 2.4[a]	146.6 ± 1.2[b]
Arabinose (%)	0.15 ± 0.01[a]	0.04 ± 0.01[b]
Glucose (%)	0.35 ± 0.02[a]	0.17 ± 0.02[b]
Fructose (%)	0.45 ± 0.02[a]	0.06 ± 0.02[b]
Sucrose (%)	7.20 ± 0.08[a]	4.61 ± 0.08[b]
Maltose (%)	0.13 ± 0.02[a]	0.12 ± 0.02[b]
Raffinose (%)	5.26 ± 0.02[a]	2.46 ± 0.02[b]
Total phenols (mM gallic acid/g)	2.061 ± 0.02[a]	2.747 ± 0.0[b]
Total antioxidant activity (mmol Trolox equiv/g)	24.1 ± 0.24[a]	32.3 ± 0.15[b]
Phytase activity[B] (U)	0.77 ± 0.02[a]	2.78 ± 0.08[b]
Ca^{++} (ppm)	405 ± 11[a]	430 ± 8[b]
Fe^{++} (ppm)	69 ± 2[a]	95 ± 3[b]
K^{+} (ppm)	8700 ± 12[a]	9400 ± 19[b]
Mg^{++} (ppm)	0.21 ± 0.01[a]	0.22 ± 0.01[a]
Mn^{++} (ppm)	180 ± 12[a]	215 ± 7[b]
Na^{+} (ppm)	66 ± 3[a]	106 ± 2[b]
Zn^{++} (ppm)	250 ± 4[a]	275 ± 5[b]
In vitro protein digestibility (%)	60.0 ± 0.4[a]	62.9 ± 0.3[b]

Data are the mean of three independent fermentations twice analysed.
[a,b]Values in the same row with different superscript letters differ significantly ($P < 0.05$).
[A] Lipase activity was defined as the minimum concentration of the crude enzyme extract that failed to give a detectable zone of hydrolysis after 24 h of incubation at 30 °C.
[B] One unit (U) of phytase activity was defined as the amount of enzyme required to liberate 1 nmol of phosphate per min under the assay conditions.

2.10. Statistical analysis

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 for Windows (Statistica 6.0 per Windows 1998).

3. Results

3.1. Chemical characterisation of raw wheat germ

The averaged values of raw wheat germ (RWG) were the following: moisture 11.11 ± 0.37%, protein ($N \times 5.70$) 28.56 ± 0.88% of dry matter (d.m.); fat 7.99 ± 0.04% of d.m.; and ash 3.77 ± 0.007% of d.m. RWG had pH of 6.34 ± 0.08 and TTA of 18.1 ± 0.23 ml of 0.1 M NaOH/10 g (Table 1). The concentration of total free amino acids was 15.711 ± 0.13 g/kg. Arg, Ser and Glu were the free amino acids found at the highest concentration (Fig. 1). Sucrose and raffinose were the carbohydrates found at the highest concentration (7.20 ± 0.08 and 5.26 ± 0.02%, respectively) (Table 1). Arabinose, glucose, fructose and maltose were also present.

3.2. Microbiological analyses

The cell density of yeasts in RWG ranged from 1.2 ± 0.4 to 3.4 ± 0.3 × 10^3 cfu/g. Enterobacteria were not found in 1 g of sample. As estimated by plating on modified MRS agar, presumptive mesophilic lactic acid bacteria varied from 2.8 ± 0.2 to 4.5 ± 0.3 × 10^5 cfu/g. Gram-positive, catalase-negative, non-motile rods and cocci able to grow at 15 °C and to acidify the MRS broth were identified by partial sequencing of the 16S rRNA. The species were the following: *P. pentosaceus* (10 isolates), *W. confusa* (21), *L. plantarum* (8) and *L. rossiae* (1). *L. plantarum* and *W. confusa* were further characterised by partial sequencing of the *recA* and *rpoB* genes, respectively.

All isolates were subjected to RAPD-PCR analysis. Primers M13, P4, and P7 generated different patterns (bands ranging from 5000 to 100 bp), and were used for clusters analysis. The reproducibility of the RAPD fingerprints was assessed by comparing the PCR products obtained from three separate cultures of the same strain. The

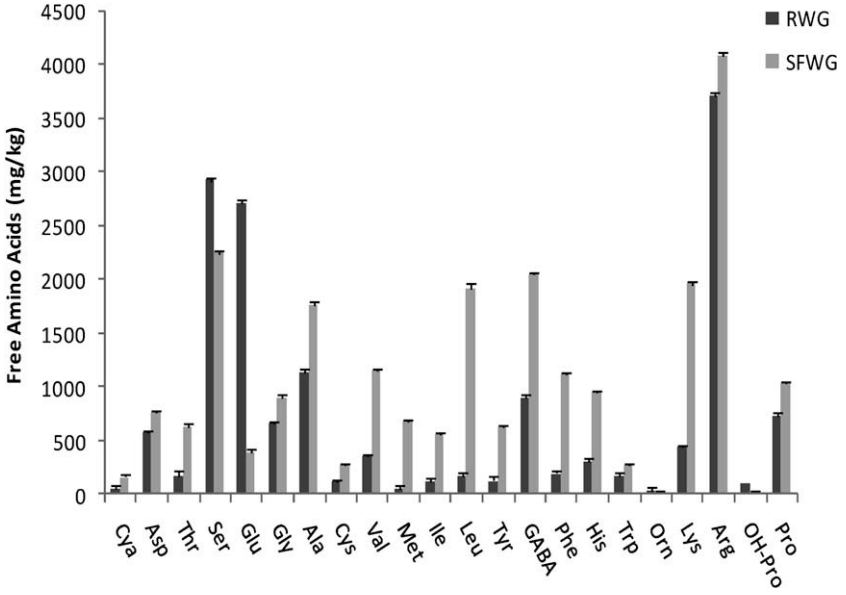

Fig. 1. Concentration of free amino acids (mg/kg) in raw (RWG) and sourdough fermented (SFWG) wheat germ. Data are the means of three independent experiments twice analysed. Bars of standard deviations are also represented.

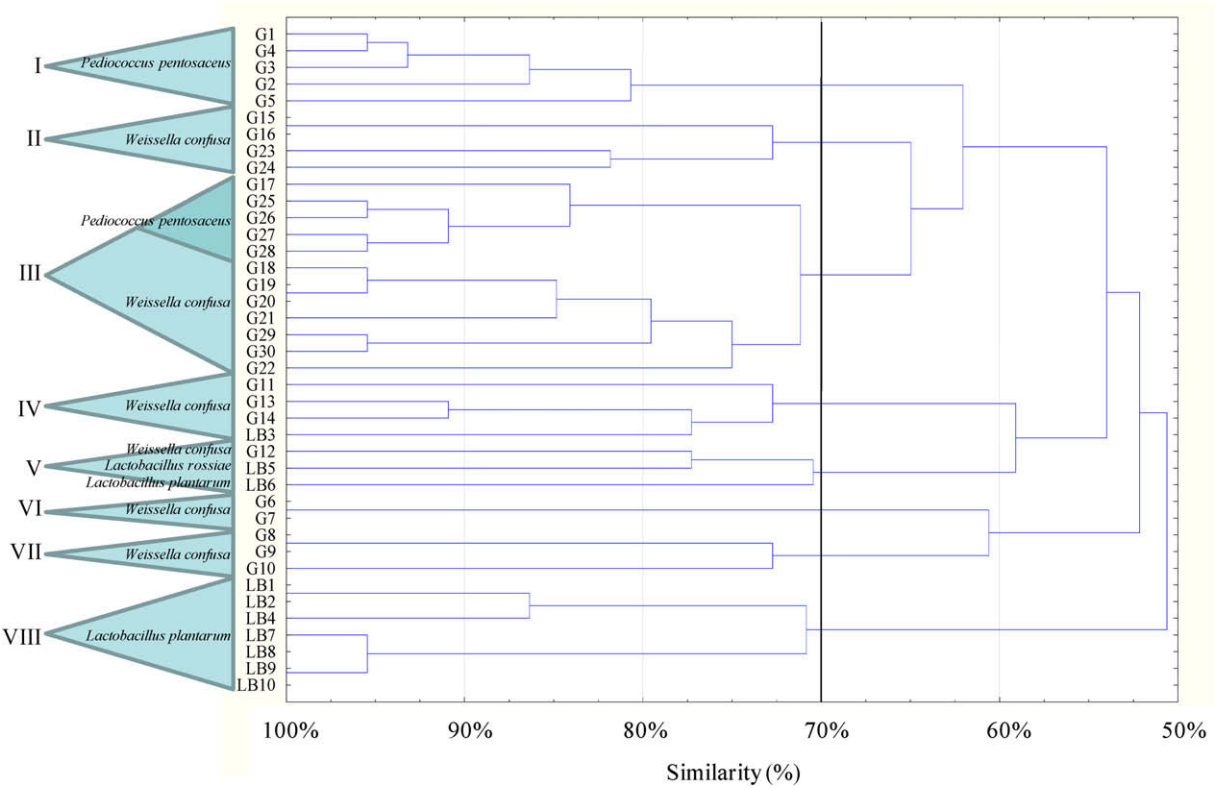

Fig. 2. Dendrogram obtained by combined random amplification of polymorphic DNA patterns for the isolates from raw wheat germ using primers M13, P4 and P7. Cluster analysis was based on the simple matching coefficient and unweighted pair group with arithmetic average.

dendrogram for the 40 isolates is shown in Fig. 2. At the similarity level of 70%, isolates were grouped into eight clusters (I–VIII). Isolates of *P. pentosaceus* did not group in a unique cluster but were separated into two different clusters (I and III). Isolates of *W. confusa* were separated in clusters II, III, IV, V, VI, and VII. Of these, only clusters III and V contained strains belonging to other species. Almost all isolates of *L. plantarum* grouped cluster VIII. Only *L. plantarum* LB6 was grouped with *W. confusa* G12 and *L. rossiae* LB5 (cluster V). Identical fingerprints were found for some isolates (G15 and G16; G19 and G20; G6 and G7; G8 and G9; LB1 and LB2; LB8, LB9, and LB10).

3.3. Selection of starters

Eight strains, each one representative of the above eight clusters, were singly used to ferment RWG at 30 °C for 8 h. After fermentation, all strains reached the cell density in the range from 2.0 ± 0.3 to $4.5 \pm 0.5 \times 10^9$ cfu/g. The values of ΔpH varied from 1.20 to 1.88 (median value of 1.34) (Fig. 3), whereas V_{max} varied from 0.27 ± 0.03 to 0.45 ± 0.02 dpH/h. The highest values of ΔpH were found for *L. plantarum* LB1 and *L. rossiae* LB5 (1.88 (0.02 and 1.79 (0.08, respectively). The same strains also showed the highest values of V_{max} (0.45 ± 0.02 and 0.34 ± 0.03 dpH/h, respectively).

3.4. Wheat germ fermentation

Selected *L. plantarum* LB1 and *L. rossiae* LB5 were used as starters to produce sourdough fermented wheat germ (SFWG). After 24 h of fermentation at 30 °C, cell densities of lactic acid bacteria ranged from 2.5 ± 0.5 to $7.5 \pm 0.4 \times 10^9$ cfu/g.

The pH of SFWG was 4.15 ± 0.05 (Table 1). TTA increased to 25.5 ± 0.11 ml of 0.1 M NaOH/10 g. The concentration of lactic and acetic acid was 0.96 ± 0.03 and 0.24 ± 0.01%, respectively. The quotient of fermentation (QF, molar ratio between lactic and acetic acids) was 2.69. After fermentation, the concentration of total free amino acids increased to 23.489 ± 0.09 g/kg. Almost all free amino acids increased. Leu, Lys, Phe, Val, His, Ala, and Met showed the major increment, reaching concentrations from 1.5- (Ala) to ca 12-fold (Leu and Met) higher than those observed in RWG. Arg, Ser, GABA and Lys were found at highest concentration in SFWG (4080 ± 45, 2242 ± 25, 2043 ± 28 and 1954 ± 27 mg/kg, respectively) (Fig. 1).

Compared to RWG, the concentration of Glu decreased from 2711 ± 28 to 394 ± 25 mg/kg. All soluble carbohydrates decreased during fermentation. In particular, the concentration of raffinose decreased to 2.46 ± 0.12% (Table 1).

3.5. Lipase activity and SPME analysis

Water/salt-soluble extracts from RWG and SFWG were used to determine the lipase activity by agar diffusion assay. After 24 h of fermentation at 30 °C, the minimum concentration of the crude enzyme extract that failed to give a detectable zone of hydrolysis was 56.7 ± 2.4 and 146.6 ± 1.2 μg/ml for RWG and SFWG, respectively.

Samples of RWG and freeze dried SFWG were stored at room temperature for 40 days and analysed by SPME/GC/MS to determine the level of lipid oxidation. During storage, the moisture of RWG and freeze dried SFWG was similar (11.02 ± 0.73 and 11.11 ± 0.37%, respectively). Table 2 shows the profile of volatile compounds according to chemical classes. Before storage, 50 compounds were identified in RWG. Alkanes/alkenes were the most abundant (27 compounds, ca. 46.4% of the total peak area). After 40 days, 138 volatile compounds were identified in stored RWG. They corresponded to 11 alcohols, 9 aldehydes, 10 ketones, 3 esters, 2 lactons, 3 oxygenous heterocyclic compounds, 53 alkanes/

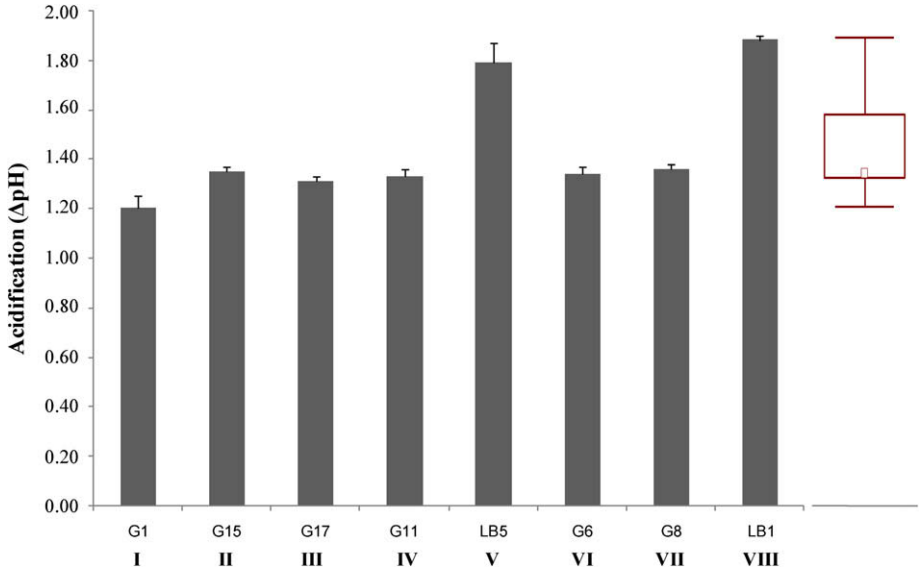

Fig. 3. Values of ΔpH of wheat germ sourdoughs started with *Pediococcus pentosaceus* G1 and G17, *Weissella confusa* G6, G8, G15, and G11, *Lactobacillus rossiae* LB5 or *Lactobacillus plantarum* LB1. Strains are representative of each of the 8 clusters (I–VIII) of Fig. 2. Data are the means of three independent experiments twice analysed, and related bars of standard deviations are represented. The aggregate data of the strains are shown in a box plot. The centre line of the box represents the median (□), the top and bottom of the box represent the 75th and 25th percentile of the data, respectively. The top and bottom of the bars represent the 5th and 95th percentile of the data, respectively.

alkenes, 24 aromatic and cyclic hydrocarbons, 1 terpen, 3 carboxylic acids, and 19 other compounds. Compared to RWG before storage, especially the area ratios of aldehydes, ketones and aromatic/cyclic compounds (9.18%, 10.26% and 1.81% of the total peak area, respectively) increased. Among the 11 alcohols identified in stored RWG, ethanol was the most abundant. High-molecular weight alcohols such as 1-hexanol and 2-undecanol were synthesised during storage. These compounds were probably synthesised from the degradation of hydroperoxides, in turn originated from unsaturated fatty acids. Aldehydes were also probably synthesised from oxidative reactions. Only benzaldehyde, 2,4-dimethyl- was identified in RWG. On the contrary, 9 aldehydes were identified in stored RWG. The most abundant was hexanal (47% of the total aldehydes). Ten ketones were identified in stored RWG, 5 of them were methylketones. Cyclobutanone, 2,2,3-trimethyl- and bicyclo[4.3.1]decan-10-one were already present in RWG before storage. After 40 days of storage, 85 volatile compounds were identified in SFWG. They corresponded to 7 alcohols, 8 aldehydes, 6 ketones, 1 ester, 2 lactons, 2 oxygenous heterocyclic compounds, 36 alkanes/alkenes, 12 aromatic and cyclic hydrocarbons, 1 terpen, 2 carboxylic acids, and 8 other compounds. Except for alkanes/alkenes, all the chemical classes showed an increase in the area ratios, lower than those found for stored RWG. The major differences were found for aldehydes (1.39% vs. 9.18%), ketones (1.06% vs. 10.26%), alcohols (3.11% vs. 9.03%), and aromatic/cyclic hydrocarbons (11.25% vs. 18.36%).

3.6. Antioxidant activities

Several studies showed that methanol is one of the most effective solvent in extracting phenols and other polar substances from cereals (Ragaee, Abdel-Aal, & Noaman, 2006). Under the conditions of this study, 80% methanol extracts of RWG and SFWG were used to determine the concentration of total phenols and antioxidant activities. RWG and SFWG significantly ($P < 0.05$) differed for the concentration of total phenols; 2.061 ± 0.02 vs. 2.747 ± 0.01 mM (Table 1).

The antioxidant properties were determined based on the scavenging activity towards DPPH and ABTS radicals. During assay, the coloured stable DPPH radical is reduced to non-radical DPPH-H when in the presence of an antioxidant or a hydrogen donor. DPPH radical without antioxidants or wheat germ extracts was stable over the time (Fig. 4). According to previous studies (Ragaee et al., 2006), the colour intensity of DPPH showed a logarithmic decline when in the presence of BHT (75 ppm). Both water/salt-soluble extracts from RWG and SFWG favoured a sharp drop of the DPPH colour intensity, indicating the rapid and high capacity to quench DPPH radical. After 10 min of reaction, the remaining colour intensity of DPPH was 38 ± 0.12% with BHT, 25.54 ± 0.08% with RWG and 21.21 ± 0.24% with SFWG.

ABTS assay is based on the formation of the ferryl myoglobin radical from metmyoglobin and hydrogen peroxide, which cause the oxidation of ABTS to $ABTS^{+}$, the chromogen radical cation. In the presence of an antioxidant agent such as Trolox (water-soluble vitamin E analog), the chromogenic reaction is suppressed. The ABTS scavenging activity of the water/salt-soluble extracts of RWG and SFWG was 24.1 ± 0.24 and 32.3 ± 0.15 μmol/g, respectively.

3.7. Phytase activity and minerals

The phytase activity contained in the water/salt-soluble extract of SFWG (2.78 ± 0.08 U) was significantly ($P < 0.05$) higher than that found in RWG (0.77 ± 0.02 U) (Table 1). The same extracts were also subjected to Atomic Absorption Spectrophotometric (AAS) analysis to determine the concentration of some free minerals (Table 1). According to the phytase activity, the water/salt-soluble extract of SFWG contained significantly ($P < 0.05$) higher concentrations of free Ca^{++}, Fe^{++}, K^{+}, Mn^{++} and Zn^{++} than those found in RWG.

3.8. In vitro protein digestibility

The *in vitro* protein digestibility (IVPD) of the wheat flour was used as the reference. Under our experimental conditions, IVDP was 67.3 ± 0.1%. The value of IVDP for RWG was 60.4 ± 0.4%. It significantly ($P < 0.05$) increased to 62.9 ± 0.3% in SFWG.

Table 2
Volatile compounds found in raw wheat germ (RWG) and sourdough fermented wheat germ (SFWG) after 40 days storage at room temperature, as determined by gas-chromatography mass spectrometry/solid-phase micro-extraction (GC–MS/SPME) analysis. The volatile compound profile of RWG before storage (t_0) is also reported. Mean values ± SD are calculated as ratio peak area/total peak area percent.

Compounds	RWG (t_0)	RWG	SFWG
Alcohols			
Ethanol	2.478 ± 0.025^{a}	2.270 ± 0.023^{a}	1.034 ± 0.010^{b}
Isopropyl alcohol	2.014 ± 0.020^{a}	1.415 ± 0.014^{b}	0.210 ± 0.002^{c}
2-Propanol, 1-(2-methoxypropoxy)-	2.784 ± 0.028^{a}	0.020 ± 0.001^{b}	–
1-Pentanol, 4-methyl-	3.529 ± 0.035^{a}	0.080 ± 0.001^{b}	–
2-Penten-1-ol, (Z)-	–	0.731 ± 0.006	–
1,5-Pentanediol, 3-methyl-	–	0.800 ± 0.008^{a}	0.050 ± 0.001^{b}
1-Hexanol	–	1.663 ± 0.017	–
1-Hexanol, 2-ethyl-	2.721 ± 0.027^{a}	1.062 ± 0.011^{ab}	0.884 ± 0.009^{b}
1-Hexanol, 5-methyl-2-(1-methylethyl)-	–	0.366 ± 0.004^{a}	0.366 ± 0.003^{a}
2-Undecanol	1.275 ± 0.013^{a}	0.060 ± 0.001^{b}	0.020 ± 0.001^{c}
Cyclobutanol	–	0.566 ± 0.006^{a}	0.546 ± 0.005^{ab}
Aldehydes			
Acetaldehyde	–	0.852 ± 0.007^{a}	0.113 ± 0.001^{b}
Pentanal	–	0.378 ± 0.004^{a}	0.050 ± 0.001^{b}
Hexanal	–	4.325 ± 0.040^{a}	0.271 ± 0.003^{b}
Heptanal	–	0.440 ± 0.005^{a}	0.020 ± 0.001^{b}
2,4-Heptadienal, (E,E)-	–	0.788 ± 0.008^{a}	0.130 ± 0.001^{b}
Octanal, 7-methoxy-3,7-dimethyl-	–	0.943 ± 0.008^{a}	0.211 ± 0.002^{b}
Nonanal	–	0.421 ± 0.004^{a}	0.101 ± 0.001^{b}
Benzaldehyde	–	0.562 ± 0.006	–
Benzaldehyde, 2,4-dimethyl-	3.187 ± 0.032^{a}	0.475 ± 0.005^{b}	0.501 ± 0.005^{b}
Ketones			
2-Butanone, 3-hydroxy-	–	1.180 ± 0.012^{a}	0.234 ± 0.002^{b}
3,3-Dimethyl-4-methylamino-butan-2-one	–	0.448 ± 0.005^{a}	0.248 ± 0.002^{b}
2-heptanone	–	0.632 ± 0.006^{a}	0.156 ± 0.002^{b}
5,9-Dodecadien-2-one, 6,10-dimethyl-, (E,E)-	–	0.759 ± 0.008	–
Cyclobutanone, 2,2,3-trimethyl-	1.960 ± 0.020^{b}	3.020 ± 0.050^{a}	0.238 ± 0.002^{c}
Ketone, 2,2-dimethylcyclohexyl methyl	–	0.365 ± 0.004	–
2(1H)-Naphthalenone, octahydro-, trans-	–	0.883 ± 0.008^{a}	0.149 ± 0.001^{b}
Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl-, (1R)-	–	0.356 ± 0.004	–
Bicyclo[4.3.1]decan-10-one	1.672 ± 0.015^{a}	0.280 ± 0.003^{b}	0.034 ± 0.001^{c}
Bicyclo[3.3.2]decan-9-one	–	0.337 ± 0.003	–
Esters			
Ethyl acetate	–	1.049 ± 0.011^{a}	0.364 ± 0.003^{b}
Pentafluoropropionic acid, octadecyl ester	–	0.527 ± 0.005	–
Malonic acid, bis 2-trimethylsilylethyl ester	–	0.402 ± 0.004	–
Lactons			
Butyrolactone	2.514 ± 0.025^{a}	0.868 ± 0.009^{b}	0.467 ± 0.005^{c}
γ-Hexalactone	–	0.291 ± 0.003^{a}	0.080 ± 0.001^{b}
Oxygenous heterocyclic compounds			
Furan, 2-butyl-	–	0.603 ± 0.006^{a}	0.085 ± 0.001^{b}
Furan, 2-pentyl-	–	0.854 ± 0.008^{a}	0.185 ± 0.002^{b}
2(5H)-Furanone, 3-methyl-	–	0.352 ± 0.004	–
Alkanes and alkenes			
2-Methyl-2,4-dimethoxybutane	–	0.622 ± 0.006^{ab}	0.762 ± 0.008^{a}
Hexane	0.500 ± 0.005^{b}	2.982 ± 0.030^{a}	0.224 ± 0.002^{c}
Heptane, 2-methyl-	0.832 ± 0.007^{b}	0.784 ± 0.008^{bc}	1.298 ± 0.013^{a}
Heptane, 2,4-dimethyl-	1.240 ± 0.012^{c}	2.563 ± 0.026^{b}	5.963 ± 0.060^{a}
Heptane, 4,4-dimethyl-	2.031 ± 0.020^{b}	2.092 ± 0.021^{b}	5.092 ± 0.051^{a}
Octane, 4-methyl-	0.855 ± 0.009^{b}	0.754 ± 0.008^{b}	1.954 ± 0.019^{a}
Octane, 2,6-dimethyl-	2.075 ± 0.021^{b}	0.535 ± 0.005^{c}	7.568 ± 0.076^{a}
Octane, 2,7-dimethyl-	0.921 ± 0.010^{a}	0.615 ± 0.006^{b}	0.817 ± 0.008^{ab}
Octane, 3,5-dimethyl-	1.536 ± 0.015^{a}	0.102 ± 0.001^{b}	–
Octane, 4,5-dipropyl-	–	0.390 ± 0.004^{b}	0.469 ± 0.005^{a}
Octane, 2,4,6-trimethyl-	1.277 ± 0.013^{a}	0.110 ± 0.001^{b}	0.020 ± 0.001^{b}
Nonane	0.82 ± 0.007^{b}	0.509 ± 0.005^{ab}	0.759 ± 0.008^{a}
Nonane, 2-methyl-	1.121 ± 0.011^{c}	3.190 ± 0.032^{b}	7.719 ± 0.078^{a}
Nonane, 3-methyl-	0.432 ± 0.005^{a}	0.255 ± 0.003^{b}	0.373 ± 0.004^{a}
Nonane, 5-butyl-	–	0.284 ± 0.003^{a}	0.400 ± 0.004^{a}
Nonane, 2,5-dimethyl-	0.900 ± 0.008^{b}	0.661 ± 0.007^{c}	2.807 ± 0.028^{a}
Nonane, 2,6-dimethyl-	1.389 ± 0.014^{a}	0.02 ± 0.001^{b}	–
Nonane, 5-(2-methylpropyl)-	–	0.798 ± 0.008	0.899 ± 0.009
Decane	3.045 ± 0.030^{a}	0.862 ± 0.009^{c}	2.262 ± 0.023^{b}
Decane, 2-methyl-	–	0.819 ± 0.008	–
Decane, 3-methyl-	–	0.793 ± 0.008	–
Decane, 4-methyl-	1.525 ± 0.014^{a}	0.704 ± 0.007^{b}	1.784 ± 0.018^{a}
Decane, 5-methyl-	–	0.468 ± 0.005^{b}	1.324 ± 0.013^{a}
Decane, 1,1'-oxybis-	–	0.491 ± 0.005	–

(*continued on next page*)

Table 2 (*continued*)

Compounds	RWG (t_0)	RWG	SFWG
Decane, 3-ethyl-3-methyl-	–	1.035 ± 0.010[b]	1.543 ± 0.015[a]
Decane, 3,6-dimethyl-	2.084 ± 0.021[c]	3.654 ± 0.037[b]	11.855 ± 0.120[a]
Decane, 3,7-dimethyl-	–	0.645 ± 0.006	–
Decane, 2,2,3-trimethyl-	–	1.099 ± 0.011	–
cis,trans-1,6-Dimethylspiro[4.5]decane	–	0.478 ± 0.005	–
Undecane	1.665 ± 0.017[a]	0.817 ± 0.008[b]	0.902 ± 0.009[ab]
Undecane, 2-methyl-	–	1.530 ± 0.015[b]	2.227 ± 0.022[a]
Undecane, 3-methyl-	–	0.953 ± 0.009	–
Undecane, 4-methyl-	–	1.072 ± 0.011[a]	0.403 ± 0.004[b]
Undecane, 5-methyl-	–	1.033 ± 0.010	–
Undecane, 6-methyl-	–	0.839 ± 0.008	–
Undecane, 2,6-dimethyl-	–	1.234 ± 0.012[ab]	1.408 ± 0.014[a]
Undecane, 4,6-dimethyl-	–	0.309 ± 0.003[ab]	0.409 ± 0.004[a]
Undecane, 4,8-dimethyl-	–	0.964 ± 0.010[b]	1.464 ± 0.015[a]
Undecane, 5,7-dimethyl-	–	1.135 ± 0.011[ab]	1.847 ± 0.018[a]
Dodecane	5.123 ± 0.049[a]	2.170 ± 0.022[b]	5.270 ± 0.053[a]
Dodecane, 2-methyl-	1.827 ± 0.016[a]	0.210 ± 0.002[b]	0.010 ± 0.001[c]
Dodecane, 2,6,10-trimethyl-	1.422 ± 0.015[b]	0.602 ± 0.006[c]	1.886 ± 0.019[a]
Tridecane, 6-methyl-	–	0.571 ± 0.006	–
Nonadecane	2.200 ± 0.021[a]	0.03 ± 0.001[b]	–
Tridecane	5.850 ± 0.060[a]	0.303 ± 0.003[c]	1.506 ± 0.015[b]
Tridecane, 7-propyl-	–	0.745 ± 0.007[b]	1.255 ± 0.012[a]
Tetradecane	–	0.150 ± 0.002[b]	0.394 ± 0.004[a]
Pentadecane	–	0.273 ± 0.003[b]	0.404 ± 0.004[a]
Heneicosane	0.050 ± 0.001[b]	0.772 ± 0.008[ab]	0.972 ± 0.010[a]
3-Hexene, 2,2,5,5-tetramethyl-, (Z)-	–	0.731 ± 0.007	–
Spiro[2.4]hepta-4,6-diene	1.291 ± 0.013[a]	0.05 ± 0.001[b]	–
2-Undecene, 3-methyl-, (Z)-	2.800 ± 0.030[a]	0.02 ± 0.001[b]	–
5-Undecene, 9-methyl-, (Z)-	1.592 ± 0.016[a]	0.03 ± 0.001[b]	–
Aromatic and cyclic hydrocarbons			
o-Xylene	–	0.917 ± 0.009	–
p-Xylene	1.286 ± 0.013[a]	0.756 ± 0.008[b]	0.210 ± 0.002[c]
Ethylbenzene	–	1.039 ± 0.010[a]	0.453 ± 0.004[b]
Benzene, 1-ethyl-3-methyl-	1.663 ± 0.015[a]	0.129 ± 0.001[c]	0.414 ± 0.004[b]
Benzene, 1-ethyl-4-methyl-	–	0.336 ± 0.003[b]	0.377 ± 0.004[a]
Benzene, 1,3-bis(1,1-dimethylethyl)-	1.854 ± 0.020[b]	3.980 ± 0.040[a]	3.298 ± 0.033[ab]
Benzene, 1,2,3-trimethyl-	2.806 ± 0.030[a]	0.07 ± 0.001[b]	–
Benzene, 1,2,4-trimethyl-	–	0.951 ± 0.009[b]	1.101 ± 0.010[a]
Benzene, 1,3,5-trimethyl-	1.264 ± 0.013[a]	0.101 ± 0.001[b]	0.120 ± 0.001[b]
Cyclopropane, propyl-	–	0.873 ± 0.009[b]	1.357 ± 0.014[a]
Cyclopentane, 1-butyl-2-propyl-	–	0.290 ± 0.003[b]	0.377 ± 0.004[a]
Cyclohexane, 1-methyl-2-pentyl-	–	1.337 ± 0.013	–
Cyclohexane, 1-methyl-4-(1-methylbutyl)-	–	0.511 ± 0.005	–
Cyclohexane, 1,2,4-trimethyl-	–	0.513 ± 0.005	–
Cyclohexane, pentyl-	–	0.499 ± 0.005	–
Cyclohexane, hexyl-	–	0.705 ± 0.007	–
Hexylidencyclohexane	–	0.347 ± 0.003	–
Cycloheptane, methyl-	–	0.854 ± 0.008	–
Cyclooctane, methyl-	1.252 ± 0.010[a]	0.090 ± 0.001[b]	–
Cyclododecane	–	0.799 ± 0.008	–
Cyclotrisiloxane, hexamethyl-	6.675 ± 0.065[a]	1.600 ± 0.016[c]	2.693 ± 0.027[b]
Tricyclo[4.4.1.0(1,6)]undecane	–	0.315 ± 0.003	–
1,3,5,7-Cyclooctatetraene	–	0.930 ± 0.009[a]	0.425 ± 0.004[b]
1,3,5-Cycloheptatriene	–	0.419 ± 0.004[a]	0.425 ± 0.004[a]
Terpens			
Limonene	–	0.844 ± 0.008[a]	0.577 ± 0.006[b]
Miscellaneous			
Ethyl ether	1.102 ± 0.012[a]	0.492 ± 0.005[b]	0.235 ± 0.002[c]
3-Oxa-6-thia-2,7-disilaoctane, 2,2,7,7-tetramethyl-	1.366 ± 0.014[a]	0.357 ± 0.004[b]	0.066 ± 0.001[c]
2-Anthracenamine	1.396 ± 0.014[a]	0.080 ± 0.001[c]	0.876 ± 0.009[b]
Trisiloxane, octamethyl-	2.143 ± 0.021[a]	0.154 ± 0.001[c]	0.698 ± 0.007[b]
5-Methyl-2-phenylindolizine	1.602 ± 0.017[a]	0.060 ± 0.001[c]	0.446 ± 0.004[b]
N-(2-Aminoethyl)-N-methylethylenediamine	–	0.160 ± 0.002[b]	1.580 ± 0.016[a]
Imidodicarbonic diamide, N-formyl-	–	0.336 ± 0.003[b]	0.536 ± 0.005[a]
2-Phenylindolizine	–	0.365 ± 0.004[b]	0.411 ± 0.004[a]
1-Propanamine, N,2-dimethyl-	–	0.342 ± 0.003	–
Acetonitrile	–	0.776 ± 0.008	–
Trichloromethane	–	0.317 ± 0.003	–
4-Trifluoroacetoxyhexadecane	–	0.486 ± 0.005	–
Hexyl octyl ether	–	0.613 ± 0.006	–
Naphthalene, decahydro-	–	0.437 ± 0.004	–
trans-Decalin, 2-methyl-	–	0.274 ± 0.003	–
Naphthalene, decahydro-2-methyl-	–	0.494 ± 0.005	–
cis-Decalin, 2-syn-methyl-	–	0.306 ± 0.003	–
1-(2-Methylallyl)azetidine	–	0.295 ± 0.003	–

Table 2 (*continued*)

Compounds	RWG (t_0)	RWG	SFWG
[1,1′-Biphenyl]-4-acetonitrile	–	0.361 ± 0.004	–
Carboxylic acids			
Butanoic acid, 2-hydroxy-	–	0.644 ± 0.006[a]	0.244 ± 0.002[b]
Butanoic acid, 4-hydroxy-	–	0.375 ± 0.004[a]	0.121 ± 0.001[b]
Mercaptoacetic acid, bis(trimethylsilyl)-	5.055 ± 0.051[a]	0.320 ± 0.003[b]	–

Data are the mean of three independent fermentations twice analysed.
[a–c]Means within a row with different superscript letters are significantly different ($P < 0.05$).

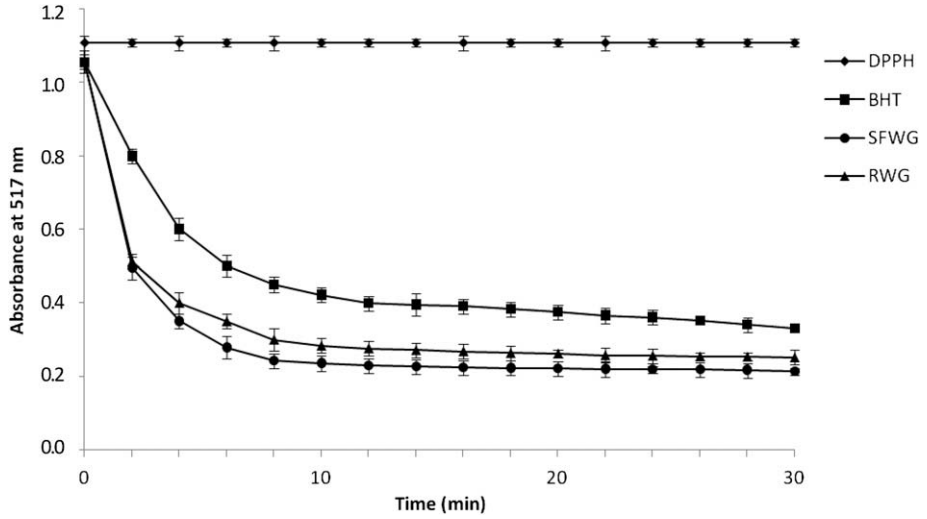

Fig. 4. Kinetics of the scavenging activity of BHT (■), raw wheat germ (RWG, ▲) and sourdough fermented wheat germ (SFWG, ●) towards DPPH radical. The concentration of DPPH in the reaction mixtures and control (DPPH, ♦) was 100 μmole. Data are the means of three independent experiments twice analysed. Bars of standard deviations are also represented.

4. Discussion

Wheat germ, corresponding to 2–3% of the total weight of wheat kernel, is almost systematically removed during commercial milling (Arrigoni et al., 2002). Although the indubitable nutritional value, wheat germ has very limited stability during storage (Kapranchikov et al., 2004). The intense activities of endogenous lipase and lipoxygenase release free fatty acids which are responsible for the appearance of the rancidity defect in baked goods containing wheat germ (Kapranchikov et al., 2004). Lipases of the wheat germ are thermally stable and maintain more than 20% of residual activity at 60–90 °C for 1 h (Kapranchikov et al., 2004). Lipases of wheat germ have an optimum of pH of ca. 8.0 and the activity markedly decreases under acidic conditions (Kapranchikov et al., 2004).

The ancient tradition of using sourdough for bread making was applied to wheat germ aiming at stabilizing the lipase activity and keeping or improving the chemical and nutritional characteristics. Two lactic acid bacteria, *L. plantarum* LB1 and *L. rossiae* LB5, were isolated from wheat germ, selected based on the capacity of acidification and used as starters for sourdough fermentation. *L. plantarum* and *L. rossiae* are considered key bacteria of type I wheat sourdough (Gobbetti, De Angelis, Di Cagno, & Rizzello, 2008). The parameters (e.g. time/temperature, dough yield, cell density) used for wheat germ fermentation were those typical of type I sourdough process. After fermentation, wheat germ had values of pH and QF similar to those found in wheat flour sourdough. Since the low pH (ca. 4.15), the lipase activity of sourdough fermented wheat germ (SFWG) was ca. 2.6-fold lower than that found in raw wheat germ (RWG). According to other studies (Mildner-Szkudlarz, Jeleń, Zawirska-Wojtasiak, & Wąsowicz, 2003), RWG before storage was characterised by relatively low number of volatile compounds. During storage, the perceived rancidity of wheat germ was attributed to the accumulation of aldehydes (e.g. pentanal, hexanal, heptanal, and octanal) from lipid oxidation (Sanches-Silva, Lopez-Hernández, & Paseiro-Losada, 2005). Indeed, hexanal markedly increased during storage of RWG and represented the most abundant aldehyde. After 40 days of storage, a very low percentage of hexanal was found in SFWG. Hexanal is considered to be an indicator of the state of lipid oxidation. Due to the low perception threshold, hexanal is one of the main volatile compounds responsible for off-flavor (Sanches-Silva et al., 2005). By comparing RWG and SFWG, similar differences were also found for pentanal. Alcohols, ketones, furanones and lactones are other volatile compounds mainly synthesised by lipid oxidation. Overall, the area ratios of several of the above compounds were markedly higher in stored RWG than in SFWG.

Recently, some studies used the fermentation of wheat germ to enhance the nutritional and functional properties. Prebiotic compounds were liberated during fermentation of wheat germ which stimulated the growth of probiotic *lactobacilli* and *bifidobacteria* (Arrigoni et al., 2002). Fermented commercial extract of wheat germ had potent anti-metastatic activities in various human malignancies (Comín-Anduix et al., 2002). As in this study, sourdough fermentation increased of ca. 50% the concentration of total free amino acids with respect to RWG (Gobbetti et al., 2008). Lys, the major limiting amino acid of wheat flour (Zhu et al., 2006), was present in SFWG at the concentration of ca. 1954 mg/kg. GABA, a non-protein amino acid, which possesses well known physiological functions such as neurotransmission, induction of hypotension, and diuretic and tranquiliser effects (Rizzello et al., 2008), also in-

creased from ca. 903 (RWG) to ca. 2043 mg/kg (SFWG). GABA is synthesised by glutamate decarboxylase (GAD) [EC 4.1.1.15], a pyridoxal 5′-phosphate (PLP)-dependent enzyme, that catalyses the irreversible α-decarboxylation of L-Glu to GABA. Overall, high GAD activity was found in wheat germ and some lactic acid bacteria strains (Rizzello et al., 2008). The safety of L-Glu and its monosodium salt is still debated. A recent report from the Federation of American Societies for Experimental Biology identified people sensitive to high daily doses of L-Glu (Populin, Moret, Truant, & Conte, 2007). After sourdough fermentation, the L-Glu concentration of the raw wheat germ decreased to ca. 15% (from ca. 2711 to ca. 363 mg/kg). This was probably due to the conversion into GABA. Protein digestibility is another important factor to assess the protein quality and nutritional status of foods or ingredients (Bilgiçli et al., 2005). As previously shown (Bilgiçli et al., 2005), wheat germ has value of IPVD lower than wheat flour. The IPVD of SFWG was higher than that of RWG (ca. 62.9% vs. ca. 60.4%). This moderately improved digestibility may be attributed to proteolysis by sourdough lactic acid bacteria and, probably, to inactivation of some anti-nutritional factors such as trypsin inhibitor (Adams, 1990). The most abundant soluble carbohydrates of wheat germ are sucrose and raffinose (Dubois et al., 1960). Sucrose is a digestible carbohydrate, while digestion of raffinose is relatively poor in mammals. This is due to the lack of the pancreatic α-galactosidase (α-Gal), responsible for hydrolysis of the α-1,6 linkages of α-galactooligosaccharides (Slominski, 1994). Lactic acid bacteria such as *L. plantarum*, *Lactobacillus fermentum*, *Lactobacillus brevis*, *Lactobacillus buchneri* and *Lactobacillus reuteri* expressed α-Gal activity and were used to remove raffinose-type sugars from soy-derived foods (Connes et al., 2004; LeBlanc et al., 2004). Compared to RWG, the concentration of raffinose in SFWG decreased to ca. 45%. Free radical chain reaction is the common mechanism to explain the direct attack of radicals towards physiologically important components of the biological systems (Yu et al., 2003, 2002). Radical scavengers react with and quench free radicals thus terminating or decreasing their potentially hazardous effects. Phenols exhibit radical scavenging capacities. They seem to have the capacity to fight free radicals in human body (Ragaee et al., 2006). The concentration of total phenols in SFWG was ca. 33% higher than that found in RWG. The increase of phenolic compounds during sourdough fermentation was previously shown. Furthermore, the increase of total antioxidant activities in the methanol extracted fraction seems to be related also to the acidification, which favours the extractability of phenolic compounds (Katina et al., 2005). Free radical (DPPH) and radical cation $ABTS^{+}$ scavenging activities observed in RWG were higher compared to those previously reported for hard and soft wheat flours (Ragaee et al., 2006); these activities also increased during sourdough fermentation of wheat germ.

Phytic acid is an anti-nutritional factor. It is an excellent chelator of minerals such as Ca^{++}, Mg^{++}, Fe^{++}, and Zn^{++}, and also complexes the basic amino acid group of proteins, thus decreasing the dietary bioavailability of these nutrients (Febles et al., 2002). In wheat kernel, phytic acid is mainly distributed in the outer layers, pericarp and germ (Febles et al., 2002). Phytase catalyses the hydrolysis of phytic acid to myo-inositol and phosphoric acid via penta- to mono-phosphates. This enzyme activity makes available phosphate and leads to a non-metal chelator compound (Martinez et al., 1996). Sourdough fermentation may result in a more suitable pH to activate flour endogenous phytases and sourdough lactic acid bacteria may also source of phytases (Di Cagno et al., 2008). The sourdough fermentation of wheat germ increased ca. 3.6-fold the phytase activity and enhanced the bioavailability of especially Ca^{++}, Fe^{++}, K^{+}, Mn^{++}, Na^{+} and Zn^{++}.

Due to its high nutritional value and palatability, wheat germ was praised as "the natural nutrient treasure-house and life source of mankind" (Ge et al., 2000). The use of the sourdough biotechnology in part stabilised and improved some nutritional characteristics of the wheat germ, making it suitable for food processing. Studies are in progress to use wheat germ for the manufacture of sourdough bread.

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