International Journal of Food Microbiology 395 (2023) 110194

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Reuse of almond by-products: Functionalization of traditional semolina sourdough bread with almond skin

Raimondo Gaglio [a], Luisa Tesoriere [b], Antonella Maggio [c], Enrico Viola [a], Alessandro Attanzio [b], Anna Frazzitta [b], Natale Badalamenti [c], Maurizio Bruno [c], Elena Franciosi [d], Giancarlo Moschetti [a], Francesco Sottile [e], Luca Settanni [a,*], Nicola Francesca [a]

[a] Dipartimento Scienze Agrarie, Alimentari e Forestali, Università degli Studi di Palermo, Viale delle Scienze 4, 90128 Palermo, Italy
[b] Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, Università degli Studi di Palermo, Via Archirafi 28, 90123 Palermo, Italy
[c] Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche, Ed. 17, Università degli Studi di Palermo, Viale delle Scienze, 90128 Palermo, Italy
[d] Research and Innovation Centre, Fondazione Edmund Mach (FEM), San Michele all'Adige, Italy
[e] Department of Architecture, University of Palermo, Viale delle Scienze, Ed. 14, 90128 Palermo, Italy

ARTICLE INFO

Keywords:
Almond by-products
Functional bread
In vitro digestion
Lactic acid bacteria
Organoleptic properties
Sourdough

ABSTRACT

Almond production generates large amounts of by-products rich in polyphenols. In this study, almond skin was explored as a valuable food ingredient in bread making. To this purpose, almond skin was used to produce functional products modifying a traditional sourdough bread recipe. The doughs were prepared replacing semolina with powdered almond skin (PAS) at 5 and 10 % (w/w). Sourdough inoculum was started with a mix of lactic acid bacteria (LAB) and propagated in semolina until reaching pH 3.7. The pH of PAS added breads was higher than that of control (CTR) breads before and after fermentation. Plate counts showed a similar evolution of LAB and total mesophilic microorganisms, but members of Enterobacteriaceae and coliform were detectable in PAS doughs. Illumina data clearly showed a dominance of lactobacilli in all trials, but PAS doughs displayed the presence of *Bacillus*. The final bread characteristics were influenced by PAS and its addition percentage; in particular, crust and crumb colour resulted darker, the alveolation decreased and, regarding sensory attributes, odour intensity increased, while bread odour diminished. In presence of PAS, bread emissions were characterized by lower percentages of alcohols and aromatic hydrocarbons and higher percentages of the other volatile compound classes, especially terpenoids like β-pinene, β-myrcene and limonene than CTR trial. After in vitro simulated digestion, the final release of phytochemicals from 10 % PAS bread was almost 100 %. Thus, PAS determined an increase of the antioxidant capacity of the breads. Phytochemicals released from digested PAS-fortified bread can provide antioxidant protection in a complex biological environment such as human intestinal-like cells. Besides the positive functional properties of PAS, this work also evidenced the hygienic issues of almond skin and, in order to avoid potential risks for the human health, highlighted the need to preserve its microbiological characteristics during storage for their reuse in bread production.

1. Introduction

Unmanaged agro-wastes and food by-products create environmental concerns (Lai et al., 2017) and have associated consistent disposal costs for producers (Pagliano et al., 2017). However, these wastes constitute a valuable source of different bioactive compounds (Szabo et al., 2018) that, once recovered, might be used to fortify different products (Kaur et al., 2021). In the past, fortification of foods was basically performed for reducing malnutrition by adding micronutrients to processed products when their levels were inadequate for a balanced diet (De Benoist et al., 2006). This practice is particularly useful to control vitamin and mineral deficiencies (Mannar and Sankar, 2004; Wimalawansa, 2013). Nowadays, the demand for food products with an added value goes far beyond the simple satisfaction of hunger or the provision of given nutrients present in low concentrations, because the correlation between diet and human health has been widely proven (Domínguez Díaz et al., 2020). Thus, the adjective "fortified" has evolved into "functional" when food products began to be fortified with special

* Corresponding author.
E-mail address: luca.settanni@unipa.it (L. Settanni).

https://doi.org/10.1016/j.ijfoodmicro.2023.110194
Received 5 October 2022; Received in revised form 1 March 2023; Accepted 25 March 2023
Available online 28 March 2023

constituents that exert physiological effects on the human body and decrease the risk of diseases (Bigliardi and Galati, 2013).

Plant and fruit derived by-products are greatly useful to develop novel functional foods (Granato et al., 2017). Nut industry generates large amounts of by-products (Caltagirone et al., 2021; Garcia-Perez et al., 2021), mainly represented by shells and skin (Chang et al., 2016). Nut by-products are particularly rich in phenolic compounds (Martínez et al., 2010); although these compounds do not represent nutritional factors, they are of relevance for the human health due to their antioxidant, anti-inflammatory, antiatherogenic, and antimutagenic properties (Carvalho et al., 2010).

Among nuts, almond (*Prunus dulcis* L.) is a crop of paramount importance for the food industry (Sottile et al., 2020). The cultivation of almond in Sicily has very ancient origins; the consumption of almonds is extremely popular and the demand for this product is on the increase all over the world. The Sicilian almond belongs to the "Mediterranean" typology, which differs from the "Californian" one for a hard or semi-hard shell, shelled rate not superior to 40–42 %, when from irrigated orchards. From a functional point of view, Mediterranean almonds are mostly used in the agro-food industry, in cosmetics and pharmaceuticals, mainly due to the higher amount of fatty acids and starch than Californian almonds (Sottile et al., 2014). Almond fruit consists of four portions: kernel, skin, middle shell and hull (Esfahlan et al., 2010). The edible part of the fruit is the kernel which is eaten alone, raw or after toasting, or as ingredient for several food formulations, especially in desserts and confectioneries, and drink preparations. As a matter of fact, almond is cultivated for the high nutritional value of kernels, while the rest of the fruit does not possess a particularly interesting potential (Barral-Martinez et al., 2021). Basically, almond by-products can be used for renewable energy production (Kaur et al., 2020) or as livestock feed (Prgomet et al., 2017). Almond skin is a source of nutrients and health promoting compounds (Barreca et al., 2020; Loizzo et al., 2021), mainly polyphenols (Mandalari et al., 2010), and for this reason it is gaining interest as a new ingredient for the food industry (Garcia-Perez et al., 2021), including the bakery sector (Barreira et al., 2019; Bartkiene et al., 2021; Pasqualone et al., 2018). In case of breads, the products enriched with almond by-products other than skin received a general positive appreciation by tasters. Regarding the application of almond skin in bread production, to our knowledge, the only work available focussed on the rheological dough properties, but the authors (Pasqualone et al., 2018) did not provide quality evidences on the final breads.

Traditional breads are produced through biological leavening performed by baker's yeast or applying sourdough technology (Ruisi et al., 2021). In particular, sourdough is a microbiologically complex environment where lactic acid bacteria (LAB) and yeasts co-exist determining several positive characteristics to the final breads (Graça et al., 2021). Indeed, sourdough biotechnology offers several nutritional and functional advantages over the use of baker's yeast only (Gobbetti et al., 2019). For this reason, sourdough fermentation is widely applied to produce bread throughout Italy (Valmorri et al., 2006).

In order to comply with the European priorities of recycling wastes, minimize and valorise food by-products, and improving food industry sustainability, the present work was aimed to test almond skin in powder form as an ingredient in semolina bread making to functionalize a traditional sourdough bread typology produced in south Italy.

2. Materials and methods

2.1. Raw materials and bacteria

Wet skin of almond cv. Tuono was separated soon after blanching at 95 °C for 3 min followed by peeling at the almond factory "Bongiovanni Almonds s.r.l.", located in Mazzarino (CL, Italy), that manages a large amount of fruit production and processing in a strategic area for almond growing in Sicily.

Almond skin was dried in the semi-industrial oven Compact Combi (Electrolux, Pordenone, Italy) at a biomass density of 2.5 kg/m^2. Drying was performed at 54 °C until reaching constant weight (almost 48 h). Dried skin was milled through a Retsch centrifugal apparatus (Mill ZM1, Haan, Germany) equipped with a 250 μm stainless steel ring sieve. The semolina (Selezione Casillo S.R.L., Corato, Italy) used for bread production was commercially available under the brand "Conad" and the labelled nutritional values (per 100 g) were: 10.5 g of proteins; 70.0 g of carbohydrates; 1 g of fats; 0.2 g of saturated fats; and 2.5 g of fibres.

Several LAB strains of sourdough origin were used to start a multiple sourdough inocula. All strains (*Lentilactobacillus diolivorans* SD4, *Fructilactobacillus sanfranciscensis* SD22, *Levilactobacillus brevis* SD46, *Lactiplantibacillus plantarum* SD96, *Weissella cibaria* SD123, *Lactiplantibacillus pentosus* SD130, *Leuconostoc citreum* SD142 and *Leuconostoc holzapfelii* SD148) belonged to the Culture Collection of the Agricultural Laboratory – University of Palermo (Italy). All strains were reactivated from −80 °C glycerol stocks and propagated twice in modified de Man-Rogosa-Sharpe (mMRS) prepared following the protocol of Corsetti et al. (2008) and incubated overnight at 30 °C.

2.2. Sourdough development

Before sourdough production in solid form, LAB strains were singly inoculated and propagated in liquid form using sterile semolina extract (SSE) broth as growth medium in which they can develop above 10^9 CFU/mL (Alfonzo et al., 2016). After three sub-cultivation steps, all LAB strains were used to prepare a multiple-strain starter culture by mixing all fermented SSEs together (Gaglio et al., 2021). A 500-g dough with a dough yield (DY = weight of the dough / weight of semolina × 100) of 160 was prepared with 312.5 g of semolina and 187.5 mL of mixed LAB suspension obtained by dilution of the SSE mixture in sterile tap H_2O in order to reach a final cell density of the co-inoculum in dough between 10^6 and 10^7 CFU/g. The dough was subjected to fermentation for 16 h at 28 °C and daily refreshed for 7 days to obtain a sourdough ready to be used in bread making (Corona et al., 2016).

2.3. Dough production and bread baking

Bread production was performed with the LAB started sourdough as unique fermenting agent. Although the absence of salt is not typical for breads produced in south Italy, salt was not added to better evaluate the effect of powdered almond skin (PAS) on the persistence of LAB inocula. Bread dough (1 kg) was produced with a DY = 175 through the following recipe for the control (CTR) trial: 571.5 g of semolina; 285.7 mL of sterile tap H_2O and 142.8 g of 7-day-mature sourdough. PAS was added at 5 or 10 % (w/w) on the weight of semolina for the experimental trials. Thus, 5-PAS and 10-PAS doughs were prepared as CTR dough but the semolina amount was 542.9 g and 514.3 g, respectively. The ingredients were mixed with the planetary mixer model XBM10S (Electrolux Professional, SpA, Pordenone, Italy) equipped with a paddle at speed 4 for 15 min.

Six 100-g doughs per trial were placed into stainless steel baking pans with the trapezoidal dimensions [143 × 79 mm (top inside), 129 × 64 mm (bottom outside), 57 mm (depth inside)] indicated by the American Association of Cereal Chemists – Method 10-10B of AACC (2000). The pans were covered with aluminum foils during fermentation performed at 28 °C for 8 h. The same fermentation conditions were applied to the remaining 400 g of dough from each formulation which were transferred into sterile glass canning jars (Vetreria Borgonovo Spa, Borgonovo Val Tidone, Italy). All bread productions were performed in duplicate (two technical repeats) and repeated twice (two independent replicates) after 1 month.

The baking process occurred with the same oven used to dry almond skin applying a 2-step program consisting of an initial exposure of the doughs under hot air/steam at 200 °C for 5 min followed by 15 min of convection heat (hot air only) at the same temperature.

2.4. Fermentation monitoring

The monitoring of the fermentation of the sourdough refreshed until inoculum and the doughs for each bread formulation occurred by determination of pH, total titratable acidity (TTA), lactic and acetic acid concentration, LAB cell density.

pH was measured at the end of each propagation of LAB in SSE, for all refreshments of the sourdough (just after inoculum and after 16 h) and during the fermentation (just after inoculum and at 2, 4, 6 and 8 h) of the three bread trials (CTR, 5-PAS and 10-PAS). The measurements were performed by immersing directly the pH-meter (XS Instruments, Carpi, Italy) probe in SSE test tubes (10 mL) or into 10 g of sourdough or bread dough collected aseptically. The pH of PAS was also measured: 10 g of powder were dispersed into 90 mL of distilled water H_2O by magnetic stirring before inserting pH-meter probe.

TTA was determined only for sourdough and bread trials and the measurements occurred at the same times of pH determinations. In particular, the same samples subjected to pH measurements were transferred into stomacher bags, added with 90 mL distilled H_2O and homogenized through the stomacher BagMixer® 400 (Interscience, Saint Nom, France) at the highest speed for 2 min before titration with NaOH 0.1 N. TTA results were expressed as mL of NaOH 0.1 N/10 g of dough.

The concentrations of (D + L) lactic acid and acetic acid were determined just after mixing and at the end of fermentation (at the 16th hour of the seventh refreshment for sourdough and at the eighth hour for CTR, 5-PAS and 10-PAS trials). Both determinations were performed by high performance liquid chromatography (HPLC) as described by Gaglio et al. (2020a) on 10 g of each dough.

In order to perform the viable count of the main group of interest during sourdough and bread dough fermentation, all samples were homogenized as reported above, but Ringer's solution (Sigma-Aldrich, Milan, Italy) was used in place of distilled H_2O. A decimal serial dilution was prepared for all cell suspensions from the homogenized samples and the appropriate dilutions were plated as follows: on plate count agar (PCA) incubated aerobically at 30 °C for 72 h to enumerate total mesophilic microorganisms (TMM); on mMRS agar, incubated anaerobically at 30 °C for 48 h for mesophilic LAB rods; on sour dough bacteria (SDB) (Kline and Sugihara, 1971) agar incubated aerobically at 30 °C for 48 h for sourdough LAB; on yeast peptone dextrose (YPD) agar added with chloramphenicol (0.1 g/L) and incubated at 28 °C for 48 h for yeasts. In addition, only for bread doughs, the investigation included also the detection of members of the Enterobacteriaceae family on violet red bile glucose agar (VRBGA), incubated at 37 °C for 24 h and total coliforms on violet red bile agar (VRBA), incubated at 37 °C for 24 h. Anaerobic conditions were obtained with jars sealed hermetically and in presence of the AnaeroGen AN25 system (Oxoid, Milan, Italy). All media were purchased from Oxoid. Microbiological analyses were performed in duplicates and the final results were expressed as Log colony forming units (CFU)/g.

2.5. DNA extraction, MiSeq library preparation, and Illumina sequencing (Franciosi)

Total genomic DNA was extracted with the Power Food Microbial DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) following manufacturer's protocol. DNAs were quantified by the Nanodrop 8800 Fluorospectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

Amplicon library preparation, quality and quantification of pooled libraries, and pair-end sequencing using the Illumina MiSeq system were carried out at Fondazione Edmund Mach (FEM, San Michele a/Adige, Italy) sequencing platform. Briefly, total genomic DNA was amplified using primers specific for the bacterial V3–V4 region (Claesson et al., 2010; Bolyen et al., 2019) of the 16S rRNA gene (*Escherichia coli* positions 341 to 805). Each sample was amplified by PCR in 25 μL reaction volume with 1 μM of each primer. PCR reactions were obtained with the GeneAmp PCR System 9700 (Thermo Fisher Scientific). Amplification products were checked on 1.5 % agarose gel and purified using the Agencourt AMPure XP system (Beckman Coulter, Brea, CA, USA), following manufacturer's instructions. Subsequently, a second PCR was performed to apply dual indices and the Illumina sequencing adapters Nextera XT Index Primer (Illumina, San Diego, CA, USA). Amplicon libraries were purified using the Agencourt AMPure XP system and the quality control was performed on a Typestation 2200 platform (Agilent Technologies, Santa Clara, CA, USA). Finally, all barcoded libraries were pooled in an equimolar ratio and sequenced on an Illumina MiSeq (PE300) platform (MiSeq Control Software 2.5.0.5 and Real-Time Analysis software 1.18.54.0).

2.6. Illumina data analysis and sequences identification by QIIME2

Raw paired-end FASTQ files were demultiplexed using idemp (https://github.com/yhwu/idemp/blob/master/idemp.cpp, accessed on 17 December 2021) and imported into Quantitative Insights Into Microbial Ecology, Qiime2, version 2020.11 (Bolyen et al., 2019). The sequences were quality-filtered, trimmed, de-noised, and merged using DADA2 (Callahan et al., 2016). Chimeric sequences were identified and removed via the consensus method in DADA2. Representative sequences were aligned with MAFFT and used for phylogenetic reconstruction in FastTree using plugins alignment and phylogeny (Katoh and Standley, 2013). Taxonomic and compositional analyses were carried by using the plugins feature classifier (https://github.com/qiime2/q2-feature-classifier, accessed on 17 December 2021). A pre-trained, accessed Naive Bayes classifier based on the Greengenes gg_13_5_otus.tar.tgz Operational Taxonomic Units (OTUs) database (http://greengenes.secondgenome.com/?prefix=downloads/greengenes_database/gg_13_5/ accessed on 17 December 2021), which had been previously trimmed to the V4 region of 16S rDNA, bound by the 341F/805R primer pair, was applied to paired-end sequence reads to the generate taxonomy tables. Data generated by Illumina sequencing were uploaded in the NCBI Sequence Read Archive (SRA) and are available under Ac. PRJNA821005.

2.7. Characteristics of breads

After baking, all breads were cooled at room temperature for 30 min before analysis of quality parameters.

Regarding the morphogeometric parameters, weight loss (WL) due to water evaporation during baking was calculated by the formula: $WL = \left(\frac{wd-wb}{wd}\right) \times 100$. The factors in this formula are as follows: wd, weight (g) of dough; wb, weight (g) of bread (Purlis and Salvadori, 2007). Bread volume was determined with the 2 L volumeter for bakery products (ErreCi s.r.l., Merate, Italy).

The colour of the breads was evaluated on crust and crumb using the Chroma Meter CR-400 (Minolta, Osaka, Japan) based on the Hunter's scale parameters (L*, a* and b*). In particular, three points of crumb were taken from the central slice, while four points of the crust were analysed on the top surface of each bread.

The texture analysis was performed measuring the resistance to compression (N/mm^2) through the Instron-5564 (Instrom Corp., Canton, MA) as reported by Corsetti et al. (2000).

Bread crumb was analysed for void fraction (the fraction of the total area corresponding to the eyes), cell density (number of cells/cm^2) and mean cell area (in mm^2). Image analysis was performed after scanning of crumb images (Epson Perfection 4180 Photo, Seiko Epson Corp., Japan) at a resolution of 350 dpi. The images, saved in TIFF format, were processed with the ImageJ software (National Institutes Health, Bethesda, Md, USA). All images were cut to a square of 207 × 207 pixels (representing 15 × 15 mm of the slice), converted to grey-level (8 bit) and binary images were obtained applying the Otsu's threshold

algorithm.

The breads were also investigated for the presence of spore-forming aerobic bacteria. Twenty-five grams of each bread were serially diluted in Ringer's solution as described for dough samples. All cell suspensions were heated at 85 °C for 15 min and aliquots of 0.1 mL were spread onto Nutrient Agar (NA) (Oxoid, Basingstoke, UK) plates which were incubated for 48 h at 32 °C (Messina et al., 2019).

2.8. Analysis of volatile organic compounds (VOCs)

The identification and quantification of the volatile organic compounds in the aroma of bread with powdered almond skin (PAS) was performed with a SPME/GC–MS method previously reported by Settanni et al. (2013), but slightly modified. A 50/30 μm divinylbenzene (DVB)/carbowax (CAR)/polydimethylsiloxane (PDMS) fibre was used, previously conditioned for 1.5 h at 250 °C, as reported by the manufacturer Supelco® (Supelco®, Bellefonte, Pennsylvania, USA). Four grams of each bread sample were triturated and placed into a 20 mL (75.5 × 22.5 mm) vial (Supelco, Bellefonte, PA, USA). All vials were added with 20 μL of the 2-octanol standard solution (0.819 g/mL). Since 2-octanol could potentially be present in bread, its absence was verified before its use as an internal standard. The samples were equilibrated at 60 °C for 10 min. The SPME fibre was exposed to the bread powder for 30 min in the headspace of the sample maintained at 60 °C. The aromatic compounds were desorbed from the fibre for 10 min and fed into the column through a splitless injector at 250 °C. All samples were prepared and tested in triplicate in standard 20 mL headspace vials. Quantitation of volatile compounds was performed using an Agilent 7000C GC system, equipped with a DB-5MS fused silica capillary column (30 m × 0.25 mm; 0.25 μm film thickness) (Santa Clara, CA, USA) coupled to an Agilent Triple Quadrupole 5973 MSD Selective Mass Detector; ionization energy 70 eV; energy of the electron multiplier 2000 V; transfer line temperature, 270 °C. Solvent delay: 0 min. Helium was used as a carrier gas (1 mL/min). The GC temperature was 40 °C for the first 2 min (during splitless injection), then 40 °C to 60 °C, increasing by 4 °C/min, 60 °C for 2 min, from 60 °C to 190 °C, increasing by 2 °C/min, from 190 °C to 230 °C, increasing by 5 °C/min and finally 230 °C for 15 min. Volatile compounds were injected automatically at 250 °C in the splitless mode. Linear retention indices were calculated using n-alkanes as reference compounds. For the analysis of the alkane solution (C_8–C_{40}) (Sigma-Aldrich, USA), the inlet mode was set to 10:1 split mode. Individual peaks were analysed using the GC-MSolution package, version 2.72. Compound identification was performed using the Adams mass spectral database (Adams, 2007), NIST 11, Wiley 9 and FFNSC 2.

2.9. In vitro digestion

In vitro digestion of the bread was carried out using the method described by Attanzio et al. (2019a). Briefly, 5.0 g samples were homogenized in a semi-micro stainless steel jar with a Waring blender (Waring, New Hartford, CT, USA) in 20 mL of a buffered solution at pH 6.8 simulating the salivary oral phase [NaCl (0.126 g), KC1 (0.964 g), KSCN (0.189 g), KH_2PO_4 (0.655 g), urea (0.200 g), $Na_2SO_4{\cdot}10\ H_2O$ (0.763 g), NH_4Cl (0.178 g), $CaCl_2{\cdot}2H_2O$ (0.228 g) and $NaHCO_3$ (0.631 g) in 1 L of distilled water]. The following gastric phase was initiated by acidifying to pH 2.0 with HCl and adding porcine pepsin (8 mg/mL; 3200–4500 units/mg, Sigma-Aldrich). The solution was stirred (100 rpm) at 37 °C for 2 h, in the dark. Digested post-gastric solution was brought to final pH 7.5 using 200 mM NaH_2PO_4/Na_2HPO_4 buffer and 5 M NaOH, porcine bile extract (2.4 mg/mL, Sigma) and pancreatin from hog pancreas (0.4 mg/mL, Sigma) were added (final volume 32 mL). The solution was incubated in the shaking water bath at 37 °C for 2 h, in the dark, simulating intestinal digestion. The enzymes were inactivated by heating (4 min at 100 °C). Post-intestinal digest was centrifuged at 167.000 ×*g* for 35 min at 4 °C (Beckman Optima TLX ultracentrifuge, Beckman Instruments, Inc., Palo Alto, CA) separating the supernatant (bioaccessible fraction) from the insoluble material. During the digestion process, aliquots (4 mL) of the oral phase were withdrawn, centrifuged at 1.500 ×*g* for 30 min at 4 °C and the supernatant collected as bread sample before digestion.

2.10. Total phenols

Samples of 1 mL of the bread before digestion and of the bioaccessible fractions obtained following in vitro digestion of bread were extracted sequentially twice using a ratio of 1:10 (v/v) with acidified ethanol solution over 2 h at room temperature. The resulting solution was centrifuged at 1000 ×*g* for 15 min at 4 °C, followed by ethanol removal by rotary evaporation. For appropriate comparison, almond skin powder samples were similarly extract using a ratio of 1:10 (w/v) with the extraction solvent. The total phenol content in the resulting aqueous solution was determined by Folin–Ciocalteu's assay (Attanzio et al., 2016) and expressed as gallic acid equivalents (GAE).

2.11. Radical scavenging activity assays

Radical scavenging activity was evaluated by carrying out $ABTS^+$ radical cationic decolorization assay as described by Attanzio et al. (2016). $ABTS^{+\bullet}$ was generated by oxidation of ABTS with potassium persulphate (Re et al., 1999).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was measured according to Brand-Williams et al. (1995).

Each sample was tested in duplicate, at three different dilutions, within the linearity range of the assay. The $ABTS^{+\bullet}$ and DPPH and radical scavenging activities of the breads samples were quantified in comparison with Trolox (Sigma), water-soluble analog of vitamin E, used for the standardization of the assays. The results of the antiradical capacity were expressed as μM Trolox equivalent g-1 bread.

2.12. Antioxidant activity in differentiated Caco-2 cells stimulated by IL-1β

Caco-2 cell line [American Type Culture Collection (ATCC), Item No. HTB-37], derived from a human colon adenocarcinoma, were cultured at 37 °C in a humidified atmosphere of CO_2/air (5/95, v/v) in Dulbecco's MEM with Glutamax supplemented with 25 mM HEPES, 10 % (v/v) heat-inactivated Fetal Bovine Serum (FBS), 1 % penicillin (1 × 103 U/mL)-streptomycin (10 mg/mL), and 1 % (v/v) non-essential amino acids (NEAA). For the experiments, Caco-2 cells, seeded at a density of 2.5 × 105 in 12-well plates (Corning Costar, Inc.), were grown in the culture medium for 18–20 days to obtain fully differentiated cells. The culture medium was changed thrice a week. Monolayers of Caco-2 at 15-days post-confluence were incubated with IL-1β 25 ng/mL in the absence or in the presence of aliquots (12.5 mg bread/100 μL) of the bioaccessible fractions of breads. Cells incubated with medium alone were considered as control. After 4 h of treatment, ROS levels were monitored by measuring fluorescence changes that resulted from intracellular oxidation of 2′,7′-dichlorofluorescin diacetate (DCFDA). Briefly, DCFDA, at 10 μM final concentration, was added to the medium 30 min before ending the treatment of the cells. The medium was then removed, and the cells were washed with PBS, resuspended in the same buffer and immediately subjected to fluorescence-activated cell sorting analysis with an Epics XLTM flow cytometer, using the Expo32 software (Beckman Coulter). At least 10.000 events per sample were evaluated (Attanzio et al., 2019b).

Cytotoxicity of the bioaccessible fractions of breads on Caco-2 cells was excluded by pilot studies using the Trypan Blue exclusion method and the MTT assay.

2.13. Sensory analysis

The final breads were subjected to a descriptive sensory analysis

performed by a panel of 17 judges, including 11 women and six men aged between 23 and 88 years old. The panellists were trained to conduct the bread attribute evaluation. During tasting section, they were asked to judge several descriptors regarding appearance, texture, and odour of breads (Comendador et al., 2012; Martins et al., 2015; Rodrigues et al., 2014). The score of each attribute was expressed on a 9-point scale (1 = extremely bad; 9 = extremely good). The judges were also asked to give an overall assessment of each bread that is a general evaluation based on the scores of all attributes considered. The analysis was carried out in single chambers following ISO 13299 guidelines (2003).

2.14. Statistical analysis

One-Way Variance Analysis (ANOVA) was applied to identify the difference among microbiological and physicochemical data. The Tukey's test was applied for multiple mean comparisons (statistical significance $p < 0.05$). Statistical processing of data was performed with the XLStat software version 7.5.2 for Excel (Addinsoft, New York, USA).

3. Results and discussion

3.1. Acidification process

The fermentation process carried out by the selected LAB strains added as starters was followed from the preparation of the liquid inoculum in SSE. The growth in SSE medium determined a decrease of the initial pH from 5.6 to an average value of 4.1 ± 0.2; the highest pH (4.4 ± 0.0) was registered for the strain *Ln. holzapfelii* SD148, while the lowest value (3.8 ± 0.1) was shown by *Lp. plantarum* SD96 and *Lp. pentosus* SD130. These data are comparable with pH evolution generally observed for LAB (basically lactobacilli, leuconostocs and weissellas) developed in flour and semolina extracts (Settanni et al., 2013; Alfonzo et al., 2016).

The eight fermented SSEs from the third propagation step were then mixed together and represented the liquid inoculum to produce the sourdough for bread production (Alfonzo et al., 2016). The sourdough developed with the eight LAB strains was characterized by an initial pH of 5.5 ± 0.1 and, from the fifth refreshment, it decreased to 3.8 ± 0.1 and remained constant until the seventh refreshment. At the last refreshment step, TTA value was 11.30 ± 0.40 mL NaOH 0.1 N/10 g. Both pH and TTA values were similar to those recorded with *F. sanfranciscensis*, *Ln. citreum* and *W. cibaria* (Alfonzo et al., 2016; Gaglio et al., 2020a) or *Lv. brevis*, *Ln. citreum* and *W. cibaria* (Gaglio et al., 2021) used in multiple-species sourdough starter inocula. Thus, after seven daily refreshments, the sourdough was considered ready to be used as leavening agent for bread production.

The results of pH and TTA measurements of the experimental doughs are reported in Table 1. Before fermentation (T_0), CTR dough displayed a pH of 5.35, while higher values were registered for the two doughs prepared mixing 5 % (pH 5.72) and 10 % (pH 5.80) of almond skin. The higher pH of PAS added trials can be explained by the pH (6.50) of almond skin which is higher than that general reported for wheat raw materials (flours and semolinas) which is in the range 5.90–6.10 (Settanni et al., 2013; Corona et al., 2016). This parameter decreased for all trials and, at the end of fermentation, were almost comparable reaching 4.20, 4.33 and 4.45 for CTR, 5-PAS and 10-PAS dough, respectively. TTA evolves inversely from pH; this acidification parameter increases linearly with pH decrease (Siepmann et al., 2019). In particular, at the end of fermentation, TTA values of PAS doughs (9.10 and 10.40 mL NaOH 0.1 N/10 g for 5-PAS and 10-PAS, respectively) were higher than that of CTR dough (7.00 mL NaOH 0.1 N/10 g).

The microorganisms with major interest during sourdough fermentation were enumerated from all three bread production trials (Table 2). LAB levels at the beginning of fermentation (T_0) were around 10^6 CFU/g both on mMRS and SDB for all trials. These data were comparable to those of TMM (5.71–5.95 Log CFU/g) confirming that LAB transferred by the mature sourdough developed in this study dominated the fermenting community of the three doughs. At the end of fermentation (T_8), LAB levels increased by almost two Log cycles in all trials, a general trend registered in sourdough bread production (Suo et al., 2021). No statistical differences were observed among CTR and PAS added doughs, indicating that at the percentages used in this study, almond peel did not negatively influence LAB development. Both at T_0 and T_8, LAB cell densities were comparable but slightly higher than TMM levels; this observation is imputable to the high nutritional requirements of LAB only partially satisfied by PCA (Alfonzo et al., 2017; Gaglio et al., 2020a). Another relevant microbial group during sourdough fermentation is represented by yeasts (Carbonetto et al., 2020). The initial levels of this population ranged between 4.15 and 4.33 and evolved to little <6.0 Log CFU/g during the 8 h of fermentation. The spontaneous development of yeasts in sourdough is a common phenomenon (Siepmann et al., 2018) and the results registered by this study are generally observed during semolina sourdough fermentation (Corona et al., 2016) and also during the fermentation of fortified traditional Sicilian sourdough breads (Gaglio et al., 2020a, 2021).

Almond skin, removed from the almond kernel by hot water blanching (Moure et al., 2007), is considered a waste by the factory "Bongiovanni s.r.l." where this raw material was collected and, as such, it does not undergo any treatment to preserve its hygienic characteristics. As a matter of fact, almond skin is treated as a waste product, left uncovered and exposed to any environmental contamination. For this reason, members of Enterobacteriaceae family and total coliforms, representing hygiene indicators (Malavi et al., 2021), were investigated in PAS and in the three dough trials. PAS was characterized by 3.00 ± 0.40 Log CFU/g of Enterobacteriaceae and 2.55 ± 0.25 Log CFU/g of

Table 1
Chemical parameters of control and experimental doughs immediately before (t_0) and after 8 h (t_8) fermentation.

Samples	t_0					t_8				
	pH	TTA	D + L Lactic acid (mg/g)	Acetic acid (mg/g)	FQ	pH	TTA	D + L Lactic acid (mg/g)	Acetic acid (mg/g)	FQ
CTR	5.35 ± 0.02^c	3.20 ± 0.10^c	0.84 ± 0.15^a	0.18 ± 0.02^a	3.12	4.20 ± 0.04^c	7.00 ± 0.10^c	3.59 ± 0.23^a	0.79 ± 0.13^c	3.03
5-PAS	5.72 ± 0.02^b	4.00 ± 0.20^b	0.82 ± 0.10^a	0.15 ± 0.02^a	3.62	4.33 ± 0.03^b	9.10 ± 0.10^b	3.48 ± 0.30^a	0.71 ± 0.20^c	3.26
10-PAS	5.80 ± 0.04^a	4.40 ± 0.10^a	0.83 ± 0.13^a	0.16 ± 0.01^a	3.42	4.45 ± 0.02^a	10.40 ± 0.20^a	3.29 ± 0.19^a	0.72 ± 0.11^c	3.03
Statistical significance	***	***	n.s.	n.s.	n.a.	***	***	n.s.	n.s.	n.a.

Results indicate mean values ± standard deviation (SD) of four determinations (carried out in two technical repeats for two independent experiments).
Data within a column followed by the same letter are not significantly different according to Tukey's test. p value: ***$p < 0.001$; n.s., not significant ($p > 0.05$).
Abbreviations: TTA, total titratable acidity; FQ, Fermentation Quotient (molar ratio between lactic and acetic acids); CTR, control semolina; 5-PAS, 5 % powdered almond skin addition, 10-PAS, 10 % powdered almond skin addition; n.a., not analysed.

Table 2
Microbial loads (Log CFU/g) of sourdough and doughs immediately before (t_0) and after 8 h (t_8) fermentation.

Samples	PCA		mMRS		SDB		YPD		VRBA		VRBGA	
	t_0	t_8	t_0	t_8	t_0	t_8	t_0	t_8	t_0	t_8	t_0	t_8
Sourdough	7.78 ± 0.31	n.a.	8.17 ± 0.29	n.a.	8.10 ± 0.39	n.a.	5.88 ± 0.21	n.a.	<1	n.a.	<1	n.a.
CTR	5.71 ± 0.20^a	7.60 ± 0.31^a	6.19 ± 0.25^a	8.00 ± 0.31^a	5.95 ± 0.22^a	8.15 ± 0.21^a	4.15 ± 0.43^a	5.94 ± 0.21^a	<1^a	<1^b	<1^b	<1^b
5-PAS	5.95 ± 0.24^a	7.79 ± 0.21^a	6.30 ± 0.34^a	8.12 ± 0.29^a	6.01 ± 0.28^a	7.91 ± 0.30^a	4.33 ± 0.21^a	5.75 ± 0.30^a	<1^a	2.25 ± 0.20^a	<1^b	2.91 ± 0.30^a
10-PAS	5.88 ± 0.35^a	7.68 ± 0.27^a	6.14 ± 0.27^a	8.19 ± 0.25^a	6.19 ± 0.24^a	8.03 ± 0.40^a	4.18 ± 0.29^a	5.90 ± 0.25^a	<1^a	2.40 ± 0.24^a	1.21 ± 0.19^a	3.17 ± 0.25^a
Statistical significance	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***	***	***

Results indicate mean values ± standard deviation (SD) of four plate counts (carried out in two technical repeats for two independent experiments).
Data within a column followed by the same letter are not significantly different according to Tukey's test. p value: ***$p < 0.001$; n.s., not significant ($p > 0.05$).
Abbreviations: CTR, control semolina; 5-PAS, 5 % powdered almond skin addition; 10-PAS, 10 % powdered almond skin addition; PCA, plate count agar for total mesophilic microorganisms; mMRS, modified de Man-Rogosa-Sharpe for mesophilic LAB rods; SDB, sour dough bacteria medium for sourdough LAB; YPD, yeast peptone dextrose for yeasts; VRBGA, violet red bile glucose agar for members of the Enterobacteriaceae family; VRBA, violet red bile agar for total coliforms; n.a., not analysed.

total coliforms. Although Enterobacteriaceae are part of the microbiome of durum wheat semolina (De Angelis et al., 2019), CTR dough did not show detectable levels of enterobacteria or coliforms at both sampling times (T_0 and T_8), but, on the contrary, both PAS trials were characterized by their presence. In particular, both groups were almost undetectable just after ingredient mixing (T_0) in 5-PAS dough as well as in 10-PAS dough, since only Enterobacteriaceae were enumerated at 1.21 in 10-PAS dough. However, they increased at the end of fermentation: coliforms were counted at 2.25 and 2.40 Log CFU/g in 5-PAS and 10-PAS doughs, respectively, while enterobacteria at 2.91 and 3.17 in 5-PAS and 10-PAS doughs, respectively. In comparison to LAB, the development of these undesired bacteria was quite limited, probably because Enterobacteriaceae are not easily cultivable in sourdough due to the low pH of the environment that is considered a key factor contributing to limit the growth of this bacterial population (Dinardo et al., 2019).

3.2. Culture-independent microbiological investigation

The DNA extracted from PAS and all doughs before and after fermentation was successfully amplified in the bacterial V3-V4 16S rRNA gene region and a total of 525,004 paired-end sequences were

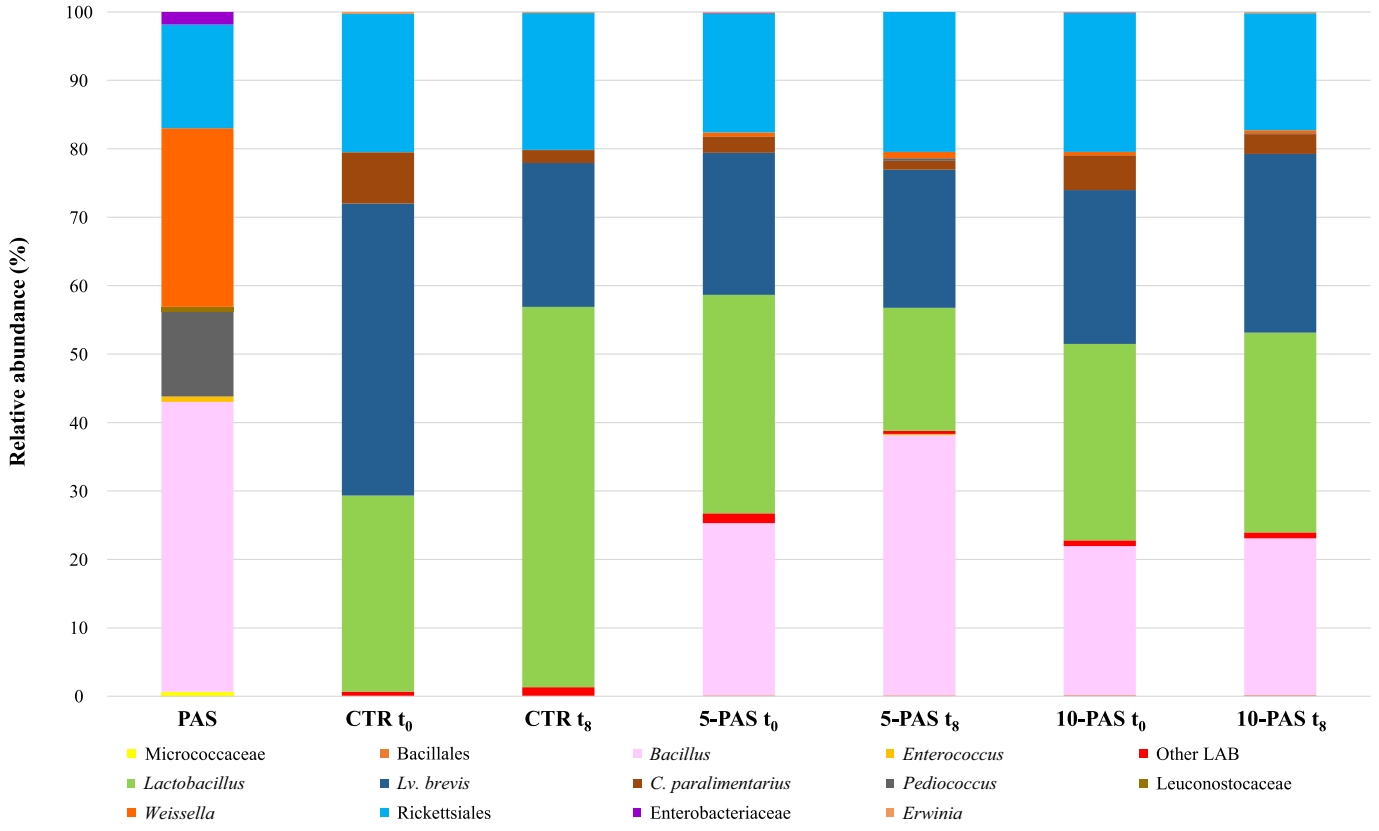

Fig. 1. Relative abundances (%) of bacterial groups identified by MiSeq Illumina in powdered almond skin and doughs immediately before (t_0) and after 8 h (t_8) fermentation. Only taxa occurring at 0.0.1 % abundance in at least one sample were included. Abbreviations: PAS, powdered almond skin; CTR, control semolina; 5-PAS, 5 % powdered almond skin addition; 10-PAS, 10 % powdered almond skin addition; *Lv.*, *Levilactobacillus*; *C.*, *Companilactobacillus*; LAB, lactic acid bacteria.

retrieved. Forty-nine operational taxonomy units (OTUs) distributed among Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Verrucomicrobia and Deinococcota phyla were identified from the doughs before and after fermentation and PAS. Fig. 1 shows only the OTUs with an individual relative abundance (RA) above 0.1 %; this level is generally fixed as the threshold for abundant communities (Logares et al., 2014). In particular, two orders, four families, six genera and two species were identified at RA > 0.1 %.

Only Rickettsiales were detected in all samples at consistent RA (15.13–20.42 %). The presence of this order is imputable to environmental contamination of plant material since members of this order are basically pathogens of vertebrate and invertebrate hosts (Yu and Walker, 2006). *Weissella* genus was also detected in all samples analysed, but the RA registered in PAS (26.08 %) was much higher than that of doughs (0.1–09 %). Weissellas are LAB members associated to wheat flours and semolinas (Alfonzo et al., 2013) and, in this work, deliberately added (*W. cibaria* SD123) to the sourdough used for leavening. However, the low RA% found after fermentation evidenced a limited role of these LAB during fermentation. Leuconostocaceae were only detected at low levels (0.69 %) in PAS and, surprisingly, not observed in any dough even though two *Leuconostoc* strains (*Ln. citreum* SD142 and *Ln. holzapfelii* SD148) were inoculated via sourdough, indicating that the leuconostocs did not persist in sourdough before its addition as dough leavening agent. Besides Leuconostocaceae, *Enterococcus*, at low RA (0.79 %), and *Pediococcus*, at high RA (12.37 %), were other LAB members found in PAS.

Lactobacillus were not found in PAS, but detected in all doughs at high levels. In particular, the highest RA of this group was recorded for CTR dough at the end of fermentation. However, the name *Lactobacillus* refers to the old classification, and therefore according to the currently effective categorization performed by Zheng et al. (2020) it includes 23 distinct genera. The only two OTUs identified at species level were *Lv. brevis* and *Companilactobacillus paralimentarius*. In particular, RA of the former ranged between 20.15 and 42.64 %. *Levilactobacillus brevis* is a typical dominating heterofermentative species of sourdough (Pino et al., 2022) and our data confirmed the persistence of this species, added as starter strain.

The genus found in PAS at the highest RA (42.34 %) was *Bacillus*. This genus as well as the corresponding order Bacillales were not detected in CTR doughs, while it ranged between 21.75 and 37.96 in PAS added doughs. The presence of *Bacillus* might represent a health concern related to pathogenic spore-forming bacteria, especially *Bacillus cereus*, an opportunistic pathogen able to grow in the human intestinal tract (Stenfors Arnesen et al., 2008). Aerobic spore-forming bacteria can be associated to several insects (Garofalo et al., 2019) and their presence in PAS is undoubtedly due to, the uncontrolled storage conditions of the almond peel mass used in this study. Micrococcaceae were also found in PAS, but its presence in doughs was not detected. On the contrary, *Erwinia* was detected in almost all doughs, but not in PAS. This member of the Enterobacteriaceae family is reported in wheat and is generally detected in semolinas (Gaglio et al., 2020b) and sourdoughs propagated with semolina (Ercolini et al., 2013).

3.3. Bread quality characteristics

Quality attributes of breads are reported in Table 3. Baking determined a WL of 14.54 % in CTR breads and, although the average value registered for 5-PAS breads (13.98 %) was not statistically different, a consistent WL was displayed by 10-PAS trial (9.88 %). A diminishing trend was observed also for the specific volume of breads; this parameter ranged between 2.11 and 2.73 cm^3/g bread with the highest value registered for CTR breads and the lowest for 10-PAS breads. Due to the lack of works reporting data on PAS added breads any direct comparison of the morphogeometric parameters of our bread with literature data is not possible. However, Pasqualone et al. (2020) used PAS to produce biscuits and noticed a decreased WL when PAS % increased, even though

Table 3
Quality parameters of breads.

Breads	Weight loss (g)	Specific volume (cm^3/g bread)	Crust colour			Crumb colour			Firmness value (N)	Void fraction (%)	Cell density (n/cm^2)	Mean cell area (mm^2)	Aerobic bacterial spores (Log CFU/g)
			L*	a*	b*	L*	a*	b*					
CTR	14.54 ± 1.70^{a}	2.73 ± 0.19^{a}	54.45 ± 3.09^{a}	12.36 ± 2.70^{a}	39.26 ± 1.50^{a}	68.01 ± 1.67^{a}	−2.05 ± 0.32^{c}	23.49 ± 1.19^{a}	22.09 ± 1.17^{c}	31.38 ± 0.38^{b}	74.81 ± 0.91^{c}	0.43 ± 0.07^{a}	<2^{b}
5-PAS	13.98 ± 1.94^{ab}	2.55 ± 0.23^{ab}	45.66 ± 2.84^{b}	12.53 ± 0.93^{a}	26.91 ± 0.79^{b}	49.16 ± 2.12^{b}	5.44 ± 0.18^{b}	18.69 ± 0.67^{b}	26.81 ± 1.01^{b}	32.83 ± 0.97^{b}	86.67 ± 0.56^{b}	0.26 ± 0.01^{b}	2.39 ± 0.19^{a}
10-PAS	9.88 ± 1.38^{b}	2.11 ± 0.14^{b}	43.31 ± 1.30^{b}	12.23 ± 0.80^{a}	23.35 ± 0.84^{b}	37.96 ± 0.86^{c}	7.04 ± 0.23^{a}	17.76 ± 0.42^{b}	31.67 ± 2.35^{a}	40.50 ± 0.39^{a}	95.55 ± 0.78^{a}	0.23 ± 0.03^{b}	2.10 ± 0.08^{a}
Statistical significance	*	*	**	n.s.	***	***	***	***	***	***	***	**	***

Results indicate mean values ± SD of four determinations (carried out in two technical repeats for two independent experiments).
Data within a column followed by the same letter are not significantly different according to Tukey's test. p value: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; n.s., not significant ($p > 0.05$).
Abbreviations: CTR, control semolina; 5-PAS, 5 % powdered almond skin addition; 10-PAS, 10 % powdered almond skin addition.

until 10 % addition those differences were negligible. Thus, in our study, the lower WL of breads registered with the increasing level of PAS can be explained by the greater hygroscopicity of fibres, which limited water migration at a higher extent than semolina alone (Pasqualone et al., 2020). Regarding specific volume, CTR breads showed an average value comparable with that registered in the work of Schoenlechner et al. (2013) who tested different percentages of millet and observed a decreasing volume.

The presence of PAS affected the colour of the breads, both in terms of L* and b*. Both parameters decreased for crust and crumb, but L* decrease was more evident for crumb, while b* for crust. a* values were not statistically different for bread crust, but impacted consistently the crumb, showing an increasing level with PAS addition. The darkening of the final products was already reported by Pasqualone et al. (2020) who

Table 4
Volatile organic compounds emitted from breads.

LRI[a]	Compounds[b]	Aroma description[c]	Odour threshold (ppb)[d]	Samples			Statistical significance
				CTR	5-PAS	10-PAS	
	ΣAlkanes			4.83 ± 0.15[c]	6.62 ± 0.20[b]	12.52 ± 0.38[a]	***
600	*n*-Hexane			2.59 ± 0.08[b]	2.12 ± 0.06[c]	6.65 ± 0.20[a]	***
1100	*n*-Undecane			0.39 ± 0.01[b]	0.73 ± 0.02[a]	0.71 ± 0.02[a]	***
1200	*n*-Dodecane			0.84 ± 0.03[c]	1.56 ± 0.05[b]	2.32 ± 0.07[a]	***
1300	*n*-Tridecane			1.01 ± 0.03[c]	2.21 ± 0.07[b]	2.84 ± 0.09[a]	***
	ΣAlcohols			5.96 ± 0.17[a]	5.51 ± 0.16[b]	4.44 ± 0.13[c]	***
983	1-Octen-3-ol	Earthy green and oily fungal, raw chicken	1	0.41 ± 0.01[a]	0.35 ± 0.01[b]	0.40 ± 0.01[a]	***
1117	Phenylethyl alcohol	Sweet, floral, fresh	750–1100	5.45 ± 0.16[a]	5.16 ± 0.15[a]	4.04 ± 0.12[b]	***
	ΣAldehydes			7.33 ± 0.22[b]	7.39 ± 0.22[b]	8.77 ± 0.26[a]	***
804	Hexanal	Fruity, grassy, green	17	1.46 ± 0.04[c]	2.14 ± 0.06[b]	3.19 ± 0.10[a]	***
903	Heptanal	Fresh, green, citrus and almond odour	3	0.57 ± 0.02[b]	0.73 ± 0.02[a]	0.72 ± 0.02[a]	***
950	(*Z*)-2-Heptenal	Soapy, mushroom	0.8–10	0.32 ± 0.01[b]	0.51 ± 0.02[a]	0.48 ± 0.01[a]	***
961	Benzaldehyde	Bitter almond, burnt sugar	350–3500	2.05 ± 0.06[a]	0.99 ± 0.03[c]	1.21 ± 0.04[b]	***
1002	Octanal	Waxy with a green peely nuance	0.7	0.81 ± 0.02[b]	0.97 ± 0.03[a]	0.74 ± 0.02[c]	***
1103	Nonanal	Citrus-like, fatty, pungent	1	1.57 ± 0.05[a]	1.33 ± 0.04[b]	1.60 ± 0.05[a]	***
1148	(*Z*)-2-Nonenal	Orris, green, cucumber with a citrus nuance	0.08–1	0.24 ± 0.01[c]	0.34 ± 0.01[b]	0.41 ± 0.01[a]	***
1205	Decanal	Sweet, waxy and citrus-like	0.1–2	0.31 ± 0.01[c]	0.38 ± 0.01[b]	0.42 ± 0.01[a]	***
	ΣEsters			0.64 ± 0.02[c]	1.21 ± 0.04[b]	1.34 ± 0.04[a]	***
1091	Methyl benzoate	Fruity		0.64 ± 0.02[c]	1.21 ± 0.04[b]	1.34 ± 0.04[a]	***
	ΣAromatic hydrocarbons			7.19 ± 0.21[a]	6.93 ± 0.21[a]	6.38 ± 0.19[b]	**
852	Ethylbenzene			0.30 ± 0.01[c]	0.34 ± 0.01[b]	0.39 ± 0.01a	***
860	1,3-Dimethylbenzene			1.93 ± 0.06[b]	1.58 ± 0.05[c]	2.33 ± 0.07[a]	***
888	Styrene	Sweet, balsamic, floral, almond	730	1.56 ± 0.05[b]	1.99 ± 0.06[a]	1.44 ± 0.04[b]	***
964	1,3,5-Trimethylbenzene			0.42 ± 0.01[a]	0.42 ± 0.01[a]	0.34 ± 0.01[b]	***
1022	*p*-Cymene	Fresh, woody and terpy-like	12	2.98 ± 0.09[a]	2.60 ± 0.08[b]	1.88 ± 0.06[c]	***
	ΣFurans			2.57 ± 0.07[c]	5.17 ± 0.17[b]	5.99 ± 0.18[a]	***
833	Furfural	Sweet, woody, almond fragrant, baked bread	3000–23,000	1.80 ± 0.05[c]	3.50 ± 0.11[b]	4.56 ± 0.14[a]	***
837	3-Hydroxymethylfuran	Strong, nutty, meaty roasty		0.48 ± 0.01[c]	1.17 ± 0.04[a]	0.96 ± 0.03[b]	***
909	2-Acetylfuran	Sweet, almondy, nutty		0.29 ± 0.01[b]	0.50 ± 0.02[a]	0.47 ± 0.01[a]	***
	ΣPyrazines			0.46 ± 0.01[c]	0.71 ± 0.02[b]	1.00 ± 0.03[a]	***
821	2-Methyl-pyrazine	Nutty, brown, musty, earthy with a slight roasted nuance	60–10,500	0.46 ± 0.01[c]	0.71 ± 0.02[b]	1.00 ± 0.03[a]	***
	ΣTerpenoids			109.98 ± 3.31[b]	153.14 ± 4.61[a]	161.28 ± 4.84[a]	***
928	*α*-Pinene	Pine, terpenic, camphoreous, with fresh herbal	6	0.22 ± 0.01[a]	0.24 ± 0.01[a]	0.23 ± 0.01[a]	n.s.
981	*β*-Pinene	Dry, woody, resinous, fresh pine	140	1.27 ± 0.04[c]	1.91 ± 0.06[a]	1.66 ± 0.05[b]	***
993	*β*-Myrcene	Terpy, herbaceous, woody with a celery nuance	13–15	5.35 ± 0.16[c]	7.30 ± 0.22[b]	8.21 ± 0.25[a]	***
1015	*α*-Terpinene	Citrusy, woody, terpy with thymol notes	120–180	0.40 ± 0.01[b]	0.57 ± 0.02[a]	0.55 ± 0.02[a]	***
1026	Limonene	Pleasant, lemon	10	99.85 ± 3.00[b]	139.20 ± 4.18[a]	146.64 ± 4.40[a]	***
1038	*β*-(*Z*)-Ocimene	floral	34	0.24 ± 0.01[a]	0.22 ± 0.01[a]	0.16 ± 0.00[b]	***
1040	*β*-Ocimene	Tropical, green, terpy and woody	34	0.58 ± 0.02[c]	0.81 ± 0.02[b]	0.98 ± 0.03[a]	***
1057	*γ*-Terpinene	Woody, citrus, pine	130–200	1.17 ± 0.04[c]	1.65 ± 0.05[a]	1.47 ± 0.04[b]	***
1066	*α*-Terpinolene	Sweet, fresh, piney citrus	200	0.44 ± 0.01[c]	0.54 ± 0.02[b]	0.69 ± 0.02[a]	***
1129	Neo-allo-ocimene	Tropical, fruity		0.32 ± 0.01[c]	0.42 ± 0.01[b]	0.53 ± 0.02[a]	***
1134	(*E*)-Limonene oxide	Grassy, lemon	12	0.14 ± 0.00[c]	0.28 ± 0.01[a]	0.16 ± 0.00[b]	***

Results are expressed in parts per million (ppm).
Data within a column followed by the same letter are not significantly different according to Tukey's test. p value: **$p < 0.01$; ***$p < 0.001$; n.s., not significant ($p > 0.05$).
Abbreviations: CTR, control semolina; 5-PAS, 5 % powdered almond skin addition; 10-PAS, 10 % powdered almond skin addition.
[a] Components listed in order of elution on an DB-5MS column.
[b] Compounds, divided into different chemical classes (alkanes, alcohols, aldehydes, esters, aromatic hydrocarbons, furans, pyrazines and terpenoids) are classified in order of LRI (Linear Retention Index) of apolar column (DB-5MS).
[c] Aroma descriptions are reported in the online database of Good Scents Company Information (http://www.thegoodscentscompany.com/) and Flavornet (http://www.flavornet.org/).
[d] Odour thresholds are reported in the online database of Leffingwell & Associates (http://www.leffingwell.com/odorthre.htm).

added PAS to produce functional biscuits. A constant increase of bread firmness was registered in presence of PAS and this observation is explained by the PAS fibres; in fact, Yao et al. (2021) reported that the dietary fibres in apricot kernel skin added to bread doughs lead to the increase of hardness. As expected, considering the reduction of specific volume, image analysis indicated a reduction of the alveolation of bread crumb with PAS addition.

Culture independent analysis of the microbial community of doughs indicated the presence of *Bacillus* in PAS added dough before baking. Thus, all final breads were also subjected to the viable counting of aerobic bacterial spores. The levels of these dormant non-reproductive bacterial structures was below the detection limit in CTR breads, while densities a little above this limit (2.0 Log CFU/g) were registered for both PAS added breads, confirming data of Illumina analysis of the corresponding doughs and indicating that baking is not able to inactivate bacterial spores transported by almond skin. One of the main concerns of the occurrence of spore-forming bacteria in bread is the potential presence of pathogenic species, especially *B. cereus* (Garofalo et al., 2019), but also *Bacillus subtilis* (Santamarta et al., 2021) and *Bacillus licheniformis* (Kirschner and von Holy, 1989). In addition to safety issues, bacilli are also spoilage agents of wheat bread due to rope formation (Thompson et al., 1993). Diseases caused by ropy breads is unlikely to happen because of the slimy appearance of the crumb, but consumption of bread containing high counts of *B. subtilis* and *B. licheniformis* showing no rope symptoms may cause diarrhoea and vomiting (Rosenkvist and Hansen, 1995).

3.4. Volatile profiles

The aroma of breads with the addition of almonds was characterized by the presence of 35 different compounds (Table 4) belonging to eight classes of compounds: alkanes, alcohols, aldehydes, esters, aromatic hydrocarbons, furans, pyrazines, and terpenoids.

No acids were identified and the percentage of alcohols (4.44–5.96 ppm), and esters (0.64–1.44 ppm) was very low, probably decreased during the cooking phase, and without statistically valid difference between the three samples. The most abundant class in the three samples analysed were terpenoids (109.98 ppm in CTR, 153.14 ppm in 5-PAS, and 161.28 ppm 10-PAS bread). Limonene is the major terpene detected in all doughs. The presence of limonene in CTR is not surprising, since generally found in sourdough breads (Corona et al., 2016; Pétel et al., 2017). The other two abundant classes were aldehydes (7.33–8.77 ppm), and aromatic hydrocarbons (6.38–7.19 ppm). Alkanes are, in order of decreasing quantity, the second class of metabolites present in the bread with 10 % almonds added (12.52 ppm). In control and in the bread added with 5 % almonds the alkanes' content is particularly lower (6.62 and 4.83 ppm, respectively).

3.5. Functional properties of PAS enriched breads

It is ascertained that almond skins are rich of bioactive phytochemicals such as flavonoids, phenolic acids and proanthocyanidins, accounting for about 70–80 % of the polyphenols in the whole almond fruit (Bolling, 2017; Mandalari et al., 2010; Shahidi et al., 2019). The total phenols content in the PAS used in our study was extracted with acidified ethanol solution and measured by Folin–Ciocalteu method with a result of 8.72 ± 0.22 mg GAE/g (n = 4). Although the phenol amount depends on the variety of the almond and on the extraction procedure, our data are in accordance with those reported in literature for roasted PAS (Garcia-Perez et al., 2021). Antioxidant and radical scavenging activity of the polyphenols is considered to underlie the health-promoting effects of these almond by-products (Chen et al., 2005; Garrido et al., 2008). We assessed the radical-scavenging ability of the PAS added breads, in comparison with unenriched bread, to evaluate the functional property of the fortified bakery product. Because polyphenols are released from the food matrix and can undergo chemical-structural changes during the digestion, we measured the phenols amount and anti-radical activity of bread samples before and after a simulated gastrointestinal digestion. Phenols released before the digestion from 5 % and 10 % PAS-enriched bread accounted for 0.73 ± 0.04 mgGAE/g and 0.88 ± 0.05 mg GAE/g (n = 5) respectively (Fig. 2). When corrected for the phenolic content of semolina (0.51 ± 0.02 mg GAE/g; unreached bread, Fig. 2) it appeared that only 50 % of the phytochemicals incorporated as PAS were released from the food matrix, probably due to interaction with other components (D'Archivio et al., 2010). Instead, after in vitro simulated digestion, post-intestinal bioaccessible fractions of 5 % and 10 % PAS-enriched bread showed an amount of phenols twice higher than that found in relevant samples before digestion (Fig. 2), with a calculated final release of the phytochemicals of almost 100 %. A lower percentage of release of phenol compounds was reported after digestion of crisp bread fortified with both natural and blanched PAS (Mandalari et al., 2016).

Using ABTS$^{\bullet}$ + decolorization assay we found that replacement of semolina with PAS powder at 5 % or 10 % (w/w) caused an increase of 1.5 and 1.8-fold of the antioxidant capacity of the bread, respectively, before digestion (Fig. 3A). Significantly higher antiradical activity was measured in the bioaccessible fractions of both the fortified breads. In terms of Trolox equivalents, antioxidant capacity of 5 % and 10 % PAS-enriched bread was 11.36 ± 0.61 μmol/g and 18.46 ± 0.7 μmol/g, respectively, exceeding by 3.5 and 5.2-fold that of unenriched bread (Fig. 3A). Very similar results were obtained using DPPH radical decolorization assay (Fig. 3B). This data demonstrated that fortification of bread with PAS improves the antioxidant potential of the product and that the digestion process leads to total release of the active compounds in the digested fraction, to be potentially available for uptake by absorptive epithelial cells.

The antioxidant effect of the bioaccessible digested fractions of the PAS-fortified breads was investigated in a cell model of oxidative stress consisting of human differentiated enterocyte-like Caco-2 cells stimulated by IL-1β (Attanzio et al., 2019a). When 15 days-post confluent Caco-2 cell monolayers were treated for 4 h with IL-1β, cytofluorimetric analysis with DCFDA showed a net increase of intracellular ROS levels compared to untreated cells (control, Fig. 4). Co-incubation of the cells with IL-1β and aliquots of the bioaccessible fraction from 5 % AS-enriched bread (equivalent to 12.5 mg of the initial sample) reduced ROS generation by about 50 % while corresponding aliquots from 10 % AS-enriched bread were able to completely prevent IL-1β -induced oxidative stress in the cells (Fig. 4). Overall the data indicated that phytochemicals released from digested PAS-fortified bread can provide antioxidant protection in a complex biological environment such as human intestinal-like cells and potentially can contribute in the healthiness of the digestive tract. In this context it is important to

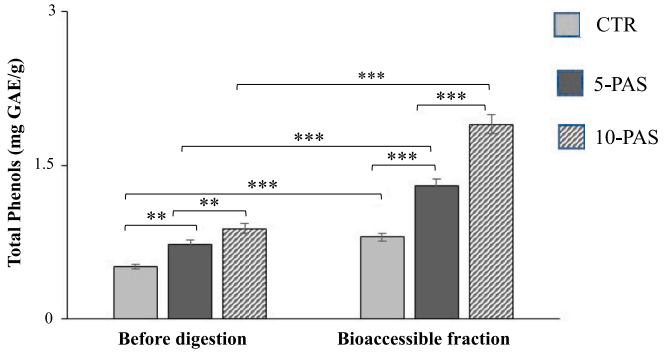

Fig. 2. Total phenol released from breads before and after in vitro digestion. Values are the mean ± SD of three triplicate experiments. Abbreviations: CTR, control semolina; 5-PAS, 5 % powdered almond skin addition; 10-PAS, 10 % powdered almond skin addition. $^{**}p < 0.01$; $^{***}p < 0.001$.

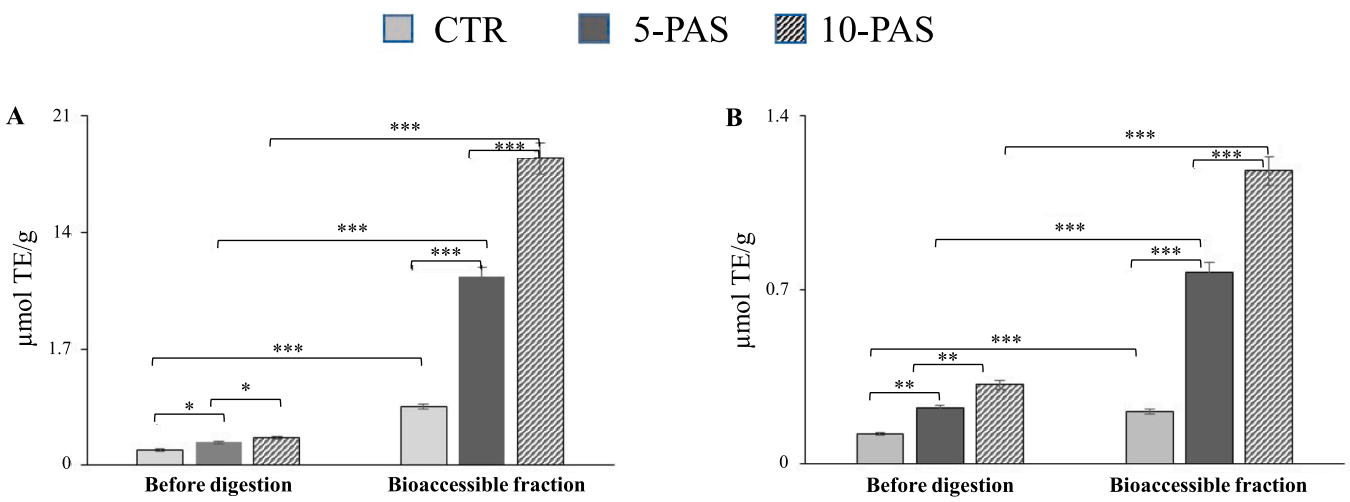

Fig. 3. Reducing activity towards ABTS+• (A) and DPPH (B) radical of breads. Abbreviations: CTR, control semolina; 5-PAS, 5 % powdered almond skin addition; 10-PAS, 10 % powdered almond skin addition. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

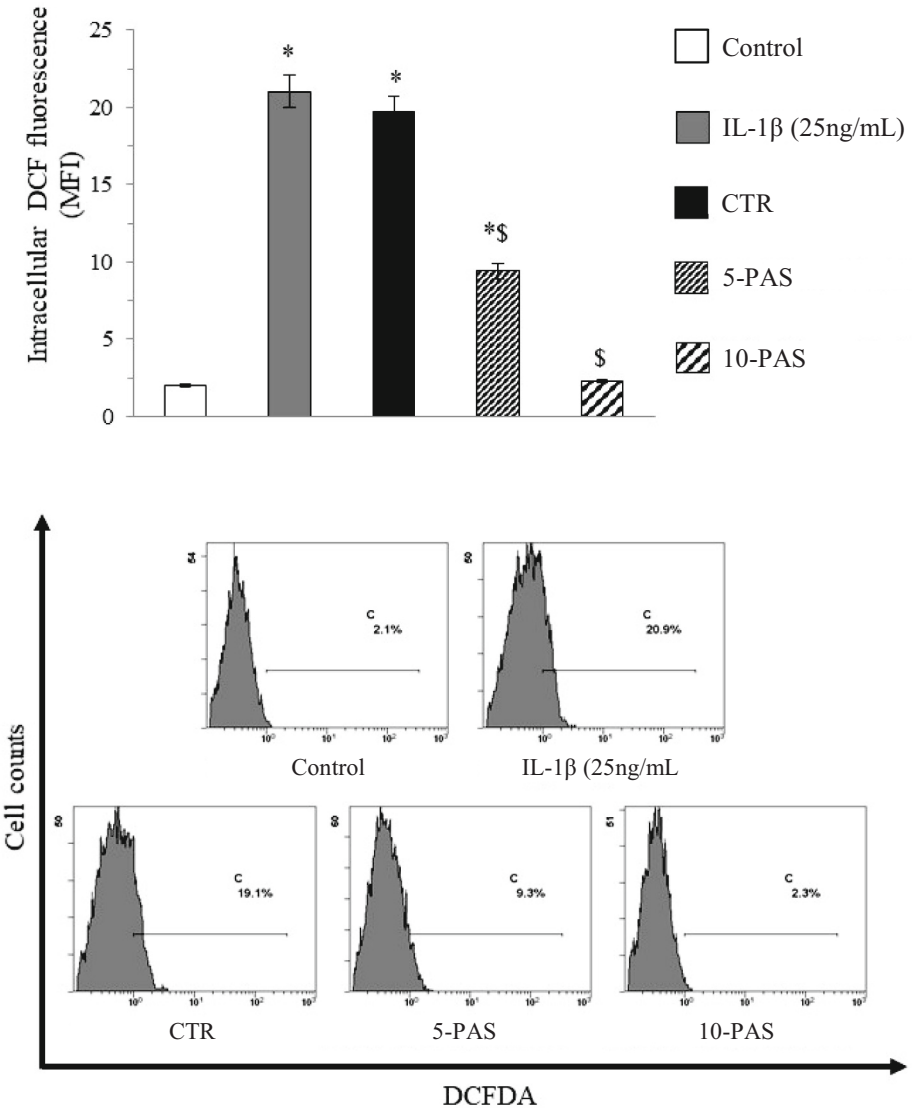

Fig. 4. Effect of the bioaccessible fraction from digestion of breads on ROS production in differentiated intestinal Caco-2 cells treated for 4 h with IL-1β. Upper panel: values are the mean ± S D of three separate experiments carried out in duplicate. * $p < 0.000$ vs control; $ $p < 0.0001$ vs IL-1 β-treated cells. Lower panel: representative cytofluorimetric image.

underline that our in vitro digestion procedure, in which 5 g of bread are processed in a total volume of 40 mL, is consistent with physiological conditions of weight/volume ratio, since a serving of bread (80 g) is considered dispersed in a total digestive volume of 640 mL (Mahé et al., 1992).

3.6. Sensory attributes of breads

Sensory analysis was limited to visual, texture and odour evaluation due to the presence of spore forming bacteria in the final breads. Generally, colour of crumb and crust and odour intensity increased with the percentage of PAS while bread odour, elasticity, porosity, alveolation and crust thickness decreased (Fig. 5). A reduction of crumb porosity has been also registered in presence of pumpkin pomace and dry tomato waste to white bread (Kampuse et al., 2015; Nour et al., 2015) and this is a common phenomenon due to the reduced percentage of flour or semolina because the percentage of gluten responsible for porosity is lower (Rathnayake et al., 2018).

4. Conclusions

The addition of PAS did not affect the development of sourdough LAB starters. Basically, the microbiological parameters during fermentation were influenced by PAS in terms of hygiene aspects for the development of coliforms in doughs and for the presence of spore forming bacteria after baking. The final bread characteristics were influenced by PAS and its addition percentage. The phytochemicals released from digested PAS added bread can provide antioxidant protection in a complex biological environment such as human intestinal-like cells. This work successfully explored the valorisation of almond by-products for the development of new added-value breads, but the results highlighted the need to keep the production and storage of almond skin under control in view of its reuse in food production. Effective hygiene measures can be realized through the modification of the line for almond skin exit, in order to let almond peel to remain inside the plant as being discharged into sanitized stainless steel containers previously treated with oxidizing solutions.

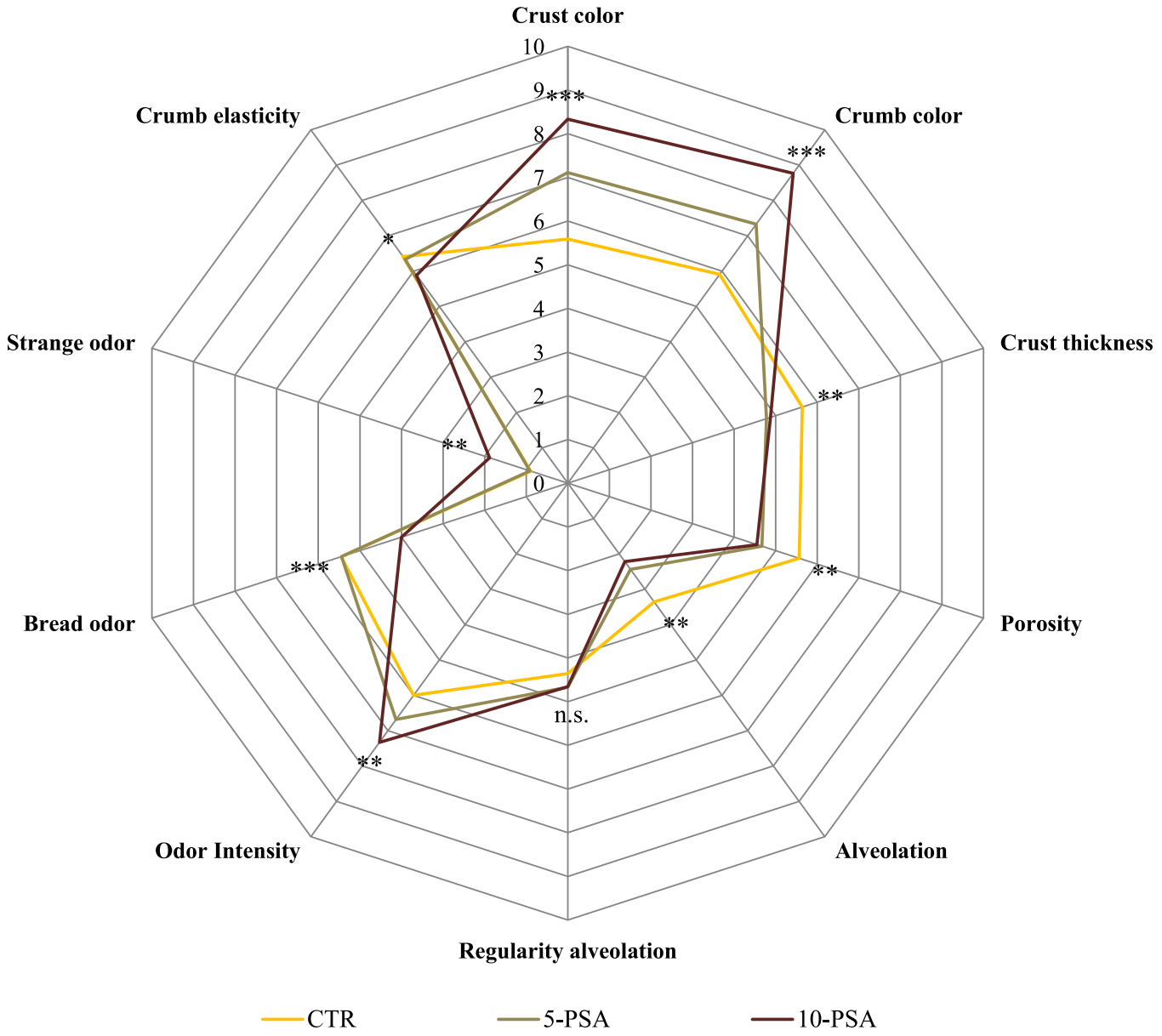

Fig. 5. Spider diagrams of descriptive sensory analysis of breads. Abbreviations: CTR, control semolina; 5-PAS, 5 % powdered almond skin addition; 10-PAS, 10 % powdered almond skin addition. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; n.s., not significant ($p > 0.05$).

Fundings

This research has been financially supported by the Ministero dello Sviluppo Economico (Italy)—Project title: "Innovazioni tecnologiche bio-based e potenziamento dell'economia circolare nella gestione degli scarti da lavorazione primaria di mandorle biologiche con elevate potenzialità agroindustriali" # F/200037/01-03/X45.

Declaration of competing interest

The authors declare that there is no conflict of interest for this research.

Data availability

Data will be made available on request.

Acknowledgements

The authors wish to thank the personnel of almond factory "Bongiovanni Almonds s.r.l." and industrial bakery "Ori di Sicilia".

References

Alfonzo, A., Ventimiglia, G., Corona, O., Di Gerlando, R., Gaglio, R., Francesca, N., Moschetti, G., Settanni, L., 2013. Diversity and technological potential of lactic acid bacteria of wheat flours. Food Microbiol. 36, 343–354. https://doi.org/10.1016/j.fm.2013.07.003.

Alfonzo, A., Urso, V., Corona, O., Francesca, N., Amato, G., Settanni, L., Di Miceli, G., 2016. Development of a method for the direct fermentation of semolina by selected sourdough lactic acid bacteria. Int. J. Food Microbiol. 239, 65–78. https://doi.org/10.1016/j.ijfoodmicro.2016.06.027.

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy, fourth ed. Allured Publishing Corporation, Carol Stream.

Alfonzo, A., Miceli, C., Nasca, A., Franciosi, E., Ventimiglia, G., Di Gerlando, R., Tuohy, K., Francesca, N., Moschetti, G., Settanni, L., 2017. Monitoring of wheat lactic acid bacteria from the field until the first step of dough fermentation. Food Microbiol. 62, 256–269. https://doi.org/10.1016/j.fm.2016.10.014.

American Association of Cereal Chemists, 2000. Approved Methods of the AACC, 10th ed. In: Method 10-10B. The Association, St. Paul, MN.

Attanzio, A., D'Anneo, A., Pappalardo, F., Bonina, F.P., Livrea, M.A., Allegra, M., Tesoriere, L., 2019. Phenolic Composition of Hydrophilic Extract of Manna from Sicilian Fraxinus angustifolia Vahl and its Reducing, Antioxidant and Anti-Inflammatory Activity in Vitro. Antioxidants 1;8 (10), 494. https://doi.org/10.3390/antiox8100494.

Attanzio, A., Diana, P., Barraja, P., Carbone, A., Spanò, V., Parrino, B., Cascioferro, S.M., Allegra, M., Cirrincione, G., Tesoriere, L., Montalbano, A., 2019b. Quality, functional and sensory evaluation of pasta fortified with extracts from Opuntia ficus-indica cladodes. J. Sci. Food Agric. 99, 4242–4247. https://doi.org/10.1002/jsfa.9655.

Attanzio, A., Tesoriere, L., Allegra, M., Livrea, M.A., 2016. Monofloral honeys by Sicilian black honeybee (Apis mellifera ssp. sicula) have high reducing power and antioxidant capacity. Heliyon 2, e00193. https://doi.org/10.1016/j.heliyon.2016.e00193.

Barral-Martinez, M., Fraga-Corral, M., Garcia-Perez, P., Simal-Gandara, J., Prieto, M.A., 2021. Almond by-products: valorization for sustainability and competitiveness of the industry. Foods 10 (8), 1793. https://doi.org/10.3390/foods10081793.

Barreca, D., Nabavi, S.M., Sureda, A., Rasekhian, M., Raciti, R., Silva, A.S., Annunziata, G., Arnone, A., Tenore, G.C., Süntar, İ., Mandalari, G., 2020. Almonds (Prunus dulcis Mill. D.A. Webb): a source of nutrients and health-promoting compounds. Nutrients 12 (3), 672. https://doi.org/10.3390/nu12030672.

Barreira, J.C., Nunes, M.A., Da Silva, B.V., Pimentel, F.B., Costa, A.S., Alvarez-Ortí, M., Pardo, J.E., Oliveira, M.B.P.P., 2019. Almond cold-pressed oil by-product as ingredient for cookies with potential health benefits: chemical and sensory evaluation. Food Sci. Hum. Wellness 8 (3), 292–298. https://doi.org/10.1016/j.fshw.2019.07.002.

Bartkiene, E., Bartkevics, V., Pugajeva, I., Borisova, A., Zokaityte, E., Lele, V., Starkute, V., Zavistanaviciute, P., Klupsaite, D., Zadeike, D., Juodeikiene, G., 2021. The quality of wheat bread with ultrasonicated and fermented by-products from plant drinks production. Front. Microbiol. 12 https://doi.org/10.3389/fmicb.2021.652548.

Bigliardi, B., Galati, F., 2013. Innovation trends in the food industry: the case of functional foods. Trends Food Sci. Technol. 31 (2), 118–129. https://doi.org/10.1016/j.tifs.2013.03.006.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28, 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5.

Bolling, B.W., 2017. Almond polyphenols: methods of analysis, contribution to food quality, and health promotion. Compr. Rev. Food Sci. Food Saf. 16, 346–368. https://doi.org/10.1111/1541-4337.12260.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Caltagirone, C., Peano, C., Sottile, F., 2021. Post-harvest industrial processes of almond (Prunus dulcis L. Mill) in Sicily influence the nutraceutical properties of by-products at harvest and during storage. Front. Nutr. 8, 243. https://doi.org/10.3389/fnut.2021.659378.

Carbonetto, B., Nidelet, T., Guezenec, S., Perez, M., Segond, D., Sicard, D., 2020. Interactions between Kazachstania humilis yeast species and lactic acid bacteria in sourdough. Microorganisms 8 (2), 240. https://doi.org/10.3390/microorganisms8020240.

Carvalho, M., Ferreira, P.J., Mendes, V.S., Silva, R., Pereira, J.A., Jerónimo, C., Silva, M. B., 2010. Human cancer cell antiproliferative and antioxidant activities of Juglans regiaL. Food Chem. Toxicol. 48, 441–447. https://doi.org/10.1016/j.fct.2009.10.043.

Chang, S.K., Alasalvar, C., Bolling, B.W., Shahidi, F., 2016. Nuts and their co-products: the impact of processing (roasting) on phenolics, bioavailability, and health benefits—a comprehensive review. J. Funct. Foods 26, 88–122. https://doi.org/10.1016/j.jff.2016.06.029.

Chen, C.Y., Milbury, P.E., Lapsley, K., Blumberg, J.B., 2005. Flavonoids from almond skins are bioavailable and act synergistically with vitamins C and E to enhance hamster and human LDL resistance to oxidation. J. Nutr. 135, 1366–1373. https://doi.org/10.1093/jn/135.6.1366.

Claesson, M.J., Wang, Q., O'sullivan, O., Greene-Diniz, R., Cole, J.R., Ross, R.P., O'Toole, P.W., 2010. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. Nucleic Acids Res. 38, e200.

Comendador, F.J., Cavella, S., Di Monaco, R., Dinnella, C., Moneta, E., Monteleone, E., Peparaio, M., Recchia, A., Sinesio, F., 2012. Il pane e altri prodotti da forno. In: Atlante Sensoriale dei Prodotti Alimentari. Tecniche Nuove, Milan, Italy, pp. 156–176.

Corona, O., Alfonzo, A., Ventimiglia, G., Nasca, A., Francesca, N., Martorana, A., Moschetti, G., Settanni, L., 2016. Industrial application of selected lactic acid bacteria isolated from local semolinas for typical sourdough bread production. Food Microbiol. 59, 43–56. https://doi.org/10.1016/j.fm.2016.05.006.

Corsetti, A., Gobbetti, M., De Marco, B., Balestrieri, F., Paoletti, F., Russi, L., Rossi, J., 2000. Combined effect of sourdough lactic acid bacteria and additives on bread firmness and staling. J. Agric. Food Chem. 48, 3044–3051. https://doi.org/10.1021/jf990853e.

Corsetti, A., Settanni, L., Braga, T.M., de Fatima Silva Lopes, M., Suzzi, G., 2008. An investigation on the bacteriocinogenic potential of lactic acid bacteria associated with wheat (Triticum durum) kernels and non-conventional flours. LWT - Food Sci. Technol. 41, 1173–1182. https://doi.org/10.1016/j.lwt.2007.07.022.

D'Archivio, M., Filesi, C., Varì, R., Scazzocchio, B., Masella, R., 2010. Bioavailability of the polyphenols: status and controversies. J. Mol. Sci. 11, 1321–1342. https://doi.org/10.3390/ijms11041321.

De Angelis, M., Minervini, F., Siragusa, S., Rizzello, C.G., Gobbetti, M., 2019. Wholemeal wheat flours drive the microbiome and functional features of wheat sourdoughs. Int. J. Food Microbiol. 302, 35–46. https://doi.org/10.1016/j.ijfoodmicro.2018.08.009.

De Benoist, B., Dary, O., Hurrell, R., 2006. In: Allen, L. (Ed.), Guidelines on Food Fortification With Micronutrients, Vol. 126. World Health Organization, Geneva.

Dinardo, F.R., Minervini, F., De Angelis, M., Gobbetti, M., Gänzle, M.G., 2019. Dynamics of Enterobacteriaceae and Lactobacilli in model sourdoughs are driven by pH and concentrations of sucrose and ferulic acid. LWT - Food Sci. Technol. 114, 108394 https://doi.org/10.1016/j.lwt.2019.108394.

Domínguez Díaz, L., Fernández-Ruiz, V., Cámara, M., 2020. The frontier between nutrition and pharma: the international regulatory framework of functional foods, food supplements and nutraceuticals. Crit. Rev. Food Sci. Nutr. 60 (10), 1738–1746. https://doi.org/10.1080/10408398.2019.1592107.

Ercolini, D., Pontonio, E., De Filippis, F., Minervini, F., La Storia, A., Gobbetti, M., Di Cagno, R., 2013. Microbial ecology dynamics during rye and wheat sourdough preparation. Appl. Environ. Microbiol. 79, 7827–7836. https://doi.org/10.1128/AEM.02955-13.

Esfahlan, A.J., Jamei, R., Esfahlan, R.J., 2010. The importance of almond (Prunus amygdalus L.) and its by-products. Food Chem. 120 (2), 349–360. https://doi.org/10.1016/j.foodchem.2009.09.063.

Gaglio, R., Alfonzo, A., Barbera, M., Franciosi, E., Francesca, N., Moschetti, G., Settanni, L., 2020a. Persistence of a mixed lactic acid bacterial starter culture during lysine fortification of sourdough breads by addition of pistachio powder. Food Microbiol. 86, 103349 https://doi.org/10.1016/j.fm.2019.103349.

Gaglio, R., Cirlincione, F., Di Miceli, G., Franciosi, E., Di Gerlando, R., Francesca, N., Settanni, L., Moschetti, G., 2020b. Microbial dynamics in durum wheat kernels during aging. Int. J. Food Microbiol. 324, 108631 https://doi.org/10.1016/j.ijfoodmicro.2020.108631.

Gaglio, R., Barbera, M., Tesoriere, L., Osimani, A., Busetta, G., Matraxia, M., Attanzio, A., Restivo, I., Aquilanti, L., Settanni, L., 2021. Sourdough "ciabatta" bread enriched with powdered insects: physicochemical, microbiological, and simulated intestinal digesta functional properties. Innov. Food Sci. Emerg. Technol. 72, 102755 https://doi.org/10.1016/j.ifset.2021.102755.

Garcia-Perez, P., Xiao, J., Munekata, P.E., Lorenzo, J.M., Barba, F.J., Rajoka, M.S.R., Barros, L., Mascoloti Sprea, R., Amaral, J.S., Prieto, M.A., Simal-Gandara, J., 2021.

Revalorization of almond by-products for the design of novel functional foods: an updated review. Foods 10 (8), 1823. https://doi.org/10.3390/foods10081823.
Garofalo, C., Milanović, V., Cardinali, F., Aquilanti, L., Clementi, F., Osimani, A., 2019. Current knowledge on the microbiota of edible insects intended for human consumption: a state-of-the-art review. Food Res. Int. 125, 108527 https://doi.org/10.1016/j.foodres.2019.108527.
Garrido, I., Monagas, M., Gómez-Cordovés, C., Bartolomé, B., 2008. Polyphenols and antioxidant properties of almond skins: influence of industrial processing. J. Food Sci. 73, C106–C115. https://doi.org/10.1111/j.1750-3841.2007.00637.x.
Gobbetti, M., De Angelis, M., Di Cagno, R., Calasso, M., Archetti, G., Rizzello, C.G., 2019. Novel insights on the functional/nutritional features of the sourdough fermentation. Int. J. Food Microbiol. 302, 103–113. https://doi.org/10.1016/j.ijfoodmicro.2018.05.018.
Graça, C., Lima, A., Raymundo, A., Sousa, I., 2021. Sourdough fermentation as a tool to improve the nutritional and health-promoting properties of its derived-products. Fermentation 7 (4), 246. https://doi.org/10.3390/fermentation7040246.
Granato, D., Nunes, D.S., Barba, F.J., 2017. An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of functional foods: a proposal. Trends Food Sci. Technol. 62, 13–22. https://doi.org/10.1016/j.tifs.2016.12.010.
ISO 13299, 2003. Sensory Analysis – Methodology – General Guidance for Establishing a Sensory Profile. International Organization for Standardization, Geneva, Switzerland.
Kampuse, S., Ozola, L., Straumite, E., Galoburda, R., 2015. Quality parameters of wheat bread enriched with pumpkin (Cucurbita moschata) by-products. Acta Univ. Cinbinesis Ser. E Food Technol. 19 (2) https://doi.org/10.1515/aucft-2015-0010.
Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.
Kaur, M., Kumar, M., Sachdeva, S., Puri, S.K., 2020. An efficient multiphase bioprocess for enhancing the renewable energy production from almond shells. Energy Convers. Manag. 203, 112235 https://doi.org/10.1016/j.enconman.2019.112235.
Kaur, S., Panesar, P.S., Chopra, H.K., 2021. Citrus processing by-products: an overlooked repository of bioactive compounds. Crit. Rev. Food Sci. Nutr. 1–20. https://doi.org/10.1080/10408398.2021.1943647.
Kirschner, L.M., von Holy, A., 1989. Rope spoilage of bread. S. Afr. J. Sci. 85, 425–427.
Kline, L., Sugihara, T.F., 1971. Microorganisms of the San Francisco sour dough process. II. Isolation and characterization of undescribed bacterial species responsible for souring activity. Appl. Microbiol. 21, 459–465. https://doi.org/10.1128/am.21.3.459-465.1971.
Lai, W.T., Khong, N.M., Lim, S.S., Hee, Y.Y., Sim, B.I., Lau, K.Y., Lai, O.M., 2017. A review: modified agricultural by-products for the development and fortification of food products and nutraceuticals. Trends Food Sci. Technol. 59, 148–160. https://doi.org/10.1016/j.tifs.2016.11.014.
Logares, R., Audic, S., Bass, D., Bittner, L., Boutte, C., Christen, R., Claverie, J.M., Decelle, J., Dolan, J.R., Dunthorn, M., Edvardsen, B., Gobet, A., Kooistra, W.H.C.F., Mahé, F., Not, F., Ogata, H., Pawlowski, J., Pernice, M.C., Romac, S., Shalchian-Tabrizi, K., Simon, N., Stoeck, T., Santini, S., Siano, R., Wincker, P., Zingone, A., Richards, T.A., de Vargas, C., Massana, R., 2014. Patterns of rare and abundant marine microbial eukaryotes. Curr. Biol. 24, 813–821. https://doi.org/10.1016/j.cub.2014.02.050.
Loizzo, M.R., Tundis, R., Leporini, M., D'Urso, G., Gagliano Candela, R., Falco, T., Piacente, S., Bruno, M., Sottile, F., 2021. Almond (Prunus dulcis cv. Casteltermini) skin confectionery by-products: new opportunity for the development of a functional blackberry (Rubus ulmifolius Schott) Jam. Antioxidants 10 (8), 1218. https://doi.org/10.3390/antiox10081218.
Mahé, S., Huneau, J.F., Marteau, P., Thuillier, F., Tomé, D., 1992. Gastroileal nitrogen and electrolyte movements after bovine milk ingestion in humans. Am. J. Clin. Nutr. 56, 410–416. https://doi.org/10.1093/ajcn/56.2.410.
Malavi, D.N., Abong, G.O., Muzhingi, T., 2021. Effect of food safety training on behavior change of food handlers: a case of orange-fleshed sweetpotato purée processing in Kenya. Food Control 119, 107500. https://doi.org/10.1016/j.foodcont.2020.107500.
Mandalari, G., Tomaino, A., Arcoraci, T., Martorana, M., Turco, V.L., Cacciola, F., Rich, G.T., Bisignano, C., Saija, A., Dugo, P., Cross, K.L., Parker, M.L., Waldron, K.W., Wickham, M.S.J., 2010. Characterization of polyphenols, lipids and dietary fibre from almond skins (Amygdalus communis L.). J. Food Compos. Anal. 23 (2), 166–174. https://doi.org/10.1016/j.jfca.2009.08.015.
Mandalari, G., Vardakou, M., Faulks, R., Bisignano, C., Martorana, M., Smeriglio, A., Trombetta, D., 2016. Matrix effects of polyphenol bioaccessibility from almond skin during simulated human digestion. Nutrients 8, 568. https://doi.org/10.3390/nu8090568.
Mannar, M.V., Sankar, R., 2004. Micronutrient fortification of foods—rationale, application and impact. Indian J. Pediatr. 71, 997–1002. https://doi.org/10.1007/BF02828115.
Martínez, M.L., Labuckas, D.O., Lamarque, A.L., Maestri, D.M., 2010. Walnut (Juglans regia L.): genetic resources, chemistry, by-products. J. Sci. Food Agric. 90 (12), 1959–1967. https://doi.org/10.1002/jsfa.4059.
Martins, Z.E., Erben, M., Gallardo, A.E., Silva, R., Barbosa, I., Pinho, O., Ferreira, I.M.P.L. V.O., 2015. Effect of spent yeast fortification on physical parameters, volatiles and sensorial characteristics of home-made bread. Int. J. Food Sci. Technol. 50, 1855–1863. https://doi.org/10.1111/ijfs.12818.
Messina, M.C., Gaglio, R., Morghese, M., Tolone, M., Arena, R., Moschetti, G., Santulli, A., Francesca, N., Settanni, L., 2019. Microbiological profile and bioactive properties of insect powders used in food and feed formulations. Foods 8, 400. https://doi.org/10.3390/foods8090400.
Moure, A., Pazos, M., Medina, I., Domínguez, H., Parajó, J.C., 2007. Antioxidant activity of extracts produced by solvent extraction of almond shells acid hydrolysates. Food Chem. 101 (1), 193–201. https://doi.org/10.1016/j.foodchem.2006.01.017.
Nour, V., Ionica, M.E., Trandafir, I., 2015. Bread enriched in lycopene and other bioactive compounds by addition of dry tomato waste. J. Food Sci. Technol. 52, 8260–8267. https://doi.org/10.1007/s13197-015-1934-9.
Pagliano, G., Ventorino, V., Panico, A., Pepe, O., 2017. Integrated systems for biopolymers and bioenergy production from organic waste and by-products: a review of microbial processes. Biotechnol. Biofuels 10 (1), 1–24. https://doi.org/10.1186/s13068-017-0802-4.
Pasqualone, A., Laddomada, B., Spina, A., Todaro, A., Guzmàn, C., Summo, C., Mita, G., Giannone, V., 2018. Almond by-products: extraction and characterization of phenolic compounds and evaluation of their potential use in composite dough with wheat flour. LWT - Food Sci. Technol. 89, 299–306. https://doi.org/10.1016/j.lwt.2017.10.066.
Pasqualone, A., Laddomada, B., Boukid, F., Angelis, D.D., Summo, C., 2020. Use of almond skins to improve nutritional and functional properties of biscuits: an example of upcycling. Foods 9 (11), 1705. https://doi.org/10.3390/foods9111705.
Pétel, C., Onno, B., Prost, C., 2017. Sourdough volatile compounds and their contribution to bread: a review. Trends Food Sci. Technol. 59, 105–123. https://doi.org/10.1016/j.tifs.2016.10.015.
Pino, A., Russo, N., Solieri, L., Sola, L., Caggia, C., Randazzo, C.L., 2022. Microbial consortia involved in traditional sicilian sourdough: characterization of lactic acid bacteria and yeast populations. Microorganisms 10 (2), 283. https://doi.org/10.3390/microorganisms10020283.
Prgomet, I., Gonçalves, B., Domínguez-Perles, R., Pascual-Seva, N., Barros, A.I., 2017. Valorization challenges to almond residues: phytochemical composition and functional application. Molecules 22 (10), 1774. https://doi.org/10.3390/molecules22101774.
Purlis, E., Salvadori, V.O., 2007. Bread browning kinetics during baking. J. Food Eng. 80 (4), 1107–1115. https://doi.org/10.1016/j.jfoodeng.2006.09.007.
Rathnayake, H.A., Navaratne, S.B., Navaratne, C.M., 2018. Porous crumb structure of leavened baked products. Int. J. Food Sci. 8187318 https://doi.org/10.1155/2018/8187318.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26 (9–10), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.
Rodrigues, Á.M.D.P., Correia, P.M.R., Guiné, R.P.F., 2014. Physical, chemical and sensorial properties of healthy and mixture breads in Portugal. J. Food Meas. Charact. 8, 70–80. https://doi.org/10.1007/s11694-013-9166-z.
Rosenkvist, H., Hansen, A., 1995. Contamination profiles and characterisation of Bacillus species in wheat bread and raw materials for bread production. Int. J. Food Microbiol. 26, 353–363. https://doi.org/10.1016/0168-1605(94)00147-X.
Ruisi, P., Ingraffia, R., Urso, V., Giambalvo, D., Alfonzo, A., Corona, O., Settanni, L., Frenda, A.S., 2021. Influence of grain quality, semolinas and baker's yeast on bread made from old landraces and modern genotypes of Sicilian durum wheat. Food Res. Int. 140, 110029 https://doi.org/10.1016/j.foodres.2020.110029.
Santamarta, S., Aldavero, A.C., Rojo, M.A., 2021. Essential oil of Cymbopogon martini, source of geraniol, as a potential antibacterial agent against Bacillus subtilis, a pathogen of the bakery industry. F1000Res. 10 (1027), 1027. https://doi.org/10.12688/f1000research.54196.1.
Schoenlechner, R., Szatmari, M., Bagdi, A., Tömösközi, S., 2013. Optimisation of bread quality produced from wheat and proso millet (Panicum miliaceum L.) by adding emulsifiers, transglutaminase and xylanase. LWT-FoodSci. Technol. 51 (1), 361–366. https://doi.org/10.1016/j.lwt.2012.10.020.
Settanni, L., Ventimiglia, G., Alfonzo, A., Corona, O., Miceli, A., Moschetti, G., 2013. An integrated technological approach to the selection of lactic acid bacteria of flour origin for sourdough production. Food Res. Int. 54, 1569–1578. https://doi.org/10.1016/j.foodres.2013.10.017.
Shahidi, F., Varatharajan, V., Oh, W.Y., Peng, H., 2019. Phenolic compounds in agri-food by-products, their bioavailability and health effects. J. Food Bioact. 5, 57–119. 10.31665/JFB.2019.5178.
Siepmann, F.B., Ripari, V., Waszczynskyj, N., Spier, M.R., 2018. Overview of sourdough technology: from production to marketing. Food Bioprocess Technol. 11 (2), 242–270. https://doi.org/10.1007/s11947-017-1968-2.
Siepmann, F.B., de Almeida, B.S., Ripari, V., da Silva, B.J., Peralta-Zamora, P.G., Waszczynskyj, N., Spier, M.R., 2019. Brazilian sourdough: microbiological, structural, and technological evolution. Eur.Food Res. Technol. 245 (8), 1583–1594. https://doi.org/10.1007/s00217-019-03254-8.
Sottile, F., Barone, E., Barbera, G., Palasciano, M., 2014. The Italian almond industry: new perspectives and ancient tradition. Acta Hortic. 1028, 401–407. 10.17660/ActaHortic.2014.1028.66.
Sottile, F., Massaglia, S., Peano, C., 2020. Ecological and economic indicators for the evaluation of almond (Prunus dulcis L.) orchard renewal in Sicily. Agriculture 10 (7), 301. https://doi.org/10.3390/agriculture10070301.
Stenfors Arnesen, L.P., Fagerlund, A., Granum, P.E., 2008. From soil to gut: Bacillus cereus and its food poisoning toxins. FEMS Microbiol. Rev. 32 (4), 579–606. https://doi.org/10.1111/j.1574-6976.2008.00112.x.
Suo, B., Chen, X., Wang, Y., 2021. Recent research advances of lactic acid bacteria in sourdough: origin, diversity, and function. Curr. Opin. Food Sci. 37, 66–75. https://doi.org/10.1016/j.cofs.2020.09.007.
Szabo, K., Cătoi, A.F., Vodnar, D.C., 2018. Bioactive compounds extracted from tomato processing by-products as a source of valuable nutrients. Plant Foods Hum. Nutr. 73 (4), 268–277. https://doi.org/10.1007/s11130-018-0691-0.

Thompson, J.M., Dodd, C.E., Waites, W.M., 1993. Spoilage of bread by Bacillus. Int. Biodeter. Biodegrad. 32, 55–66. https://doi.org/10.1016/0964-8305(93)90039-5.

Valmorri, S., Settanni, L., Suzzi, G., Gardini, F., Vernocchi, P., Corsetti, A., 2006. Application of a novel polyphasic approach to study the lactobacilli composition of sourdoughs from the Abruzzo region (central Italy). Lett. Appl. Microbiol. 43 (3), 343–349. https://doi.org/10.1111/j.1472-765X.2006.01942.x.

Wimalawansa, S.J., 2013. Rational food fortification programs to alleviate micronutrient deficiencies. J. Food Process. Technol. 4, 257–267. https://doi.org/10.4172/2157-7110.1000257.

Yao, J.L., Zhang, Q.A., Liu, M.J., 2021. Effects of apricot kernel skins addition and ultrasound treatment on the properties of the dough and bread. J. Food Process. Preserv. 45, e15611 https://doi.org/10.1111/jfpp.15611.

Yu, X.J., Walker, D.H., 2006. The order Rickettsiales. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., Stackebrandt, E. (Eds.), The Prokaryotes. Springer, New York, pp. 493–528. https://doi.org/10.1007/0-387-30745-1_20.

Zheng, J., Wittouck, S., Salvetti, E., Franz, C.M., Harris, H., Mattarelli, P., O'Toole, P.W., Bruno, P., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G.E., Gänzle, M. G., Lebeer, S., 2020. A taxonomic note on the genus Lactobacillus: description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. Int. J. Syst. Evol. Microbiol. 70, 2782–2858. https://doi.org/10.1099/ijsem.0.004107.