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Valorization of avocado (cv. Hass) waste powder in industrial-scale sourdough "ciabatta" bread production: Impact on microbial dynamics, quality attributes, and phenolic bioaccessibility

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ABSTRACT

This study investigates the incorporation of avocado waste powder (AWP), derived from peel, seeds, and discarded Hass avocados, into traditional Sicilian ciabatta bread to enhance its functional properties and promote sustainable food innovation. AWP was added at 5% and 10% levels (AWP5, AWP10), and sourdough fermentation was conducted using *Fructilactobacillus sanfranciscensis*, *Weissella cibaria*, and *Leuconostoc citreum*. Despite the initial microbial diversity in raw ingredients, fermentation effectively selected beneficial lactic acid bacteria, with AWP addition potentially supporting their growth. Technological parameters such as weight loss, volume, and firmness remained unaffected, while AWP significantly influenced crumb structure and color. Sensory analysis revealed a different color intensity, aroma, and taste persistency in AWP added breads, though higher AWP levels introduced off-notes. Volatile organic compound profiling showed increased terpenes and sesquiterpenes in AWP breads, enhancing aromatic complexity.

Phenolic compound content was determined before and after in vitro digestion (INFOGEST protocol) in fortified breads. A significant increase in phenolic content with the incorporation of AWP compared to CTR bread was observed, with further enhancement after in vitro digestion. Higher bioaccessible polyphenols, particularly in AWP10 were catechin, gallic acid, and ellagic acid. These findings highlight ciabatta bread as a promising vehicle for functional ingredients, supporting circular economy principles and the valorization of agro-industrial by-products, while maintaining or improving key quality traits.

1. Introduction

Bread is a staple food in Europe, with an average annual consumption of approximately 57 kg per capita (Sadowski et al., 2024). Among the various types of bread, sourdough products have gained increasing popularity due to their perceived health benefits compared to leavened with baker's yeast breads (Garnweidner-Holme et al., 2022). This growing demand has driven the industrialization of sourdough bread production (Yazar & Tavman, 2012), although fermentation processes in specialized industries still largely resemble artisanal practices. The quality of sourdough bread depends on multiple factors, including raw materials, process conditions, and fermenting agents (Brandt, 2019).

Sourdough is a complex microbial ecosystem dominated by lactic acid bacteria (LAB) and yeasts, whose synergistic activity drives fermentation. LAB primarily contribute to dough acidification and aroma development, while yeasts are responsible for leavening. The dominance of specific strains imparts unique characteristics to the final product, resulting in a wide diversity of regional breads (Valmorri et al., 2006). In recent years, sourdough technology has been increasingly applied to incorporate non-traditional raw materials, including agri-food by-products, to improve nutritional and functional properties while promoting sustainability (Pontonio et al., 2024).

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Avocado (*Persea americana* Mill.), particularly the Hass cultivar, is widely appreciated for its nutritional richness and bioactive compounds. However, avocado processing generates significant amounts of waste and by-products (W&BP), such as peel, seeds, and non-compliant fruits, which are rich in valuable compounds including polyphenols, pectin, and starch (Bruno et al., 2020; Salazar-López et al., 2020; Tesfaye et al., 2021). Previous studies have explored the use of dried avocado peel in functional beverages and its antioxidant role in meat preservation (Calderón-Oliver & López-Hernández, 2022). Similarly, avocado peel and seed powders have been proposed as functional ingredients in bakery products such as bread, cakes, and cookies (Bangar et al., 2022; Dziki et al., 2014). Despite these promising applications, research on the combined use of peel and seed by-products in bread formulations remains limited.

Recent investigations demonstrated that incorporating avocado AWP into semolina sourdough bread can enhance its nutritional profile by increasing phenolic content and antioxidant activity without compromising sensory quality (Viola et al., 2023). Building on these findings, the present study aims to valorize avocado waste powder (AWP), obtained from peel, seeds, and discarded fruits, by incorporating it into industrial-scale sourdough ciabatta bread production. Ciabatta, a traditional Italian bread widely consumed as part of the Mediterranean diet (Capurso & Capurso, 2020), represents an ideal vehicle for introducing functional ingredients to consumers. This approach addresses two key objectives: (i) improving the nutritional and functional properties of bread through the addition of bioactive compounds, and (ii) promoting circular economy principles by reducing agro-industrial waste.

To achieve these goals, semolina-based ciabatta breads were produced using sourdough technology and fortified with AWP at two inclusion levels (5% and 10% *w*/w). The study evaluated the impact of AWP addition on microbial dynamics during fermentation, technological and sensory attributes, volatile organic compound profiles, and phenolic compound bioaccessibility after in vitro digestion. These findings provide insights into the feasibility of scaling up functional bread production while maintaining quality standards and enhancing sustainability.

2. Materials and methods

2.1. Raw materials

The avocado waste powder (AWP) was obtained from *Persea americana* Mill. fruits of the Hass cultivar, harvested from the experimental orchard of the Department of Agricultural, Food, and Forestry Sciences at the University of Palermo. Fruits were collected at the commercial maturity stage, determined based on the dry matter (DM) content, which represents a reliable indicator of harvest readiness for the Hass cultivar. Only fruits with a DM content of ≥20.8%, corresponding to the minimum commercial maturity threshold (Lee et al., 1983; Whiley et al., 2002), were selected. After harvest, the fruits were allowed to ripen under controlled conditions (approximately 20 ± 5 °C) until reaching the overripe stage, as indicated by hue angle values ≥45 ± 7 h° (Sánchez-Quezada et al., 2021). These overripe fruits, normally considered processing waste, were instead utilized as a raw material for AWP production, contributing to product valorisation. Prior to further processing, fruits were sanitized by immersion in a chlorinated water solution (2% *w/v*) for 10 min.

Avocado fruits were processed to obtain powders from different parts of the fruit, considering the distinct initial characteristics of seeds, peel, and pulp. Seeds and peels were dehydrated at 60 °C for 4 h, while the pulp was dried at 75 °C for 28 h, according to protocols adapted from Viola et al. (2023) (Table 1). Prior to drying, seeds were washed and their outer coats manually removed. Drying was performed using a tray dryer (Ausla, 1000 W, Milan, Italy). These conditions allowed the moisture content of all fractions to fall below 12–13%, ensuring reduced microbial growth and preservation of bioactive compounds (Misra et al., 2021).

Table 1
Drying parameters and moisture content (Rs - %).

Sample	Drying Temperature	Drying Time	Moisture Content (%Rs)
Avocado Seed	60 °C	4 h	11 ± 1.0
Avocado Peel	60 °C	4 h	13 ± 0.7
Avocado Pulp	75 °C	28 h	12 ± 1.2

Results of Rs indicate mean values ± S.D. (standard deviation).

Moisture content (%Rs) was calculated using Eq. 1 (Roppolo et al., 2024):

$$\%\mathbf{Rs} = (\mathbf{c} - \mathbf{a})(\mathbf{b} - \mathbf{a})\mathbf{x100}\# \quad (\mathbf{1})$$

where:

- a = weight of the empty tray;
- b = weight of the tray with the sample before drying;
- c = weight of the tray with the sample after drying.

The dried materials were subsequently milled into a fine powder using an ultracentrifugal mill (Fritsch, Pulverisette 14, Lainate, Italy) at 700 rpm for 10 s, achieving a particle size of 1.5–2 mm. The resulting powder, combining all fruit fractions, was referred to as AWP.

To formulate the dough-fortification mix, a constant dry-weight ratio was applied: 50% dehydrated pulp, 25% seed-derived powder, and 25% peel-derived powder. These proportions were set in alignment with the functional-composition benchmarks originally defined by Viola et al. (2023).

The commercial semolina (Conad, Bologna, Italy) used in this study for sourdough preparation had the following nutritional composition (per 100 g): 11.0 g of proteins, 1.0 g of fats, 0.2 g of saturated fats, 70.0 g of carbohydrates, 1.0 g of fermentable sugars, and 2.5 g of fibres. The semolina used for industrial bread dough production was purchased from "Molino Gaetano Roccasalva s.r.l." (Modica, Italy) and had the following composition (per 100 g): 11.7 g of proteins, 0.8 g of fats, 0.2 g of saturated fats, 74.0 g of carbohydrates, 1.8 g of fermentable sugars, and 2.9 g of fibres.

2.2. Starter cultures and sourdough preparation

The sourdough starter cultures, *Fructilactobacillus sanfranciscensis* RC-UNIPASAAFM01100, *Weissella cibaria* RC-UNIPASAAFM01109, and *Leuconostoc citreum* RC-UNIPASAAFM01118, from the culture collection of the SAAF Department at the University of Palermo, were used to prepare the sourdough inoculum for industrial bread production. Although *W. cibaria* does not currently possess QPS status, it is naturally widespread in sourdough ecosystems and cereal fermentations. Its use in this work reflects its technological relevance and frequent occurrence in breadmaking environments. The industrial trials aim to assess process scalability and performance under real production conditions, rather than regulatory readiness. All strains were defrosted from the −80 °C glycerol stocks and revitalized in modified de Man-Rogosa-Sharpe (mMRS) medium (Corsetti et al., 2008) after incubation at 30 °C for 24 h. After that, the cell suspensions of each LAB strain contained approximately 10^9 CFU/mL, as determined by plate counts on mMRS agar. The multi-species starter was prepared as a liquid culture (Alfonzo et al., 2016). For this purpose, sterile semolina extract (SSE) broth was inoculated to reach a final concentration of 10^6 CFU/mL for the first inoculum. After incubation at 30 °C for 24 h, the broth cultures were used to prepare a second inoculum (1% *v*/v) in SSE, which was fermented for an additional 24 h. This propagation process was repeated

for two further sub-cultivations, resulting in four consecutive inocula. Subsequently, the LAB strains were combined to create a multi-strain inoculum (Gaglio et al., 2021).

A 500 g sourdough was then prepared using the multi-strain starter culture as inoculum. To achieve this, the mixed LAB suspension was diluted in sterile tap water to a final volume of 187.5 mL and combined with 312.5 g of semolina. The resulting dough had a dough yield (DY = weight of dough / weight of semolina × 100) of 160, and the LAB concentration ranged from 10^6 to 10^7 CFU/g, as confirmed by plate counts. Following the protocol by Corona et al. (2016), the fermentation process involved seven daily refreshments and a long fermentation at 28 °C for 16 h. After this period, the sourdough was deemed mature and ready for industrial baking.

2.3. Monitoring of acidification of doughs

Sourdough and bread doughs underwent an evaluation of the acidification process during fermentation. pH and total titratable acidity (TTA) were measured in 25 g of dough samples. For pH measurement, the pH-meter Russell RL060P (Thermo Fisher Scientific, Beverly, MA, USA) was directly inserted into the 25 g dough. The same dough from each trial was homogenized in 225 mL of sterile distilled water using an Omni Mixer Homogenizer GLH 850 (Omni International, Kennesaw, GA, USA) and then titrated with 0.1 N NaOH, with results expressed in mL of 0.1 N NaOH).

2.4. Microbiological analysis of doughs

Sourdough and dough samples were analyzed to determine the levels of various microbial groups. A 25 g portion from each sample was aseptically collected and homogenized under sterile conditions. The samples were placed in sterile plastic bags (Interscience, Saint Nom, France) and mixed with 225 mL of Ringer's solution (Sigma-Aldrich, Milan, Italy). They were then homogenized using the stomacher Bag-Mixer® 400 (Interscience) at the highest speed for 2 min. The total number of mesophilic microorganisms (TMM) was determined by spread plating on plate count agar (PCA) and incubating the plates aerobically at 30 °C for 72 h. Lactic acid bacteria (LAB) were pour plated on modified mMRS agar and incubated anaerobically at 30 °C for 48 h. Yeasts were spread plated on yeast peptone dextrose (YPD) agar with chloramphenicol (0.1 g/L) and incubated at 28 °C for 48 h. Enterobacteriaceae and coliforms were inoculated micro-anaerobically by pour plated on double-layered violet red bile glucose agar (VRGBA) and violet red bile agar (VRBA), respectively, and incubated at 37 °C for 24 h. All microbiological counts were performed in duplicates, and the results were expressed as Log colony forming units (CFU)/g.

In addition, the raw materials (semolinas and AWP) were also subjected to microbiological analysis. Alongside the enumeration of TMM, LAB, yeasts, Enterobacteriaceae, and coliforms, further assessments were conducted on 10 g samples of each ingredient. Specifically, *Pseudomonas* spp. was enumerated on *Pseudomonas* Agar Base (PAB) following incubation at 25 °C for 48 h; coagulase-positive staphylococci (CPS) were detected using Baird Parker (BP) agar supplemented with rabbit plasma fibrinogen, incubated at 37 °C for 48 h. The presence of *Listeria monocytogenes* and *Salmonella* spp. was assessed in accordance with ISO 11290-1:2017 and ISO 6579-1:2017 protocols, respectively. Spore-forming aerobic bacteria were quantified by heating the cell suspension at 85 °C for 15 min, followed by surface plating on Nutrient Agar (Oxoid, Basingstoke, UK) and incubation at 32 °C for 48 h. All Petri dishes were incubated under aerobic conditions. Unless otherwise specified, all culture media were obtained from Lickson s.r.l. (Vicari, Italy). All microbiological analyses were performed in duplicate.

2.5. Culture-independent profiling of bacterial communities

Microbial DNA was extracted from raw materials and fermented doughs using the DNeasy PowerFood Microbial Kit (QIAGEN, Hilden, Germany), following the standard protocol. For each trial, DNA from replicate samples was pooled to form composite samples. DNA integrity and concentration were evaluated via agarose gel electrophoresis and UV/Vis spectrophotometry. The V3–V4 hypervariable regions of the bacterial 16S rRNA gene were amplified using primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GACTACNVGGGTWTC-TAATCC-3′). PCR reactions (25 μL total volume) included 2× KAPA Hifi HotStart Ready Mix, 1 μM of each primer, 2 μL of template DNA (10 ng/μL), and nuclease-free water. Amplification was performed using a Verity™ 96-well Thermal Cycler under the following conditions: initial denaturation at 95 °C for 5 min; 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 40 s; and a final extension at 72 °C for 5 min. Amplicons were verified by gel electrophoresis and purified using the Agencourt AMPure XP system (Beckman Coulter, Brea, CA, USA). Indexing and library preparation followed Illumina's 16S Metagenomic Sequencing Library protocol, with Nextera XT Index Primers. Final libraries were cleaned and sequenced on an Illumina® MiSeq platform (2 × 300 bp paired-end reads), using MiSeq Control Software v2.0.5 and Real-Time Analysis Software v1.16.18 (Illumina, San Diego, CA, USA).

2.6. Bioinformatic processing and taxonomic assignment

Raw sequencing reads were demultiplexed using the idemp tool (https://github.com/yhwu/idemp) and processed in QIIME2 (version 2018.2). Quality control, trimming, denoising, and merging of paired-end reads were performed using the DADA2 pipeline (Callahan et al., 2016), which filters reads based on expected error rates and removes chimeric sequences using a consensus-based approach. High-resolution sequence variants were aligned with MAFFT and used to construct phylogenetic trees via FastTree. Taxonomic classification was carried out using the QIIME2 feature-classifier plugin, employing a pre-trained Naive Bayes model based on the Greengenes 13_8 database (99% OTUs), trimmed to match the V3–V4 region targeted by the 341F/806R primers. Taxa with a relative abundance of ≥0.1% were considered significant, following the threshold suggested by Logares et al. (2014). All sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) under accession number PRJNA1353661.

2.7. Bread production

In this study, the traditional ciabatta bread recipe from Sicilian bakeries was used to produce breads with varying percentages of AWP. At the bakery "I frutti del grano" (Naro, AG, Italy), 5 kg dough were prepared for each of the following trials: CTR (control bread production), AWP5 (experimental bread with 5% AWP), and AWP10 (experimental bread with 10% AWP). The bread-making recipes are detailed in Table S1. The choice of 5% and 10% (*w*/w) AWP was based on literature indicating that substitution of wheat flour or semolina with fruit- or vegetable-based products rarely exceeds 10% (Gómez & Martinez, 2018). Additionally, these levels allow trend comparison and were previously tested in bread production at laboratory scale (Viola et al., 2023), providing a reference for the present industrial-scale study.

The ingredients were mechanically mixed using the spiral mixer model IFA/80 (Aldegheri Forni s.r.l, San Bonifacio, Italy). The kneading program consisted of spiral rotation at the first speed for 10 min, followed by spiral rotation at the second speed for 2 min, and a final reverse rotation at the first speed for 2 min. Ciabatta doughs, each weighing 330 g were manually shaped by the bread maker. The leavening process occurred in two stages: in the first stage, the entire 5 kg doughs were left at room temperature in dough proofing boxes for 5 h; in the second stage, the 330 g doughs were left at room temperature on the loader for the deck oven for 90 min. Baking was carried out at 230 °C for 20 min in the electric oven model E080 (Aldegheri Forni s.r.l), with an initial steam exposure for 3 s. Three independent bread productions, representing three experimental replicates, were performed at 2-week

intervals.

2.8. Characteristics of breads

Thirty minutes after baking, the breads were kept at room temperature in the bakery and then weighed to determine weight loss (WL) using the formula:

$$WL = \left(\frac{\text{weight of dough (g)} - \text{weight of bread (g)}}{\text{weight of dough (g)}}\right) \times 100$$ (Purlis & Salvadori, 2007).

The breads were then placed in paper bags and transported at ambient temperature to the Agricultural Microbiology laboratories. Upon arrival, the volume of each bread was measured using a volumeter for bakery products (ErreCi s.r.l., Merate, Italy) following the rapeseed displacement method as per the American Association of Cereal Chemists method 55–50.01 (AACC, 2000). Firmness was assessed using the Instron-5564 (Instron Corp., Canton, MA, USA), which measures resistance to compression (N/mm^2). Additionally, the crust and crumb color, void fraction (total area of bubbles), cell density (number of cells/cm^2), and mean cell area (mm^2) were analyzed according to the methodologies described by Gaglio et al. (2021).

2.9. Extraction of free polyphenols from bread

Phenolic compounds were analyzed by the HPLC-UV method, as described by Cetó et al. (2018), with slight modifications. Two grams of each bread sample were solubilized in 40 mL 80% methanol/water solution and the extraction of phenolic compounds was promoted in ultrasonic bath for 1 h. After, the mixture was centrifuged at 5000 rpm and supernatant was evaporated with a rotary evaporator at 45 °C. The residue was redissolved in 2 mL of methanol and filtered through 0.45 μm PTFE syringe filter. Solutions containing free polyphenols were placed into glass vials prior to UHPLC-UV analysis.

2.10. In vitro static digestion of bread

The harmonized INFOGEST protocol was applied to simulate the human digestive process.

(Minekus et al., 2014). For the oral phase, 5 g of each bread sample were soaked with 4 mL of a salivary fluid (SSF; pH = 7.0), mixed with ceramic mortar and pestle. The mixture was transferred in a 50 mL glass tube and 0.500 mL of α-amylase 75 U/mL, 25 μL $CaCl_2(H_2O)_2$, 0.475 mL H_2O, were added (A1031; Sigma-Aldrich; Milan, Italy). The mixture was incubated at 37 °C for 2 min. Then, 20 mL of a gastric fluid at pH 3.0 plus pepsin (P7012; Sigma-Aldrich; 2.000 U/mL) was added. This gastric phase lasted for 120 min at 37 °C. For the small intestine phase, 40 mL of an intestinal fluid at pH = 7.0 plus pancreatin (P7545; Sigma-Aldrich; Milan, Italy; 100 U/mL) and bile salts (B8631; Sigma-Aldrich; Milan, Italy; 10 mM) were added to the chyme and samples were further incubated for 120 min at 37 °C. In addition, HCl (1 M) and NaOH (1 M) were used for pH regulation. The composition of each simulated fluid was detailed by Minekus et al. (2014). After digestion, the mixtures were centrifuged using Amicon® Ultra-15 centrifugal filter units (Sigma-Aldrich). The filtrate obtained after centrifugation was collected and used for subsequent analyses of phenolic compounds and their bioaccessibility. Analyses were run in triplicate.

2.11. Phenolic profiling by HPLC-UV of bread samples before and after in vitro digestion process

Bread samples were analyzed before and after digestion protocol to determine main phenolic compounds and their bioaccessibility. The HPLC-UV analysis was performed on Thermo Scientific Vanquish system, equipped with a quaternary pump (Vanquish™ Quaternary Pump C, VC-P20-A01), an automatic injector (Vanquish™ Split Sampler C, VC-A13-A-02) and a UV detector (Vanquish™ Variable Wavelength Detector, VC-D40-A-01) (Thermo Scientific, USA). The chromatographic separation was achieved using a ReproSil Saphir 100 C18 (200 mm × 4.0 mm, 5 μm stationary phase, Dr. Maisch), equipped with pre-column. Gradient separation using 0.1% formic acid in water (*v*/v) (solvent A) and methanol (solvent B) as mobile phases was as follows: 0–3 min, linear gradient from 5 to 25% B; 3–6 min, at 25% B; 6–9 min, from 25 to 37% B; 9–13 min, at 37% B; 13–18 min, from 37 to 54% B; 18–22 min, at 54% B; 22–26 min, from 54 to 95% B; 26–29 min, at 95% B; 29–29.15 min, back to initial conditions at 5% B; and from 29.15 to 36 min, at 5% B. The initial conditions were maintained for 3 min to equilibrate the column. The column was maintained at 25 °C and the flow rate was 1 mL/min. The volume of injection was 25 μL. After a series of tests of different literature-based wavelengths, values of 280 nm and 360 nm were determined optimal for obtaining signals with maximum intensity (Georgiev et al., 2019). For quantitative analysis, calibration curves were constructed for the identified compounds using standards with concentrations ranging from 10 μg/mL to 50 μg/mL. Systematic control data collection and analysis were performed using the Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System software.

2.12. Analysis of volatile organic compounds of bread

The volatile organic compounds (VOCs) in AWP, control bread, and breads enriched with AWP (AWP5 and AWP10) were analyzed using solid-phase microextraction (SPME) coupled with gas chromatography–mass spectrometry (GC–MS). For extraction, a 30 μm DVB/CAR/PDMS SPME fiber was exposed to the sample headspace for 60 min at 25 °C. Desorption of VOCs was carried out at 250 °C for 5 min via the GC injection port. The GC oven temperature was programmed to increase from 40 °C to 230 °C at a rate of 4 °C/min, followed by an isothermal hold at 230 °C for 40 min. Separation was achieved using a DB-624 capillary column (Agilent Technologies; 60 m × 0.25 mm i.d., 1.40 μm film thickness). Mass spectra were acquired in full scan mode (*m/z* 40–400), with the interface temperature set at 230 °C. Each bread sample was analyzed in triplicate. Identification of major VOCs was performed by comparing the obtained mass spectra with those in the NIST05 library. Results were expressed as relative percentages, calculated by normalizing individual peak areas to the total area of all significant peaks.

2.13. Sensory analysis of bread

The breads produced at the bakery were transported to the Laboratory of Sensory Evaluation of the Department of Agriculture, Food and Forestry Sciences at the University of Palermo for a descriptive sensory analysis. Bread transport took approximately two and a half hours. A panel of 19 judges, consisting of 12 women and seven men aged between 20 and 64, was recruited for this purpose. The judges underwent training to familiarize themselves with bread attributes using a commercial bread. Following this, they participated in the tasting session of the AWP-added breads produced in this study. Slices of bread, each 2 cm thick (Panirani et al., 2023), were cut and served to the panellists at room temperature within 5 min. The slices were presented on plastic plates labelled with three-digit codes (Moretton et al., 2023). The judges did not see the entire bread shapes before tasting. They were asked to evaluate the appearance, texture, odor, and taste of the breads, using descriptors from previous studies (Martins et al., 2015; Rodrigues et al., 2014). Each attribute was scored on a 9-point scale (1 = extremely bad; 9 = extremely good). Additionally, an overall assessment of the breads was provided, considering the scores of all evaluated attributes. The sensory tests were conducted in single chambers, following ISO 13299 guidelines (2003).

2.14. Statistical analysis

Statistical evaluations were performed using one-way analysis of

variance (ANOVA) to assess differences between control and experimental samples. Pairwise comparisons were subsequently conducted using Tukey's post hoc test, with a significance threshold set at $p < 0.05$. All statistical computations were carried out using XLStat software (version 2020.3.1; Addinsoft, New York, NY, USA), integrated within Microsoft Excel.

3. Results and discussion

3.1. Dehydration process

The use of powder derived from AWP proved highly effective for enriching ciabatta bread, thanks to a hot air convective drying process that allowed a significant reduction in moisture while preserving pigments, phenolic compounds, and antioxidant activity. The results confirmed that moderate drying conditions, based on the combination of low temperature and short time, are effective in preventing the degradation of bioactive components and maintaining the nutritional profile of the materials (Oliveira et al., 2016). Specifically, seeds and peel were treated at 60 °C for 4 h, reaching a moisture content of 11 ± 1.0% and 13 ± 0.7%, respectively, and pulp was treated at 75 °C for 28 h, with a final moisture content of 12 ± 1.2%. These final moisture values are also optimal for ensuring the microbiological safety and nutritional stability of the dried products (Viola et al., 2023). After dehydration, the dried materials were milled into a fine powder, obtaining particle sizes of 1.5–2 mm. Previous studies have shown that particle size can influence the functional and sensory properties of powders; in general, fine milling improves solubility, reactivity, and uniform distribution in food products (Famuwagun et al., 2016; Mohammed et al., 2022). This approach allowed the production of a fine, stable green powder with a high content of bioactive compounds, demonstrating that hot air convective drying is an effective technique for the sustainable valorisation of avocado waste in the food industry.

3.2. Acidification process

Acidification kinetics were evaluated in a 7-day mature sourdough fermented with three selected LAB strains. The sourdough reached a pH of 3.59 ± 0.03 and a total titratable acidity (TTA) of 16.40 ± 0.71 mL NaOH 0.1 N/10 g, indicating a slightly higher degree of acidification compared to values previously reported by Alfonzo et al. (2016) and Gaglio et al. (2021) for sourdoughs fermented with multi-strain consortia including *Fructilactobacillus sanfranciscensis*, *Leuconostoc citreum*, and *Weissella cibaria*.

Doughs supplemented with AWP exhibited marginally higher pH values than the control trial throughout fermentation, a trend that persisted until the end of the process (Fig. 1). Nevertheless, all samples experienced a progressive pH decline, reaching approximately 3.7 by the conclusion of the second leavening stage. These pH values reflect a robust acidification process and suggest that the inclusion of AWP did not interfere with fermentation kinetics, even under industrial processing conditions. This observation aligns with previous laboratory-scale findings reported by Viola et al. (2023).

Consistent with trends observed in sourdough fermentation (Siepmann et al., 2018), TTA values demonstrated an inverse correlation with pH across all experimental trials (Fig. 1). Notably, doughs enriched with AWP exhibited higher TTA values than the control, both at the beginning and at the end of fermentation. Specifically, TTA values at the end of monitoring reached 13.75 and 14.10 mL NaOH 0.1 N/10 g in the AWP-supplemented doughs, compared to 12.45 mL in the control trial. This observation appears paradoxical given the slightly elevated pH recorded in the AWP trials. This discrepancy may be attributed to the protein content of this AWP ingredient (Araújo et al., 2018), which could exert a buffering effect (Mennah-Govela et al., 2020). Such buffering capacity may modulate acid release and retention, contributing to the observed TTA elevation despite minimal pH variation.

3.3. Microbiological evolution

Microbiological analyses were conducted throughout the dough production process, beginning with the individual raw materials (Table 2) and continuing through to the dough samples collected at both the initial stage (start of the first leavening step) and the final stage of fermentation (end of the second leavening step) (Table 3).

The microbiological analysis revealed distinct differences in microbial populations across the tested raw materials and the mature sourdough (Table 2). TMM and LAB were detected in both semolina samples, with slightly higher counts in the semolina “Molino Gaspare Roccasalva”

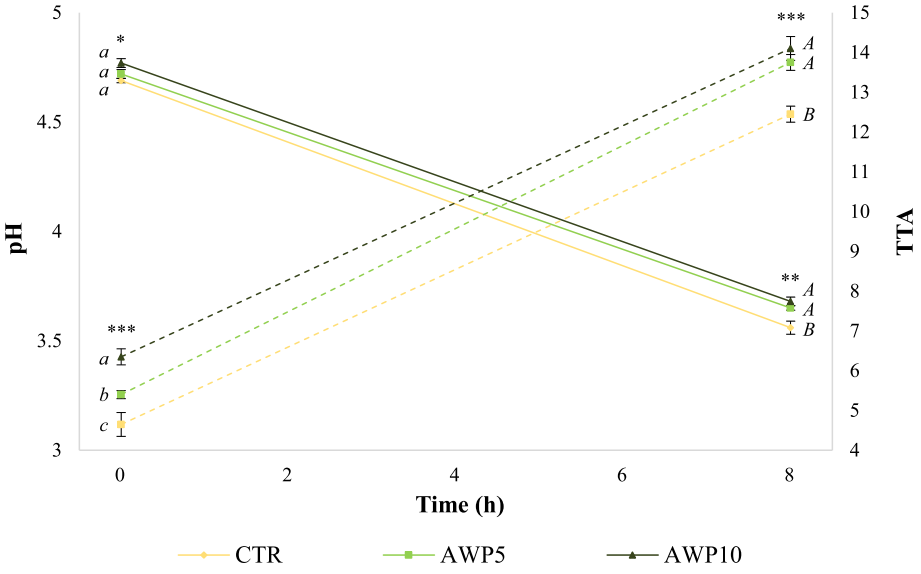

Fig. 1. Kinetics of acidification of control and experimental doughs during fermentation. Solid lines: pH. Dotted lines: TTA. Results indicate mean values ± standard deviation of six determinations (performed in duplicate for three independent productions). Different letters (lowercase for t_0 and uppercase for t_8) indicates significant differences among the samples as measured by one-way ANOVA followed by Tukey's post hoc test. p value: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Abbreviations: TTA, total titratable acidity; CTR, control dough; AWP5, experimental dough containing 5% (*w*/w) of Avocado Waste Powder (AWP); AWP10, experimental dough containing 10% (w/w) of AWP.

Table 2
Microbial loads (log CFU/g) of raw materials.

Microbial group	Raw materials				SEM	*p* value
	Semolina (Conad)	Semolina (Roccasalva)	Avocado waste power	Sourdough		
TMM	2.18 ± 0.21 b	2.69 ± 0.32 b	b.d.l. c	8.93 ± 0.29 a	0.71	<0.0001
LAB	2.48 ± 0.34 b	2.85 ± 0.20 b	b.d.l. a	9.10 ± 0.45 a	0.72	<0.0001
Yeasts	b.d.l. c	2.10 ± 0.10 b	b.d.l. c	7.56 ± 0.23 a	0.66	<0.0001
Enterobacteriaceae	b.d.l.	b.d.l.	b.d.l.	b.d.l.	n.e.	n.e.
Coliforms	b.d.l.	b.d.l.	b.d.l.	b.d.l.	n.e.	n.e.
Pseudomonas spp.	b.d.l.	b.d.l.	b.d.l.	n.d.	n.e.	n.e.
CPS	b.d.l.	b.d.l.	b.d.l.	n.d.	n.e.	n.e.
L. monocytogenes	b.d.l.	b.d.l.	b.d.l.	n.d.	n.e.	n.e.
Salmonella spp.	b.d.l.	b.d.l.	b.d.l.	n.d.	n.e.	n.e.
Spore forming bacteria	b.d.l.	b.d.l.	b.d.l.	b.d.l.	n.e.	n.e.

Results indicate mean values ± S.D. (standard deviation) of two plate counts (carried out in duplicate).
Abbreviations: TMM, total mesophilic microorganisms; LAB, lactic acid bacteria; CPS, coagulase positive staphylococci; *L.*, *Listeria*; b.d.l., below detection limit; n.d. = not determined; n.e. not evaluated.

Table 3
Microbial loads (log CFU/g) of doughs.

Microbial group	Fermentation	Samples			SEM	*p* value
		CTR	AWP5	AWP10		
TMM	start	7.45 a	7.65 a	7.53 a	0.05	0.621
	end	8.22 a	8.16 a	8.09 a	0.04	0.754
LAB	start	7.70 a	7.48 a	7.31 a	0.07	0.343
	end	8.48 a	8.54 a	8.32 a	0.04	0.388
Yeasts	start	6.12 a	6.02 a	6.26 a	0.04	0.396
	end	6.95 ± 0.23	7.08 ± 0.25	6.88 ± 0.18	0.05	0.566
Enterobacteriaceae	start	b.d.l.	b.d.l.	b.d.l.	n.e.	n.e.
	end	b.d.l. b	b.d.l. b	2.18 ± 0.12 a	n.e.	<0.0001
Coliforms	start	b.d.l.	b.d.l.	b.d.l.	n.e.	n.e.
	end	b.d.l.	b.d.l.	b.d.l.	n.e.	n.e.

Results indicate mean values of six plate counts (performed in duplicate for three independent productions). Data within a line followed by the same letter are not significantly different according to Tukey's test. Abbreviations: CTR, control dough; AWP5, experimental dough containing 5% (w/w) of Avocado Waste Powder (AWP); AWP10, experimental dough containing 10% (w/w) of AWP; SEM, standard error of mean, TMM, total mesophilic microorganisms; LAB, lactic acid bacteria; b.d.l., below detection limit; n.e. not evaluated.

(2.69 log CFU/g and 2.85 log CFU/g, respectively) compared to the semolina "Conad" (2.18 log CFU/g and 2.48 log CFU/g, respectively). These values suggest a modest native microbial load, likely influenced by differences in milling processes, storage conditions, or grain origin. AWP showed no detectable microbial counts across all tested groups, indicating either a highly hygienic processing method or the presence of antimicrobial compounds naturally occurring in avocado W&BP. Indeed, avocado seeds show a high spectrum of antimicrobial activity (Kupnik et al., 2023). This absence of microbial load supports its safe inclusion in food formulations from a microbiological standpoint. The mature sourdough exhibited significantly higher microbial counts, with TMM and LAB reaching 8.93 log CFU/g and 9.10 log CFU/g, respectively. These high levels are consistent with active fermentation and the proliferation of LAB, which are essential for sourdough development (Suo et al., 2021). Yeasts were also present at high levels (7.56 log CFU/g), confirming their contribution during sourdough development. Moreover, the ratio between LAB and yeast populations was approximately 100:1, a proportion considered optimal for achieving desirable sourdough characteristics (Calvert et al., 2021). Importantly, no Enterobacteriaceae, coliforms, *Pseudomonas* spp., CPS, *Listeria monocytogenes*, *Salmonella* spp., or spore-forming bacteria were detected in any of the samples. This absence indicates good microbiological quality and safety of both raw materials and the final sourdough product. The lack of pathogenic or spoilage organisms is particularly relevant for industrial applications, where microbial stability and safety are critical (Noshirvani & Abolghasemi Fakhri, 2025).

The microbiological analysis of dough fermentation revealed distinct trends across CTR and AWP-supplemented doughs (Table 3). TMM showed a consistent increase from the start to the end of fermentation in all samples, indicating active microbial growth. Initial TMM levels were slightly higher in AWP5 (7.65 log CFU/mL) compared to CTR (7.45 log CFU/mL), but by the end of fermentation, CTR reached the highest count (8.22 log CFU/mL). LAB were present in high numbers from the beginning. CTR had the highest initial LAB count (7.70 log CFU/mL), while AWP5 and AWP10 showed slightly lower values (7.48 and 7.31 log CFU/mL, respectively). LAB counts increased in all samples during fermentation. The consistent overlap between TMM and LAB data clearly indicates that LAB dominated the fermentation process. Yeast populations followed a similar trend to TMM and LAB, though at lower levels. Initial yeast counts were comparable across samples, with AWP10 slightly higher (6.26 log CFU/mL) than CTR and AWP5. By the end of fermentation, yeast counts had increased in all samples. These results suggest that AWP supplementation does not inhibit overall microbial proliferation. Although a slight reduction in final counts was observed at higher AWP concentrations, this effect can be considered negligible.

Regarding hygiene indicators, Enterobacteriaceae and coliforms were below detection limits at the start of fermentation in all samples. At the end, CTR and AWP5 remained below this limit, while AWP10 showed detectable levels of Enterobacteriaceae (2.18 log CFU/mL). This confirms that when a microbial group is below the detection limit, it does not necessarily mean it is absent (Lambert et al., 1991). Coliforms remained undetectable throughout, further supporting the sanitary quality of the process. Microbiological data suggest that the sourdough fermentation process selectively promotes beneficial microbial groups such as LAB and yeasts even in presence of AWP (Gobbetti et al., 2020). This supports the inclusion of AWP as a promising functional ingredient in sourdough bread production.

3.4. Microbial community composition

Illumina sequencing results for the three bread trials (CTR, AWP5, and AWP10) and the raw materials (semolina and AWP), as shown in Fig. 2, highlight a pronounced shift in microbial communities during bread production. Semolina was primarily composed of Alphaproteobacteria [92.3% relative abundance (RA)], with smaller proportions of unassigned bacteria (6.8% RA), *Erwinia*, and other Enterobacteriaceae, while *Lactobacillus* and other LAB were nearly absent. The detection of Enterobacteriaceae may indicate suboptimal hygiene in the raw materials (Sahoo et al., 2022). In AWP, Alphaproteobacteria remained dominant (94.8% RA), but there was a small presence of *Lactobacillus* (0.7% RA) and other LAB (2.7% RA), suggesting that AWP may introduce or support these beneficial groups. Following dough fermentation, *Lactobacillus* became the dominant genus in CTR bread (85.7% RA), with Alphaproteobacteria decreasing to 14.2% RA, and no unassigned, *Vibrio*, *Erwinia*, or other Enterobacteriaceae detected. In breads with AWP, *Lactobacillus* remained high, 82.5% RA in AWP5 and 96.9% RA in AWP10, while Alphaproteobacteria dropped further and other groups were undetectable. This transition from Alphaproteobacteria to *Lactobacillus* dominance is characteristic of fermentation, where LAB, particularly lactobacilli, outcompete other bacteria due to the acidic environment (Zhang et al., 2019). Although AWP introduces some microbial diversity (such as other LAB, *Corynebacterium*, and *Vibrio*), these groups do not persist in the final bread. Moreover, increasing the AWP concentration from 5% to 10% further enhanced *Lactobacillus* abundance, indicating that AWP may promote LAB growth or survival during fermentation. The prevalence of lactobacilli in all bread samples underscores the importance of LAB in sourdough fermentation, contributing to improved safety, as evidenced by the reduction of unassigned and potentially undesirable groups like Enterobacteriaceae, along with longer shelf-life and appreciable sensory qualities (Di Biase et al., 2025).

3.5. Impact of AWP on bread properties

The incorporation of AWP at 5% and 10% levels into bread formulations (AWP5 and AWP10) was evaluated against CTR bread to assess its impact on technological and visual quality parameters (Table 4). Weight loss, specific volume, and firmness did not differ significantly among the three formulations ($p > 0.05$). This suggests that AWP inclusion, even at 10%, does not adversely affect moisture retention during baking, loaf expansion, or textural resistance. The slight numerical decrease in weight loss and specific volume with increasing AWP may be

Table 4
Bread attributes.

Attributes		Samples			SEM	*p* value
		CTR	AWP5	AWP10		
Weight loss (g)		16.84 a	15.68 a	15.10 a	0.25	0.161
Specific volume (cm^3/g bread)		5.65 a	5.35 a	5.15 a	0.07	0.135
Firmness (N/mm^2)		0.048 a	0.053 a	0.061 a	0.003	0.431
Crust color	*L**	64.56 a	53.99 ± b	48.70 b	1.79	0.003
	*a**	2.03 b	4.32 ± a	5.14 a	0.42	<0.0001
	*b**	26.07 a	25.38a	24.72 a	0.23	0.256
Crumb color	*L**	69.62 a	62.25 b	52.54 c	1.19	<0.0001
	*a**	−3.36 c	−0.80 b	0.72 a	0.42	<0.0001
	*b**	22.59 a	17.75 b	16.45 b	0.67	<0.0001
Void fraction (%)		39.76 c	45.00 b	47.00 a	0.78	<0.0001
Cell density (n/cm^2)		109.41 c	129.48 b	137.64 a	2.98	<0.0001
Mean cell area (mm^2)		0.60 a	0.48 ab	0.46 b	0.02	0.023

Results indicate mean values of six determinations (performed in duplicate for three independent productions). Data within a line followed by the same letter are not significantly different according to Tukey's test. Abbreviations: CTR, control bread; AWP5, experimental bread containing 5% (w/w) of Avocado Waste Powder (AWP); AWP10, experimental bread containing 10% (w/w) of AWP; SEM, standard error of mean. On the row: a, b, c = $p < 0.05$.

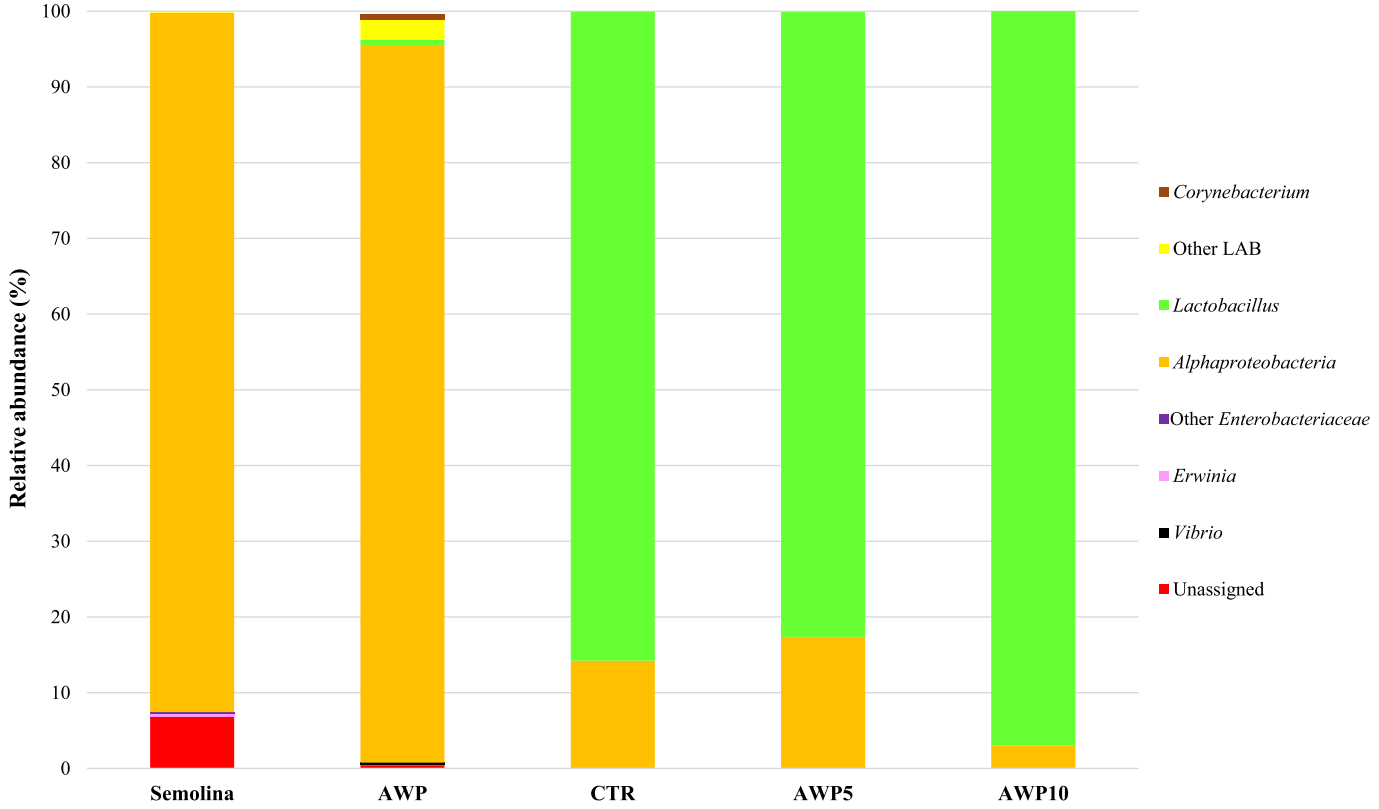

Fig. 2. Relative abundances (%) of bacterial community identified by MiSeq Illumina. Abbreviations: AWP, Avocado Waste Powder, CTR, control dough; AWP5, experimental dough containing 5% (w/w) of AWP; AWP10, experimental dough containing 10% (w/w) of AWP; LAB, Lactic Acid Bacteria.

attributed to the fiber content of AWP, which could influence water binding and gas retention (Martins et al., 2017), but these trends were not statistically significant. Similarly, the slight increase in firmness in AWP10 compared to CTR was not significant, indicating that the structural integrity of the crumb remains largely unaffected by AWP addition.

Color parameters were significantly influenced by AWP inclusion. Crust lightness (L*) decreased markedly with AWP addition ($p = 0.003$), indicating a darker crust in AWP5 and AWP10. This darkening is likely due to the natural pigments in AWP and enhanced Maillard reactions during baking (Zuñiga-Martínez et al., 2022). Crust redness (a*) increased significantly ($p < 0.0001$), suggesting a shift toward warmer hues, while crust yellowness (b*) remained statistically unchanged. In the crumb, all color parameters were significantly affected. Lightness (L*) decreased progressively from CTR to AWP10 ($p < 0.0001$), confirming a darker internal appearance. Redness (a*) increased from negative values in CTR (greenish hue) to positive values in AWP10, indicating a clear shift in color balance. Yellowness (b*) also decreased significantly ($p < 0.0001$), suggesting a reduction in the typical yellowness of wheat-based crumb. These changes reflect the strong influence of natural colorants of AWP and its interaction with the dough matrix during baking, a phenomenon similarly observed in breads added with mango peel powder (Chulibert et al., 2024; Hasan et al., 2024).

The void fraction and cell density increased significantly with AWP addition ($p < 0.0001$), indicating a more aerated and finely structured crumb in AWP5 and AWP10. This could be due to the water-holding capacity and fiber content of AWP, which may influence gas retention during fermentation (Chen et al., 1988). Conversely, the mean cell area decreased significantly ($p = 0.023$), confirming that the crumb structure became finer and more uniform with AWP inclusion. These structural changes are generally favourable, as they contribute to a lighter mouthfeel and improved crumb texture (Rathnayake et al., 2018).

3.6. Determination of phenolic compounds before and after in vitro digestion process

The inclusion of AWP in bread formulations results in a nutritional enhancement of the final product. The powder provides phenolic compounds and other bioactive constituents naturally present in avocado by-products, contributing to an increased antioxidant potential and an overall improvement of the functional nutritional profile. These components influence the characteristics of the enriched bread, particularly in terms of its nutritional value and potential health-related benefits.

Table 5 and Table 6 show the main phenolic compounds (mg/100 g) identified in crude and digested bread samples. Analyses were conducted before and after in vitro digestion, following INFOGEST protocol (Minekus et al., 2014). The results demonstrated a significant increase in phenolic content with the incorporation of AWP compared to CTR bread, with further enhancement observed after digestion, indicating improved polyphenol bioaccessibility. Statistical analyses included one-way ANOVA with Tukey's HSD for enrichment effects (pre- and post-digestion, Table 5) and paired *t*-tests for digestion effects within each formulation (Table 6), with significance set at $p < 0.05$.

Several studies have investigated the effect of in vitro gastrointestinal digestion on phenolic composition, generally reporting increased concentrations in digested samples (Carrasco-Chávez et al., 2024; Jara-Palacios et al., 2018; Scrob et al., 2022). Among digested samples, catechin, gallic acid, ellagic acid, and protocatechuic acid were the most abundant compounds. In DG_AWP10, catechin reached 1050.22 mg/100 g, followed by gallic acid (1037.83 mg/100 g), protocatechuic acid (500.49 mg/100 g), and ellagic acid (453.32 mg/100 g), while the CTR showed the lowest values in quantitative terms and was free of phenolic compounds detectable only in the digested experimental bread.

Although digestion increased polyphenol levels in CTR, concentrations remained far lower than in AWP breads, confirming the poor phenolic profile of conventional bread and the potential of AWP to enhance nutritional quality. For instance, ellagic acid showed a growth from 9.35 mg/100 g to 39.72 mg/100 g in CTR sample after digestion (DG_CTR), but in AWP breads it increased from 40.13 mg/100 g (AWP5) and 125.56 mg/100 g (AWP10) to 146.35 mg/100 g and 453.32 mg/100 g, respectively. Gallic acid showed a similar trend, increasing from 13.16 to 47.87 mg/100 g in CTR and from 294.81 to 1037.83 mg/100 g in AWP10. Catechin was not detected in CTR sample, either before or after digestion, but reached 243.82 and 1050.22 mg/100 g in DG_AWP5 and DG_AWP10.

In contrast, chlorogenic acid and quercetin derivatives decreased in digested experimental bread, suggesting instability in gastrointestinal conditions. Some flavonoids (e.g., naringenin, kaempferol, apigenin) were only detected in DG_AWP10 sample.

Overall, INFOGEST protocol promoted greater release of phenolic acids in AWP breads, while flavonoids decreased. These findings strongly indicate that AWP supplementation, particularly at 10%, significantly improves the phenolic profile of bread, with digestion further increasing its bioavailability. This highlights both the nutritional

Table 5
Single phenolic compounds in experimental bread and in digested experimental bread, identified by HPLC-UV.

Polyphenols	Samples			SEM	*p* value	Samples			SEM	*p* value
	CTR	AWP5	AWP10			DG_CTR	DG_AWP5	DG_AWP10		
Ellagic Acid	9.35 a	40.13 b	125.56 c	21.30	<0.0001	39.72 a	146.46 b	453.32 c	76.06	<0.0001
Gallic Acid	13.16 a	174.46 b	294.81 c	50.05	<0.0001	47.87 a	429.31 b	1037.83 c	179.59	<0.0001
Protocatechuic Acid	13.66 a	46.84 b	176.65 c	30.53	<0.0001	54.67 a	194.15 b	500.49 c	82.27	<0.0001
Catechin	n.d. c	73.39 a	298.75 b	55.21	<0.0001	n.d. c	243.82 a	1050.22 b	194.84	<0.0001
Chlorogenic Acid	n.d. c	38.27 a	100.88 b	18.01	<0.0001	n.d. c	6.21 a	33.75 b	6.39	0.0001
Caffeic Acid	n.d. c	68.48 a	76.27 b	14.92	0.156	6.51 a	82.39 b	159.86 c	27.58	<0.0001
Vanillic Acid	n.d. c	14.02 a	18.81 b	3.46	0.0003	n.d. c	17.76 a	38.77 b	6.87	<0.0001
Epicatechin	n.d. c	4.10 a	7.66 b	1.36	<0.0001	n.d. c	11.18 a	45.59 b	8.48	0.0004
Syringic Acid	3.23 a	4.42 ab	6.10 b	0.58	0.010	6.95 a	28.92 b	47.94 c	7.33	<0.0001
Sinapic Acid	2.39 a	2.45 a	2.42 a	0.02	0.288	n.d. c	1.56 a	1.90 b	0.36	0.027
p-Coumaric Acid	n.d. c	19.09 a	63.68 b	11.56	<0.0001	n.d. c	3.63 a	37.05 a	8.43	0.053
Quercetin-3-glucoside	n.d. c	24.53 a	41.82 b	7.44	<0.0001	n.d. c	5.62 a	13.52 b	2.47	0.008
Quercetin-3-rhamnoside	n.d. c	20.20 a	34.96 b	6.21	<0.0001	n.d. c	0.48 a	7.64 b	1.56	0.002
Naringenin	n.d.	n.d.	n.d.	n.e.	n.e.	n.d. b	n.d. b	5.54 a	1.22	<0.0001
Kaempferol	n.d.	n.d.	n.d.	n.e.	n.e.	n.d. c	6.64 a	9.57 b	1.73	<0.0001
Apigenin	n.d.	n.d.	n.d.	n.e.	n.e.	n.d. c	0.18 a	1.13 b	0.22	<0.0001

Results as expressed in mg/100 g of DM and indicate mean values of six determinations (carried out in triplicate for two independent productions). Abbreviations: CTR, control bread; AWP5 experimental bread enriched with 5% (w/w) of Avocado Waste Powder (AWP); AWP10 experimental bread enriched with 10% (w/w) of AWP; SEM, standard error of the mean; DG_CTR, digested control bread; DG_AWP5, digested experimental bread enriched with 5% (w/w) of AWP: DG_AWP10, digested experimental bread enriched with 10% (w/w) of AWP; n.d. not detected; n.e. not evaluated. On the row: a, b, c = $p < 0.05$.

Table 6
Paired t-tests between each crude and digested sample to evaluate differences in polyphenolic profile.

Polyphenols	Samples					
	CTR vs DG_CTR		AWP5 vs DG_AWP5		AWP10 vs DG_AWP10	
	p value	Significance	*p* value	Significance	*p* value	Significance
Ellagic Acid	0.001	**	0.0002	***	0.001	**
Gallic Acid	<0.0001	***	0.008	**	0.014	*
Protocatechuic Acid	<0.0001	***	0.0002	**	0.017	*
Catechin	n.d.	n.d.	0.022	*	0.001	**
Chlorogenic Acid	n.d.	n.d.	0.0001	***	0.114	n.s.
Caffeic Acid	<0.0001	***	0.616	ns	0.019	*
Vanillic Acid	n.d.	n.d.	0.010	*	0.001	**
Epicatechin	n.d.	n.d.	0.033	*	0.005	**
Syringic Acid	0.001	**	0.006	**	0.003	**
Sinapic Acid	<0.0001	***	0.006	**	0.001	**
p-Coumaric Acid	n.d.	n.d.	0.016	*	0.172	n.s.
Quercetin-3-glucoside	n.d.	n.d.	0.007	**	0.003	**
Quercetin-3-rhamnoside	n.d.	n.d.	0.001	**	0.001	**
Naringenin	n.d.	n.d.	n.e.	n.e.	0.050	*
Kaempferol	n.d.	n.d.	<0.0001	***	<0.0001	***
Apigenin	n.d.	n.d.	<0.0001	***	<0.0001	***

Comparison between crude and digested samples (paired t-test). Abbreviations: CTR, control bread; DG_CTR, digested control bread; AWP5 experimental bread enriched with 5% (w/w) of Avocado Waste Powder (AWP); DG_AWP5, digested experimental bread enriched with 5% (w/w) of AWP; AWP10 experimental bread enriched with 10% (w/w) of AWP; DG_AWP10, digested experimental bread enriched with 10% (w/w) of AWP. *$p \leq 0.05$; **$p \leq 0.01$; **$p \leq 0.001$; n.d., not detected; n.s., not significant; n.e. not evaluated.

potential of avocado by-products and the importance of evaluating bioaccessibility when assessing functional ingredients. These results are consistent with previous studies reporting that INFOGEST digestion facilitates the release of phenols bound by plant matrices (Rocchetti et al., 2020; Tagliazucchi et al., 2010). These findings support the hypothesis that gastrointestinal conditions promote the release of bound phenolic forms, enhancing their solubility and detection. Consequently, the significant post-digestive increases observed in fortified bread samples, particularly for catechin, gallic acid, and ellagic acid, are likely due to improved bioaccessibility, consistent with the broader literature on polyphenol behaviour during digestion.

The phenolic profile assessed in this study mainly comprised plant-derived phenolic acids and flavonoids (e.g., chlorogenic, caffeic, p-coumaric acids; quercetin and kaempferol derivatives), which primarily represent substrates and central intermediates rather than the typical end products of LAB-driven catabolism. Classic microbial metabolites of phenolics (e.g., decarboxylated or reduced derivatives such as 4-vinyl phenols or dihydrocaffeic acid) were not specifically targeted in our analytical panel. Therefore, the absence of such compounds in our data set likely reflects the targeted nature of the method rather than the lack of microbial metabolism of phenolic substrates.

3.7. Volatile organic compounds emitted from baked breads

The volatile organic compounds profile was comprehensively analyzed in AWP before addition to doughs and in all bread trials (Table 7). In CTR, the identified VOCs included alcohols, aldehydes, carboxylic acids, furans, and ketones. Among these, carbonyl compounds were the most abundant, with hexanal, heptanal, and benzaldehyde being particularly prominent. Phenylethanol emerged as the dominant alcohol, while 2-pentylfuran, a typical Maillard reaction product (Martínez-Anaya, 1996), was also detected. The VOC profile of CTR was consistent with previous findings in similar bread formulations (Chulibert et al., 2024; Gaglio et al., 2021; Giannone et al., 2018), confirming the reliability of the analytical approach.

In contrast, the AWP-enriched breads exhibited a more complex and diverse VOC profile. Terpenes, sesquiterpenes, and additional carboxylic acids, absent in CTR, were identified, clearly originating from the AWP ingredient. Notably, sesquiterpenes accounted for 55% of the total VOCs in AWP and were present in significant proportions in the enriched breads: 18.7% in AWP5 and 31.4% in AWP10. Among these, α-caryophyllene and bergamotene were the most abundant, aligning with their known presence in avocado tissues (Galvão et al., 2016). Furthermore, the detection of oleic and palmitic acids in both AWP and AWP-enriched breads highlights the contribution of avocado seed components to the lipid profile. Palmitic acid was particularly notable, representing 18.6% of total VOCs in AWP and appearing at 8.91% and 10.07% in AWP5 and AWP10, respectively. These findings are consistent with previous reports on the fatty acid composition of avocado seeds (Soledad et al., 2021).

Overall, the incorporation of AWP significantly altered the volatile profile of breads, with a proportional increase in terpenes, sesquiterpenes, and carboxylic acids observed in AWP10. Terpenes are commonly found in fruit-enriched bakery products (Garofalo et al., 2024), and their presence in AWP-enriched breads may contribute to enhanced aromatic complexity. Additionally, the inclusion of avocado seeds introduces lipophilic compounds that not only enrich the sensory characteristics of the bread but may also offer functional benefits. This compositional enhancement may potentially influence consumer perception and acceptance, as aroma plays a crucial role in food quality and preference. The enriched VOC profile, particularly in AWP10, suggests that AWP can serve as a valuable ingredient in functional bakery products, aligning with current trends in sustainable food innovation and valorization of agro-industrial by-products (Bett-Garber & Lea, 2013; Du & Rouseff, 2014; Muchlinski et al., 2020).

3.8. Sensory evaluation

The sensory evaluation of the three bread samples revealed that the incorporation of AWP significantly influenced several organoleptic attributes (Fig. 3). Both AWP5 and AWP10 breads exhibited higher crust and crumb color scores compared to the control (CTR), reflecting the natural pigments present in avocado peel and seed components. Aroma intensity and taste persistency were also enhanced in AWP-enriched breads, which is consistent with the enriched volatile profile observed in these samples. In particular, the presence of terpenes and sesquiterpenes, absent in CTR but abundant in AWP, is likely associated with the more complex aromatic notes detected by the panel (Garofalo et al., 2024).

However, the sensory panel also reported increased perception of atypical odor, astringency, and bitterness, especially in AWP10. These undesirable attributes may be associated with the VOC analysis, which

Table 7
Volatile organic compound emitted from breads.

Compounds	Samples				SEM	*p* value
	AWP	CTR	AWP5	AWP10		
Aldehydes						
Heptanal	n.d.	6.44 a	4.92 a	2.58 b	0.44	0.006
Benzaldehyde	1.09	5.95 a	4.84 ab	3.46 b	0.28	0.007
Benzacetaldehyde	n.d.	4.72 a	4.50 a	1.96 b	0.33	0.001
2-Octenal	n.d.	2.35 a	1.22 b	0.88 b	0.17	0.001
Nonanal	2.99	4.71 a	6.51 a	5.11 a	0.21	0.072
Decanal	0.93	2.54 a	2.09 ab	1.65 b	0.11	0.026
Nonadienal	n.d.	0.91 a	n.d. b	n.d. b	0.12	<0.0001
Hexanal	n.d.	17.65	10.43	8.87	1.01	0.001
Hexadecanal	1.25	n.d. b	n.d. b	1.06 a	0.13	<0.0001
Undecanal	5.26	n.d. c	1.92 b	2.43 a	0.26	<0.0001
Trans-2-decenal	5.04	n.d. b	2.27 a	3.05 a		<0.0001
Carboxylic acids						
Benzoic acid	n.d.	2.51 a	n.d. b	n.d. b	0.30	<0.0001
Octanoic acid	1.63	2.38 a	1.95 a	1.04 b	0.15	0.004
Nonanoic acid	n.d.	4.49 a	1.91 b	1.74 b	0.33	0.001
Decanoic acid	n.d.	5.08 a	3.25 b	2.95 b	0.25	0.002
Oleic acid	3.86	n.d. c	2.36 a	1.06 b	0.24	<0.0001
Palmitic acid	18.6	n.d. bc	8.91 a	10.07 a	1.17	0.001
Alcohols						
1-Octanol	n.d.	1.61 a	n.d. b	n.d. b	0.19	<0.0001
Phenethyl alcohol	n.d.	19.97 a	12.09 b	10.56 b	1.12	0.003
3-Methyl-1-butanol	n.d.	7.74 a	3.43 b	2.43 b	0.60	0.001
Ketones						
Acetophenone	n.d.	1.70 a	n.d. b	n.d. b	0.20	<0.0001
Furans						
2-Pentylfuran	n.d	9.26 a	6.73 b	4.93 b	0.43	0.002
Terpenes						
α-Pinene	1.49	n.d. c	1.04 b	1.76 a	0.19	<0.0001
Limonene	2.95	n.d. b	0.93 a	1.00 a	0.12	<0.0001
Sesquiterpenes						
α-Cubebene	5.16	n.d. c	1.66 b	3.25 a	0.34	<0.0001
γ-Cadinene	1.91	n.d.	n.d.	n.d.	n.e.	n.e.
α-Copaene	4	n.d. c	1.77 b	2.92 a	0.30	<0.0001
Germacrene	1.24	n.d. b	n.d. b	0.91 a	0.11	<0.0001
α-Caryophyllene	15.75	n.d. c	6.17 b	9.54 a	1.00	<0.0001
β-Caryophyllene	3.01	n.d. b	n.d. b	1.60 a	0.19	<0.0001
Bergamotene	12.3	n.d. c	5.04 b	8.57 a	0.89	<0.0001
Farnesene	1.05	n.d.	n.d.	n.d.	n.e.	n.e.
Cadrene	3.03	n.d. c	1.25 b	1.64 a	0.18	<0.0001
α-Bisabolene	0.92	n.d.	n.d.	n.d.	n.e.	n.e.
β-Bisabolene	4.92	n.d. b	2.81 a	3.05 a	0.36	<0.0001
Muurolene	1.66	n.d. b	n.d. b	0.92 a	0.11	<0.0001

Data are reported as percentage (peak area of each compound/total area of significant peaks) x100 of six determinations (performed in duplicate for three independent productions). Abbreviations: AWP, avocado waste powder CTR-bread, bread produced; AWP5 experimental bread enriched with 5% (w/w) of AWP; AWP10 experimental bread enriched with 10% (w/w) of AWP; n.d., not detected; n.e. not evaluated. On the row: a, b, c = $p < 0.05$.

revealed a proportional increase in sesquiterpenes and carboxylic acids at higher AWP inclusion levels. Sesquiterpenes such as α-caryophyllene and bergamotene, while contributing to aromatic complexity, can impart resinous or herbal notes that may be perceived as off-flavors. Similarly, the detection of oleic and palmitic acids, originating from avocado seeds, may influence mouthfeel and bitterness. This interplay between volatile composition and sensory perception highlights the dual effect of AWP: enhancing aroma complexity while introducing challenging flavor notes at higher concentrations.

Overall acceptability scores confirmed these trends: CTR bread achieved the highest rating, while AWP5 offered a favourable compromise, improving color and aroma without substantially reducing consumer appeal. In contrast, AWP10, despite its richer volatile profile, introduced off-flavors that negatively impacted acceptance. These findings underscore the importance of optimizing AWP concentration to balance functional benefits with sensory quality. Although the incorporation of AWP may slightly reduce overall acceptability, the growing consumer interest in functional and sustainable foods supports further development of such formulations. Future strategies could include refining AWP processing to mitigate bitterness or combining AWP with complementary ingredients to mask off-notes, thereby maximizing the potential of avocado by-products in innovative bakery products (Marra et al., 2024; Zarzycki et al., 2024).

4. Conclusions

This study supports the strategic goals of the National Recovery and Resilience Plan (NRRP), demonstrating how the AWP production from fruit waste and its use can contribute to sustainable food innovation. Despite initial microbial diversity in raw ingredients, fermentation effectively selected beneficial lactobacilli, with AWP addition potentially favoring their growth. Technologically, AWP at 5% and 10% did not compromise bread quality, while enhancing crumb structure and visual appeal. Sensory analysis revealed a characteristic color, aroma, and taste persistence in AWP supplemented breads, though higher AWP levels introduced off notes. Volatile profiling confirmed the presence of terpenes and fatty acids from AWP, enriching aromatic complexity. Phenolic analysis showed significantly increased polyphenol content and bioaccessibility post-digestion, especially in AWP10, highlighting its functional potential. Analyses related to nutrient bioaccessibility were performed on digested bread, as they require simulated gastrointestinal conditions, whereas VOC profiling and sensory assessment were

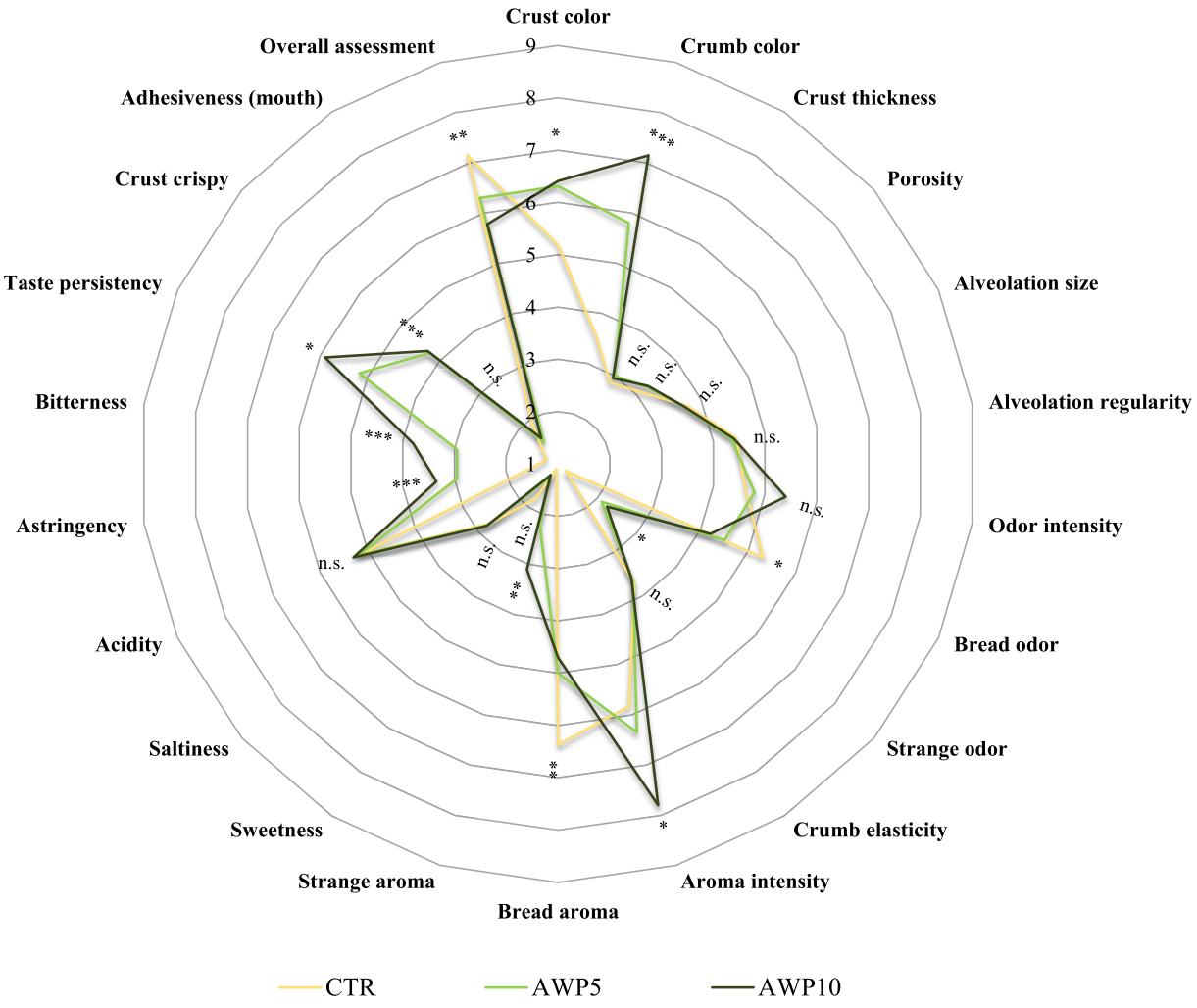

Fig. 3. Spider diagrams of descriptive sensory analysis of breads. Abbreviations: CTR, control bread; AWP5, experimental bread containing 5% (w/w) of Avocado Waste Powder (AWP); AWP10, experimental bread containing 10% (w/w) of AWP. *p* value: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; n.s., not significant.

conducted on the undigested product because these attributes are expressed prior to digestion and directly reflect technological and consumer-relevant qualities. Overall, AWP supplementation enhances nutritional and aromatic profiles without major technological drawbacks, aligning with circular economy principles and offering a promising route for valorizing agri-food waste in functional bakery products.

CRediT authorship contribution statement

Raimondo Gaglio: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Marcella Barbera:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Lino Sciurba:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Angela D'Amico:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Elena Franciosi:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Carla Buzzanca:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Vittorio Farina:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Vita Di Stefano:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ilenia Tinebra:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Luca Settanni:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2026.118346.

Data availability

Data will be made available on request.

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