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Reuse of almond by-products: Scale-up production of functional almond skin added semolina sourdough breads

Enrico Viola [a], Natale Badalamenti [b,c], Maurizio Bruno [b,c,d], Rosa Tundis [e], Monica Rosa Loizzo [e], Giancarlo Moschetti [a,c], Francesco Sottile [d,f], Vincenzo Naselli [a], Nicola Francesca [a], Luca Settanni [a,*], Raimondo Gaglio [a]

[a] Dipartimento Scienze Agrarie, Alimentari e Forestali, Università degli Studi di Palermo, Viale delle Scienze 4, 90128 Palermo, Italy
[b] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Viale delle Scienze, Palermo, 90128, Italy
[c] NBFC, National Biodiversity Future Center, Palermo, 90133, Italy
[d] Centro Interdipartimentale di Ricerca "Riutilizzo bio-based degli scarti da matrici agroalimentari" (RIVIVE), Università di Palermo, Italy
[e] Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036 Rende CS, Italy
[f] Department of Architecture, University of Palermo, Viale delle Scienze, Ed. 14, 90128, Palermo, Italy

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ABSTRACT

The present work reports the application of powdered almond skin (PAS) for industrial bread production. Three trials were conducted involving seven bread shapes, including control production (CTR), and two experimental productions with PAS addition [5–10 % (w/w), 5-PAS and 10-PAS, respectively]. Sourdough inoculum determined the acidification of all doughs and the levels of lactic acid bacteria increased. Spore-forming aerobic bacteria, members of the Enterobacteriaceae family, and total coliforms were not detected until the end of the fermentation process. PAS addition determined a lower weight loss, an increase of firmness, a diminution of specific volume, and a different sensory profile of the breads. Mafalda was the most appreciated bread shape and was subjected to photothermal aging. 10-PAS sample, after nine-day stress, still showed a significant total phenolic compound TPC content (111.0 mg GAE/g extract). The radical scavenging potential increased with PAS with a final IC_{50} of 103.3 μg/mL in 10-PAS breads. Experimental breads exhibited a notable enhancement in protection against lipid peroxidation. Mold-free shelf life assessment showed a 10-day shelf life for CTR breads, while a 12-day shelf life in presence of PAS. Collectively, the data suggest that PAS holds significant promise as a functional additive for industrial production of bread.

1. Introduction

Food industry by-products and agri-food waste are being currently used as functional food ingredients (Melini et al., 2020) thanks to their bioactive compounds, such as antioxidant and antimicrobial agents (Zainal Arifin et al., 2023). Upcycling of waste and by-products through novel food productions generates final products with a high added value (Mirosa and Bremen, 2023). To this purpose, fruit by-products represent most raw materials used to produce some functional foods (Comunian et al., 2021). Several works are available on the health properties of fruit by-products (Das et al., 2021; Wall-Medrano et al., 2020; Teshome et al., 2023) and foods processed with their inclusion, such as fruit-based beverages, cheese, and, especially, baked goods (Gaglio et al., 2021; Rodríguez et al., 2021; Gómez and Martinez, 2018). Among fruit derived by-products, nut peel is particularly rich in phenolic compounds (Martínez et al., 2010) exerting health promoting effects (Barreca et al., 2020; Loizzo et al., 2021) and, for this reason, applied in bakery (Barreira et al., 2019; Bartkiene et al., 2021; Pasqualone et al., 2018; Gaglio et al., 2023).

Bread is a staple food in many countries (Lockyer and Spiro, 2020) contributing consistently to the daily energy and nutrient intake (FAO, 2017). Due to the low levels of essential amino acids, among all lysine (Dhinda et al., 2011), bread is historically subjected to the fortification process to enhance its nutritional value (Settanni, 2017). As a matter of fact, people are quite accustomed to seeing other ingredients in addition to the four common basic ingredients of bread: wheat or rye flour, water, salt, and a leavening agent. As a result, bread consumers may become acquainted with functional breads that are made by incorporating fruit

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

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by-products during processing.

Gaglio et al. (2023) focused on the functionalization of traditional semolina sourdough bread with almond skin, demonstrating that powdered almond peel allowed a consistent release of phytochemicals from enriched breads, after *in vitro* simulated digestion. Thus, the work showed a high potential of almond skin added breads to provide antioxidant protection in human intestine. However, the work also evidenced serious hygienic issues of almond skin used in bread production, mainly due to the presence of detectable levels of Enterobacteriaceae, especially coliforms, in doughs, *Bacillus* operational taxonomic units within total DNA of doughs, and aerobic bacterial spores after baking. Spore-forming bacilli, such as *Bacillus cereus, B. subtilis* and *B. licheniformis* can be opportunistic human pathogens (Stenfors Arnesen et al., 2008; Santamarta et al., 2021; Kirschner and von Holy, 1989). When bacilli are also agents of wheat bread ropiness (Thompson et al., 1993), they are hardly responsible for health issues due to the slimy appearance of the breads, but diarrhoea and vomiting have been associated to the consumption of products containing *B. subtilis* and *B. licheniformis* with no appreciable ropiness (Rosenkvist and Hansen, 1995).

Indeed, before testing the functional potential of almond skin (Gaglio et al., 2023), this by-product was simply considered a waste by the producing factory. Thus, it was discharged outside the production facility where it was piled up, left uncovered and exposed to any environmental contamination. As part of this study, we made adjustments to the peeling process and successfully incorporated PAS into bread production at an industrial level. Hygienic aspects, physicochemical characterization, sensory measurements and impact of shelf life on phytochemical content and health properties were evaluated on the final breads in order to validate the reuse of almond by-products at bakery level.

2. Materials and methods

2.1. Production of almond skin powder

The almond factory "Bongiovanni Almonds s.r.l.", located in Mazzarino (CL, Italy), faced PAS hygiene issues, firstly through the modification of the peeling line (Fig. 1). Modifications included the collection of almond skin inside the facility; the run of the skin after almond peeling was reduced with a shorter stainless-steel pipe and the piling up occurred into sanitized stainless-steel containers. The entire peeling system, including the discharging pipe and the containers were treated with an oxidizing agent for food industries, specifically 0.50 % (*v/v*) peracetic acid water solution ACIPER 5 (Golmar Italia S.p.A., Torino, Italy) applied at room temperature for 30 min.

Peeling of almonds from the cultivars "Genco" and "Tuono" was obtained after blanching the skin covered seeds at 95 °C for 3 min. Wet skin of each cultivar was collected separately, transferred into polyethylene bags for food matrices and kept frozen (−20 °C) until use.

Almond skin from the two almond cultivars was mixed (50 % each) and the resulting mass was dried in a natural convection laboratory oven (mod. E34 WTB-Binder, Tuttlingen, Germany) at 54 °C until constant weight. A layer of skin biomass weighing 2.5 kg/m^2 was evenly spread across the oven tray. The residual activity water (a_w) of dried skin was measured with the Rotronic Hygropalm HC2-AW (Rotronic AG, Bassersdorf, Switzerland). The Fritsch Mill Pulverisette 14 centrifugal apparatus (Fritsch GmbH, Idar-Oberstein, Germany) was used to mill dried almond skin at 250 μm. Finally, PAS from the two almond cultivars were stored under dark at room temperature in sterile PolySilk®

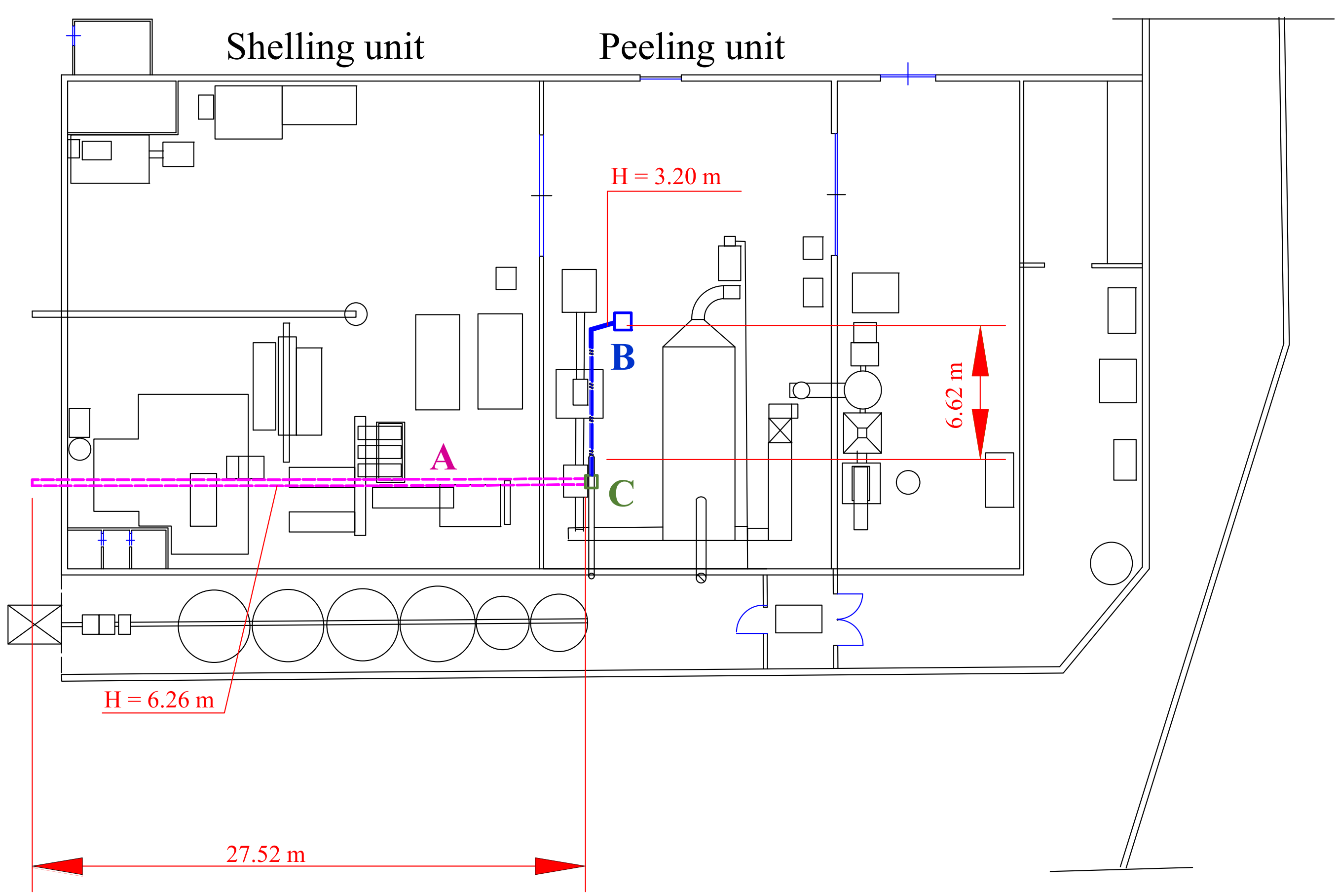

Fig. 1. Schematic view of the almond processing plant: A, almond skin exit pipe before plant modification; B, almond skin exit pipe after plant modification; C, by-pass and inspection/cleaning valve.

BagLight® 400 bags (Interscience, Saint Nom, France).

2.2. Bread making

Three bread making recipes (Table 1) were realized in the industrial plant of the bakery "Ori di Sicilia" (Mazzarino, CL, Italy) using the commercial Linea Rossa semolina (Molino di Mino s.r.l., San Cataldo, Italy) and a mix (50:50) of PAS from the cultivars Genco and Tuono: CTR, control dough without PAS addition; 5-PAS, dough prepared with 5 % (*w/w*) PAS on the weight of semolina; 10-PAS, dough prepared with 10 % (*w/w*) PAS on the weight of semolina. The fermenting agents used for leavening were a 30-year artisanal sourdough starter propagated in the same bakery; and the commercial baker's yeast Maestro Lievito (AB Mauri Italy S.p.A., Casteggio, Italy). Cooking salt was added because this ingredient is typical for breads produced in south Italy and tap water was used for kneading. To this purpose, all ingredients were put in the mechanical stainless steel AISI 304 mixer mod. Twist 80S (Sottoriva S.p.A., Marano Vicentino, Italy) equipped with a spiral hook. Kneading was obtained applying the following program: reverse rotation in first speed for 2 min; first speed spiral rotation for 10 min; second speed spiral rotation for 2 min. Seven 200 g traditional bread shapes of central Sicily (Ciambellina, Galletto, Sfilatino, Lunetta, Pagnottella, Mafalda and Chiocciolina) were manually realized by the bread maker. Leavening occurred in the Maturpan s. 180 chamber (Colip S.r.l., San Vincenzo Galliera, Italy) at 28 °C with 80 % relative humidity for 60 min. Bread baking was performed in a rotating rack oven mod. RT 80 rotor (Aldegheri Forni s.r.l, San Bonifacio, Italy) at 220 °C for 18 min, preceded by an initial steam exposure for 5 s. Four independent bread productions (experimental replicates) were performed at 3-week distance in triplicate (technical repeats).

2.3. Analysis of sourdough and bread doughs

Sourdough starter (10 kg) was propagated by daily back-slopping with 20 kg of tender flour type "0" (Industria Molitoria Denti s.r.l., Vicofertile, Italy) and 9 L of tap water at the dough yield (DY = weight of the dough/weight of semolina × 100) 195. Kneading was performed in the same mixer reported for bread dough production for 20 min at the first speed spiral rotation. Sourdough was fermented at 25 °C for 3 h and then kept under refrigeration until its use in bread production (generally 18 h). Sourdough starter just before addition in bread making (for each of the four independent productions) was subjected to pH measurement by the portable pH-meter Russell RL060P (Thermo Fisher Scientific, Beverly, MA, USA). Sourdough samples (150 g) were also aseptically collected, put into 200 mL volume sterile cups (Anicrin, Scorzé, Italy) and transported in a portable fridge containing reusable ice packs to the laboratory of Agricultural Microbiology of University of Palermo where they were analysed for total titratable acidity (TTA), LAB and yeasts cell densities following the methodology reported by Gaglio et al. (2023). Lactic and acetic acid concentrations were determined by high performance liquid chromatography (HPLC) as reported by Gaglio et al. (2020). All media, supplement and anaerobic kit were purchased from Oxoid Basingostoke, UK). Microbiological analyses were performed in duplicates for each production and the results expressed as Log colony forming units (CFU)/g. Physicochemical and microbiological analyses were also performed on bread doughs following the same methodology applied on sourdough samples as described by Gaglio et al. (2023).

Table 1
Dough recipes.

Dough (5 kg)	Composition					
	Semolina (g)	PAS (g)	Sourdough (g)	Baker's yeast (g)[a]	Salt (g)[a]	H_2O (mL)
CTR	2857.5	0	714	50	60	1428.5
5-PAS	2714.5	143	714	50	60	1428.5
10-PAS	2571.5	286	714	50	60	1428.5

Abbreviations: PAS, powdered almond skin; CTR, control dough; 5-PAS, experimental dough enriched with 5 % (*w/w*) of PAS; 10-PAS, experimental dough enriched with 10 % (*w/w*) of PAS.

[a] g added to the 5 kg-dough.

2.4. Hygienic characteristics of almond skin powder, doughs, and breads

PAS, doughs at the end of fermentation and breads were microbiologically investigated for some undesired groups. All samples were analysed for spore-forming aerobic bacteria. To this purpose, 25 g of each PAS, dough and bread sample were serially diluted in Ringer's solution. Following the methodology described by Messina et al. (2019), all cell suspensions were heated for 15 min at 85 °C and 0.1 mL were then spread onto Nutrient Agar (NA) (Oxoid) and incubated at 32 °C for 48 h. Further PAS and dough sample aliquots (10 g) were homogenized as reported above and investigated for the levels of TMM as already described and for 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. Cell suspensions on both VRBGA and VRBA were pour plated on double-layered agar. Analyses were performed in duplicate.

2.5. Quality of breads

After 30 min from baking, the breads kept at the bakery at room temperature were weighted to calculate weight loss (WL) (Purlis and Salvadori, 2007) as follows:

$$\mathrm{WL} = \left(\frac{\text{weight of dough (g)} - \text{weight of bread (g)}}{\text{weight of dough (g)}}\right) \times 100.$$

All breads were then transferred into paper bags and transported at ambient temperature to the laboratories of Agricultural Microbiology. At arrivals, all breads were subjected to volume determination using a volumeter for bakery products (ErreCi s.r.l., Merate, Italy) applying rapeseed replacement method of the American Association of Cereal Chemists method 55–50.01 (AACC, 2000). Firmness evaluation was conducted by means of the Instron-5564 (Instrom Corp., Canton, MA, USA) that measures the resistance to compression (N/mm^2). The analysis of crust and crumb colour, void fraction (fraction of total area of bubbles), cell density (number of cells/cm^2), and mean cell area (in mm^2) was conducted following the methodologies described by Viola et al. (2023).

2.6. Sensory analysis

The breads produced at the industrial facility were transported within 2 h from baking at the Department of Agriculture, Food and Forestry Sciences – Laboratory of Sensory Evaluation – University of Palermo to perform a descriptive sensory analysis. To this purpose, 22 judges were recruited; the evaluation panel included 13 women and nine men, and their ages ranged from 23 to 66. After training to acquire familiarity with bread attributes using a commercial bread, the panel participated to the tasting section using PAS added breads produced in this work. Two-centimetre thick slices were cut from each bread (Panirani et al., 2023), approximately 5 min before tasting and presented on plastic plates with three-digit codes at room temperature (Moretton et al., 2023). The panellists did not visualize the entire bread shapes before tasting. The panel was asked to judge appearance, texture, odour and taste, descriptors of breads (Comendador et al., 2012; Martins et al., 2015; Rodrigues et al., 2014) expressing a score on a 9-point scale (1 = extremely bad; 9 = extremely good) for each attribute. A general assessment of the breads considering the scores of all attributes evaluated was also provided. ISO 13299 (2003) guidelines were followed for conducting sensory tests which were performed in single chambers.

2.7. Visual preference

In order to select the most appreciated shapes for high-volume industrial production, 100 untrained consumers (mainly University students) were asked to provide an independent personal visual preference on the bread shapes exclusively based on their empathy, consumption habits, and convenience of use (e.g., dressing with sliced ham or cheese). The judges were asked to express a preference value, from 1 (least preferred) to 10 (most preferred), for purchasing each bread shape.

2.8. Degradation testing and extration procedures

Tests were performed only on the Mafalda samples, breads with a more particular shape, presenting a greater surface area than the other samples and that obtained the highest score in the preference test.

2.8.1. Photostability test

Photostability test was simulated by UV–visible ray irradiation using SUNTEST XLS +II (Atlas®, URAI, Assago, MI, Italy) for 24 h; SUNTEST instrument was set up in according to standard European procedures (UNI EN ISO 7730:2006), with the following parameters: time: 4.5 h corresponding to 216 h solar light; irradiation control: 300 - 800 nm; irradiation (W/m^2): 750; room temperature: 18 - 26 °C; black standard temperature (BST): 45 °C; humidity: 45 – 65 %. Samples of almond skin, and Mafalda breads (CTR, 5-PAS, and 10-PAS) were taken from the photothermal chamber at 1.5 h, 3 h, and 4.5 h corresponding, respectively, to 3, 6, and 9 days of solar light exposition. One sample each of PAS and Mafalda breads (CTR, 5-PAS, and 10-PAS) was not subjected, instead, to thermophotometric stress (day 0).

2.8.2. Extraction procedure on almond skin and Mafalda bread

To evaluate the antioxidant power, a slightly modified extraction procedure reported by Sicari et al. (2023) was used. One gram of almond skin and enriched bread (control, 5-PAS, and 10-PAS), already subjected to photothermal stress, were extracted using ultrasound-assisted extraction in hydroalcoholic ethanol solution ($EtOH/H_2O$ 80:20) in a 1:3 ratio (sample:solvent) (3 cycles, ultrasonic frequency of 35 kHz) in a water bath (Branson 5200, Milan, Italy) at room temperature for 30 min. Before the analysis, the samples were filtered through a PTFE 0.45 μm Millipore filter (BGB, USA) and freeze-dried.

2.8.3. Evaluation of total phenol content in almond skin and Mafalda bread

For the evaluation of total phenol content (TPC) the methodology previously published was applied (Loizzo et al., 2021). Briefly, extract was mixed with Folin–Ciocâlteu reagent, H_2O, and 5 % Na_2CO_3. Then the mixture was heated at 40 °C for 20 min, and the TPC was determined and expressed as mg gallic acid equivalent (GAE)/g extract.

2.9. Evaluation of antioxidant activity on Mafalda bread

To perform 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activities the methodologies previously described was adopted (Loizzo et al., 2021). Briefly, in DPPH test, sample at different concentrations (1–1000 μg/mL) were mixed with DPPH solution. The bleaching of DPPH was spectrophotometrically determined at $\lambda = 517$ nm.

To perform ABTS assay a solution of ABTS radical was prepared and left in the dark for 12 h. Sample at different concentrations (1–400 μg/mL) were mixed with diluted ABTS solution. After 6 min of incubation at 25 °C the absorbance was measured at $\lambda = 734$ nm. Ascorbic acid was used as the positive control in both assays.

The protection of lipid peroxidation was assessed using β-carotene bleaching test (Loizzo et al., 2021). Briefly, Tween 20, β-carotene and linoleic acid were mixed with samples at different concentration (5–100 μg/mL) after 30- and 60-minutes incubation at 45 °C the absorbance was read at $\lambda = 470$ nm. Propyl gallate was used as a positive control.

2.10. Mold-free shelf life test

Mafalda breads were tested for fungal appearance as described by Syrokou et al. (2022). For this specific test, the breads were cooled at room temperature for 3 h and, then, sliced. Each slice of approximately 20 g was packed into sterile BagLight® 400 Multilayer® bags (Interscience, Saint Nom, France), made in PolySilk®, thermally sealed with a hot bar (Laica VT3112, Vicenza, Italy). All packed slices were incubated at 25 °C (Ju et al., 2020) and daily monitored for detecting visible mold presence. The experiment was performed in triplicate.

2.11. Statistical analysis

Physicochemical and microbiological parameters of doughs were analysed statistically using the generalised linear model (GLM) procedure in SAS 9.2 (SAS, 2010) to evaluate the effect of time of fermentation (T: 0 and 1 h), trials (TR: CTR, 5-PAS and 10-PAS) and T*TR interaction. Quality attributes of different bread shapes were analysed by one-way variance analysis (ANOVA) using XLStat software version 7.5.2 for Microsoft Excel (Addinsoft, New York, NY, USA). The inhibitory concentration 50 % (IC_{50}) was calculated by using Prism GraphPad Prism version 4.0 for Windows (GraphPad Soft-ware, San Diego, CA, USA). Linear regression, assessment of repeatability, calculation of average, relative standard deviation (SD), and Pearson's correlation coefficient (r) were calculated by using Microsoft Excel 2010 software (Redmond, USA). The results were expressed as means of three different experiments ± SD.

3. Results and discussion

3.1. Physicochemical characteristics of sourdough and bread doughs and microbial evolution

Sourdough inoculum used for bread production was characterized by a pH value of 3.77 ± 0.03 and TTA 14.00 ± 0.5 ml NaOH 0.1 N. Lactic and acetic acid were 4.98 ± 0.27 and 1.24 ± 0.23 mg/g, respectively, giving a Fermentation Quotient (FQ = molar ratio between lactic and acetic acids) of 2.68. FQ indicates the impact of lactic and acetic acids on the aroma profile of dough (Francesca et al., 2019), and should be comprised in the range 1.5 - 4 to exert a positive influence on the aromatic/textural properties breads (Spicher, 1983), better yet in the restricted range 2.0 - 2.7 (Hammes and Gänzle, 1998). The levels of LAB in sourdough were 9.1 ± 0.3 Log CFU/g, while yeasts were more than three orders of magnitude lower (6.0 ± 0.2 Log CFU/g). The parameters registered for the sourdough used in this study are commonly displayed by sourdoughs propagated in Sicily region to produce traditional breads (Ventimiglia et al., 2015).

Physicochemical analysis of bread doughs is reported in Table 2. Except for $D + L$ lactic acid and acetic acid, which did not differ between trials, all other characteristics evaluated were influenced by the addition of PAS. However, no statistically significant differences ($p > 0.05$) were found for the interaction T*TR. In detail, the initial pH value of control trial was 5.16, while higher values were registered for PAS added doughs. The same trend was previously observed by Gaglio et al. (2023) who tested PAS addition in bread production carried out at laboratory scale level and explained the behaviour recorded with the pH of almond skin that is higher than that of common bread making raw materials, such as flour and semolina or their combinations (Alfonzo et al., 2013; Ruisi et al., 2021). After 1 h of fermentation the pH values of the same doughs decreased negligibly. A different observation was made for TTA that increased more consistently than pH diminution after fermentation. However, TTA values displayed by PAS added doughs, both at the beginning and at the end of fermentation, were higher than that of control dough, even though their pHs were higher. The elevated TTA observed in doughs with added PAS cannot be attributed to a higher density of LAB cells and their subsequent increased activity. Instead, it is

Table 2
Chemical parameters of control and PAS added doughs.

Parameters	t_0			t_1			*p* value		
	CTR	5-PAS	10-PAS	CTR	5-PAS	10-PAS	Time (T)	Trial (TR)	T*TR
pH	5.16 ± 0.04 c	5.55 ± 0.02 ab	5.65 ± 0.03 a	5.11 ± 0.06 c	5.48 ± 0.08 b	5.61 ± 0.05 a	0.045	< 0.0001	0.874
TTA	4.20 ± 0.15 c	4.50 ± 0.15 b	4.70 ± 0.10 b	4.70 ± 0.10 b	5.10 ± 0.10 a	5.30 ± 0.25 a	< 0.0001	0.0001	0.807
$D + L$ Lactic acid (mg/g)	0.44 ± 0.07 b	0.42 ± 0.04 b	0.43 ± 0.06 b	0.82 ± 0.14 a	0.69 ± 0.09 a	0.74 ± 0.10 a	< 0.0001	0.373	0.573
Acetic acid (mg/g)	0.10 ± 0.01 c	0.11 ± 0.02 c	0.10 ± 0.01 c	0.15 ± 0.02 b	0.15 ± 0.01 b	0.18 ± 0.02 a	< 0.0001	0.284	0.115
FQ	2.93	2.55	2.87	3.64	3.07	2.74	n.a.	n.a.	n.a.

Results indicate mean values ± S.D. (standard deviation) of twelve determinations (carried out in three technical repeats for four independent experiments). Data within a column followed by different small letters are significantly different. Data within a row followed by different letters are significantly different.
Abbreviations: CTR, control dough; 5-PAS, experimental dough enriched with 5 % (*w/w*) of Powdered Almond Skin (PAS); 10-PAS, experimental dough enriched with 10 % (*w/w*) of PAS; n.a., not analysed; TTA, total titratable acidity; FQ, Fermentation Quotient (molar ratio between lactic and acetic acids).

likely due to the acidic content present in almond by-products (Loizzo et al., 2021). By-products with a complex chemical composition show a buffering effect (Mamma et al., 2008) and a similar buffering effect responsible for the lower pH decrease can be supposed for PAS. A similar buffering behaviour was registered by Villanueva et al. (2018) who studied the impact of acidification and protein fortification on bread quality using starch from different sources. The increase of TTA value was also explained by the increase of both $D + L$ lactic acid and acetic acid concentrations. After barely 1 h of fermentation, the level of $D + L$ lactic acid almost doubled (from 0.44 to 0.82 mg/g) in CTR sample. Despite PAS doughs exhibiting a more modest increase in this compound, the rise remained in line with the increase in total titratable acidity (TTA). Further validation of this significance came from the analysis of variance. Acetic acid levels increased across all trials. However, while the control (CTR) and 5-PAS trials did not show a significant increase, the 10-PAS trial did, reaching the highest absolute value recorded at 0.18 mg/g. This organic acid can better explain the negligible pH decrease despite a more consistent TTA increase in all doughs; acetic acid contributes to TTA rather than altering pH (Galal et al., 1978). Molar ratio of lactic/acetic acid indicated that FQs of all doughs, before and after fermentation, as reported above, were within the range impacting positively the final breads. The result of the plate counts of the doughs are reported in Table 3. No statistically significant differences were observed for any of the microbial groups object of investigation to the T, TR and T*TR effects. Sourdough load was 8.56 ± 0.44 Log CFU/g of TMM. A higher level was detected on mMRS for presumptive LAB (9.10 ± 0.39 Log CFU/g), while yeasts accounted for more than three log cycles (6.10 ± 0.61 Log CFU/g) lower than LAB. After dough preparation following the recipes of Table 1, all doughs showed LAB in the range 7.39 - 7.64 Log CFU/g. Cell densities of yeasts were at the same order of magnitude of LAB (7.10 - 7.34 Log CFU/g) thanks to the addition of baker's yeast. After 1 h of fermentation LAB slightly increased for all doughs, while the increase of yeasts was more pronounced (until 7.80 Log CFU/g) in control dough. Gaglio et al. (2023) already stated the suitability of PAS with LAB fermentation and the present study clearly indicated that this almond by-product does not negatively influence the growth of yeasts when added in bread production.

3.2. Safety of almond skin and processed breads

In a previous study, Gaglio et al. (2023) registered hygiene issues related to the production of PAS due to the presence of members of Enterobacteriaceae family, including coliforms, and especially *Bacillus* spp. In particular, the latter species, due to the spore formation, can survive the baking process and were detected in the final breads. Based on the results of Gaglio et al. (2023), PAS, doughs, and the resulting breads (Ciambellina, Galletto, Sfilatino, Lunetta, Pagnottella, Mafalda, and Chiocciolina) were investigated for the presence of spore-forming aerobic bacteria, members of Enterobacteriaceae family and total coliforms. None of the samples analysed showed the presence of these groups (results not shown). These results clearly indicated the high efficiency of the implementations of the almond processing plant (Fig. 1) to ensure the hygienic safety of almond skin, keeping the main undesirable microbial groups below the detection limit. The hygiene characteristics of by-products are of paramount importance to convert these matrices form wastes to be disposed into profitable products to be marketed (Lau et al., 2021; Ravindran et al., 2016). Almond producers have successfully overcome this critical challenge, which serves as a fundamental milestone in the development of food ingredients for human consumption using almond by-products.

3.3. Quality attributes of the final breads

The seven bread shapes (Ciambellina, Galletto, Sfilatino, Lunetta, Pagnottella, Mafalda, and Chiocciolina) obtained without and with (5 and 10 %) PAS addition is reported in Fig. 2. Weight loss, specific volume, firmness, and colour are generally considered as main quality parameters of final breads (Keskin et al., 2004). These parameters were then measured for all seven bread shapes produced at industrial level in this work and their values are summarized in Table 4. The findings from Gaglio et al. (2023) on laboratory-scale bread-making experiments using PAS were corroborated in our study across nearly all bread shapes. PAS addition determined a lower weight loss, an increase of firmness and a diminution of specific volume. The results align with patterns observed in the study by Gómez and Martinez (2018). Their research revealed that incorporating escalating quantities of powdered vegetable by-products into baked goods led to a firmer crumb and decreased weight loss.

Table 3
Microbiological analysis of doughs.

Media	t_0			t_1			*p* value		
	CTR	5-PAS	10-PAS	CTR	5-PAS	10-PAS	Time (T)	Trial (TR)	T*TR
PCA	7.64 ± 0.39	7.51 ± 0.42	7.39 ± 0.32	7.78 ± 0.41	7.75 ± 0.34	7.51 ± 0.37	0.367	0.493	0.957
mMRS	7.44 ± 0.43	7.76 ± 0.31	7.61 ± 0.40	7.99 ± 0.46	7.91 ± 0.39	7.73 ± 0.51	0.194	0.786	0.626
YPD	7.10 ± 0.43	7.27 ± 0.35	7.34 ± 0.40	7.80 ± 0.44	7.75 ± 0.49	7.72 ± 0.37	0.101	0.9419	0.796

Results indicate mean values ± S.D. (standard deviation) of twelve plate counts (carried out in three technical repeats for four independent experiments). Data within a column followed by different small letters are significantly different. Data within a row followed by different letters are significantly different.
Abbreviations: PCA, plate count agar; mMRS, modified de Man, Rogosa, and Sharpe agar; YPD, yeast extract peptone dextrose agar; CTR, control dough; 5-PAS, experimental dough enriched with 5 % (*w/w*) of Powdered Almond Skin (PAS); 10-PAS, experimental dough enriched with 10 % (*w/w*) of PAS.

Fig. 2. Different bread shapes processed with powdered almond skin at industrial level: A, Ciambellina; B, Galletto; C, Sfilatino; D, Lunetta; E, Pagnottella; F, Mafalda; G, Chiocciolina.

Similarly, Yao et al. (2021) documented enhanced firmness in fortified breads post-baking, likely due to the influence of these by-products on gluten network formation. The differences with control trials were consistently higher for trials 10-PAS. The highest weight loss was registered for trial CTR of Galletto shape (23.49 %), while the lowest weight loss was displayed by trial 10-PAS of Chiocciolina shape (11.22 %). The statistical analysis of this parameter revealed a clear separation between the control and the two PAS-added treatments for three of the seven shapes (Pagnottella, Mafalda and Chiocciolina), while for the other three shapes (Ciambellina, Galletto and Sfilatino), the 5-PAS treatment was statistically grouped in a middle position between the control and the 10-PAS ones. The only bread shape that did not exhibit statistically significant differences between samples was Lunetta. The last trial (10-PAS Chiocciolina) also showed the highest firmness (0.02053 N/mm^2), while the lowest value (0.01222 N/mm^2) for this parameter was found for trail CTR of Ciambellina shape. With regard to the firmness attribute, the statistical trend observed across the various shapes was consistent, with the highest values recorded for 10-PAS and the lowest for the control treatment. The shape of bread also influenced specific volume with the highest value recorded for trial CTR of Mafalda shape (3.77 cm^3/g) and the lowest (2.53 cm^3/g) for both Mafalda and Chiocciolina bread shapes. The statistical analysis of variance revealed a comparable pattern for the previously examined parameters. In detail, five out of seven shapes (Ciambellina, Galletto, Lunetta, Mafalda and Chiocciolina) exhibited a clear statistical separation between the control and the PAS-added treatments. In contrast, the remaining two shapes (Sfilatino and Pagnottella) demonstrated that the 5-PAS treatments exhibited specific volume values in a middle statistical position between the control and the 10-PAS treatments. Furthermore, the addition of PAS determined a change in the colour parameters of both the crust and crumb of the breads. With regard to the crust, the parameter regarding lightness (L*) showed a progressive decrease in values, indicating a darkening of the crust with the percentage increase in PAS addition. A similar pattern was observed for the yellowness (b*) parameter, which recorded the highest values for the CTR of the Lunetta shape (38.04), while the lowest was registered for the 10-PAS of the Mafalda shape (22.22). The redness (a*) parameter did not show any statistically significant differences between treatments, with the exception of Chiocciolina shape breads, which exhibited the highest value for CTR (16.14) and the lowest for 5-PAS (12.31). The colour analysis of crumb revealed a similar trend to the crust regarding the L* parameter. In contrast to the crust analysis, the b* parameter did not demonstrate any statistically significant differences, while the a* parameter exhibited a consistent trend across all shapes, with higher and positive values observed for the 10 PAS treatments and negative values observed in the CTR treatments. Finally, these results showed that lightness of crust and crumb and yellowness of crust decreased with increasing percentages of PAS, while redness of crumb showed an opposite trend as previously reported by Gaglio et al. (2023).

3.4. Sensory evaluation and consumers' preference

The spider plots resulting from sensory evaluations of control and PAS added breads are reported in Fig. 3. The addition of both percentages of PAS to semolina undoubtedly generated final products with sensory traits quite distant from those of control breads, especially for crust and crumb colour, porosity, alveolation, bread odour, and aroma, odour and aroma intensity, taste persistence, bitter and astringency. In particular, the highest differences among trials were registered for crumb colour with a colour intensity increasing consistently from a score of 4.80 characterizing CTR until 8.26 in 10-PAS trial. In general, colour intensity of both bread sections analysed (crust and crumb) increased notably with PAS percentage added. A similar trend was registered for odour intensity, bitter, and taste persistency. Regarding aroma intensity, a consistent difference was observed among CTR and the other trials, but both 5- and 10-PAS trials were characterized by almost superimposable score (6.63 and 6.79, respectively). Indeed, similar findings were found for crispness of the crust, because CTR trial received a score of 4.78,

Table 4
Quality attributes of different bread shapes processed with PAS addition.

Bread shape	Weight loss (%)	Firmness (N/mm^2)	Specific volume (cm^3/g bread)	Crust colour			Crumb colour		
				L*	a*	b*	L*	a*	b*
Ciambellina									
CTR	22.45 ± 2.11 a	0.01222 ± 0.00136 b	3.37 ± 0.25 a	58.27 ± 3.01 a	13.91 ± 0.78	37.37 ± 2.11 a	75.95 ± 3.27 a	−2.12 ± 0.31 c	18.75 ± 1.51
5-PAS	21.24 ± 2.25 ab	0.01719 ± 0.00182 ab	2.73 ± 0.19 b	51.20 ± 2.42 b	13.21 ± 1.15	29.96 ± 1.72 b	54.66 ± 2.24 b	5.35 ± 0.70 b	17.88 ± 0.55
10-PAS	17.39 ± 1.61 b	0.01937 ± 0.00352 a	2.61 ± 0.12 b	44.57 ± 1.67 c	14.05 ± 1.02	24.22 ± 1.15 c	46.43 ± 2.56 c	8.26 ± 0.53 a	17.14 ± 1.14
p value	0.049	0.028	0.006	0.001	0.572	0.0001	< 0.0001	< 0.0001	0.295
Galletto									
CTR	23.49 ± 2.34 a	0.01356 ± 0.00212 b	3.42 ± 0.21 a	56.17 ± 2.75 a	14.50 ± 2.02	35.78 ± 2.56 a	75.30 ± 3.43 a	−2.09 ± 0.44 c	17.28 ± 0.32
5-PAS	20.42 ± 2.02 ab	0.01689 ± 0.00186 ab	2.90 ± 0.23 b	47.29 ± 2.10 b	14.86 ± 0.82	28.49 ± 0.95 b	53.95 ± 1.19 b	5.75 ± 0.91 b	17.89 ± 1.09
10-PAS	16.74 ± 1.73 b	0.01865 ± 0.00163 a	2.77 ± 0.15 b	46.44 ± 2.37 b	13.04 ± 1.41	24.70 ± 1.77 b	47.20 ± 1.75 c	8.48 ± 0.80 a	19.14 ± 1.43
p value	0.019	0.041	0.016	0.005	0.354	0.001	< 0.0001	< 0.0001	0.130
Sfilatino									
CTR	21.11 ± 2.09 a	0.01324 ± 0.00151 b	3.51 ± 0.26 a	53.57 ± 2.16 a	15.23 ± 0.77	34.47 ± 2.03 a	74.06 ± 2.20 a	−2.08 ± 0.39 c	18.51 ± 0.73
5-PAS	19.39 ± 2.21 ab	0.01712 ± 0.00159 ab	3.05 ± 0.18 ab	51.38 ± 2.34 ab	13.06 ± 3.03	29.57 ± 1.24 b	53.10 ± 1.45 b	5.86 ± 0.75 b	18.35 ± 0.60
10-PAS	16.03 ± 1.36 b	0.01839 ± 0.00257 a	2.93 ± 0.21 b	46.17 ± 1.84 b	13.00 ± 2.21	24.07 ± 0.69 c	46.98 ± 1.60 c	8.20 ± 0.97 a	18.56 ± 0.29
p value	0.045	0.040	0.039	0.013	0.425	0.0001	< 0.0001	< 0.0001	0.897
Lunetta									
CTR	21.74 ± 2.15	0.01248 ± 0.00204 b	3.69 ± 0.27 a	60.56 ± 3.09 a	13.16 ± 0.84	38.04 ± 3.30 a	74.75 ± 2.63 a	−2.09 ± 0.24 c	17.34 ± 0.37
5-PAS	19.65 ± 2.27	0.01563 ± 0.00174 ab	3.06 ± 0.23 b	52.34 ± 2.01 b	12.40 ± 1.13	29.28 ± 0.70 b	53.32 ± 1.69 b	5.66 ± 0.63 b	18.01 ± 1.19
10-PAS	17.26 ± 1.43	0.01710 ± 0.00110 a	2.79 ± 0.18 b	45.70 ± 1.93 c	12.73 ± 1.97	23.65 ± 0.96 c	47.53 ± 2.24 c	8.03 ± 0.86 a	18.57 ± 1.01
p value	0.085	0.037	0.008	0.001	0.807	0.0001	< 0.0001	< 0.0001	0.333
Pagnottella									
CTR	17.79 ± 1.99 a	0.01487 ± 0.00145 b	3.44 ± 0.19 a	51.20 ± 1.82	15.60 ± 0.53	32.50 ± 1.55 a	71.54 ± 3.09 a	−2.03 ± 0.40 c	18.64 ± 0.25
5-PAS	13.23 ± 1.33 b	0.01865 ± 0.00118 ab	3.05 ± 0.25 ab	50.98 ± 2.13	13.27 ± 2.12	29.36 ± 2.10 a	55.46 ± 0.93 b	5.36 ± 0.78 b	17.73 ± 0.33
10-PAS	11.70 ± 1.54 b	0.02014 ± 0.00183 a	2.86 ± 0.20 b	47.79 ± 1.59	12.97 ± 1.18	25.38 ± 0.61 b	46.81 ± 1.30 c	8.12 ± 0.71 a	18.52 ± 0.71
p value	0.010	0.013	0.041	0.116	0.123	0.004	< 0.0001	< 0.0001	0.110
Mafalda									
CTR	18.40 ± 2.04 a	0.01512 ± 0.00050 b	3.77 ± 0.24 a	48.71 ± 1.70 a	16.39 ± 1.61	32.31 ± 1.44 a	74.39 ± 2.51 a	−2.01 ± 0.19 c	18.83 ± 1.11
5-PAS	13.85 ± 1.57 b	0.01919 ± 0.00284 ab	2.86 ± 0.15 b	48.15 ± 2.55 a	14.34 ± 1.70	30.00 ± 1.13 a	56.59 ± 1.40 b	5.52 ± 0.54 b	18.55 ± 0.54
10-PAS	12.39 ± 1.68 b	0.02047 ± 0.00132 a	2.53 ± 0.13 b	41.68 ± 1.78 b	15.09 ± 0.89	22.22 ± 1.72 b	46.49 ± 0.92 c	8.38 ± 0.99 a	18.49 ± 0.30
p value	0.014	0.027	0.0001	0.010	0.288	0.0001	< 0.0001	< 0.0001	0.837
Chiocciolina									
CTR	18.09 ± 1.84 a	0.01428 ± 0.00269b	3.30 ± 0.20 a	50.23 ± 2.33 a	16.14 ± 2.16 a	33.14 ± 1.86 a	71.98 ± 3.13 a	−2.02 ± 0.36 c	18.42 ± 0.42
5-PAS	12.90 ± 1.63 b	0.01827 ± 0.00180 ab	2.82 ± 0.11 b	51.71 ± 2.25 a	12.31 ± 0.61 b	29.60 ± 3.05 ab	55.33 ± 1.22 b	5.87 ± 0.78 b	18.72 ± 0.24
10-PAS	11.22 ± 1.39 b	0.02053 ± 0.00161 a	2.53 ± 0.16 b	44.85 ± 1.80 b	13.91 ± 0.90 ab	24.73 ± 0.87 b	45.52 ± 2.05 c	8.27 ± 0.62 a	18.02 ± 0.78
p value	0.005	0.028	0.003	0.018	0.041	0.008	< 0.0001	< 0.0001	0.737

Results indicate mean values ± S.D. (standard deviation) of twelve determinations (carried out in three technical repeats for four independent experiments). Data within a column followed by different letters are significantly different. Abbreviations: CTR, control breads; 5-PAS, experimental breads enriched with 5 % (*w/w*) of Powdered Almond Skin (PAS); 10-PAS, experimental breads enriched with 10 % (*w/w*) of PAS.

while 5- and 10-PAS trials 5.25 and 5.36, respectively. Regarding porosity, alveolation, bread odour, and crust elasticity, an inverse trend was observed: the scores consistently decreased with increasing PAS percentage. Interestingly, the aroma of bread remained quite similar between the 5 % and 10 % PAS trials. Additionally, the scores for alveolation regularity, acidity, saltiness, and adhesiveness were highly similar across all trials. None of PAS added breads resulted sweet. Unexpectedly, 5-PAS breads, like CTR breads, were not scored astringent and were not characterized by strange odours, while 10-PAS breads received 2.85 score for astringency and 2.53 for strange odours. The panellists were also asked to score their overall judgement on the breads considering all attributes and their evaluation. Even though CTR breads received the highest scores, 5-PAS breads were generally highly appreciated, while 10-PAS breads were characterized by a consistently lower acceptability than CTR breads.

The previous work conducted on PAS added breads produced at laboratory scale level (Gaglio et al., 2023) reported a sensory evaluation limited to visual, texture and odour sensations, because the microbiological analysis of processed breads revealed the presence of spore forming bacteria. Independently on bread shape, the breads produced at

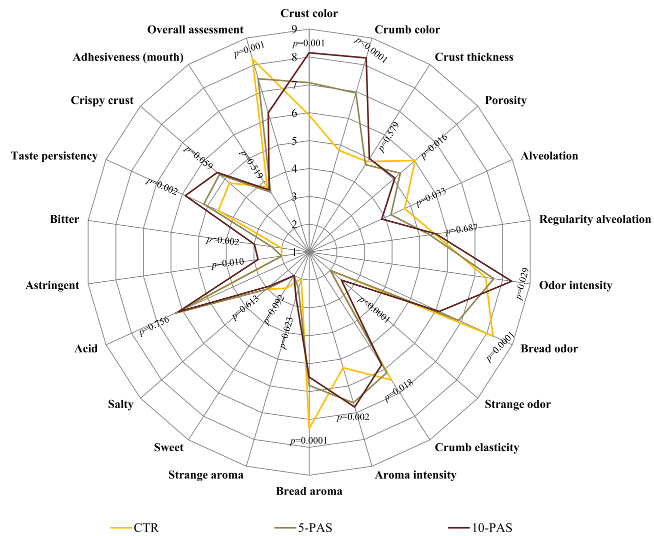

Fig. 3. Spider diagrams of descriptive sensory analysis of breads. Abbreviations: CTR, control breads; 5-PAS, experimental breads enriched with 5 % (*w/w*) of Powdered Almond Skin (PAS); 10-PAS, experimental breads enriched with 10 % (*w/w*) of PAS; *p, p* value.

the industrial facility in the present work confirmed the sensory trends registered for the experimental breads with the trapezoidal shape imparted by the stainless-steel baking pans of the dimensions indicated by the Method 10–10B of AACC (2000). This work also provided a full description of the taste attributes of PAS breads. The decrease of crumb porosity displayed by PAS containing breads is a general phenomenon observed when the percentage of flour/semolina decreases and is consequent to the reduction of dough gluten that determines the network of bubbles during dough rise (Mills et al., 2003; Rathnayake et al., 2018). The higher astringency and bitter observed after PAS addition is due to the phenolic compounds of the almond skin. The concentration of total phenols is generally associated to these attributes (Siliani et al., 2006) and phenolic compounds are responsible for bitter and astringent taste of several foods (Uğurlu et al., 2020). The higher crispness perception of PAS added breads can be considered a positive feature. Water absorption during baking causes the bread crust to lose its crispness (Meinders and van Vliet, 2011). This softening of the crust is linked to bread staling (Gao et al., 2015).

Based on previous results showed for experimental breads exerting antioxidant protection of human intestine (Gaglio et al., 2023), before inserting the new functional bread production line with PAS addition, the baking industrial facility required a preference study on the bread shapes to be successfully launched on the market. Considering the overall assessment of the descriptive sensory analysis, only 5-PAS breads were subjected to the preference test. The results of the visual preference conducted with 100 untrained consumers is reported in Fig. 4. This analysis undoubtedly showed the preference of the panel towards Mafalda and Sfilatino with an average score of 9.10 and 8.79, respectively. Interestingly, the Ciambellina, Lunetta, and Galletto bread shapes received notably low preference scores (ranging from 4.02 to 4.48). This suggests that introducing these shapes to the market would carry significant risk. The second stage of sensory evaluation is crucial for gauging the potential consumer interest in a specific food product, including bread (Lyon et al., 2012).

3.5. Total phenolic content (TPC) in almond skin and enriched Mafalda bread

Several research evidenced that almond skin is rich of bioactive phytochemicals particularly phenols (Bolling, 2017). Total phenolic content was evaluated on almond skin and Mafalda breads subjected to

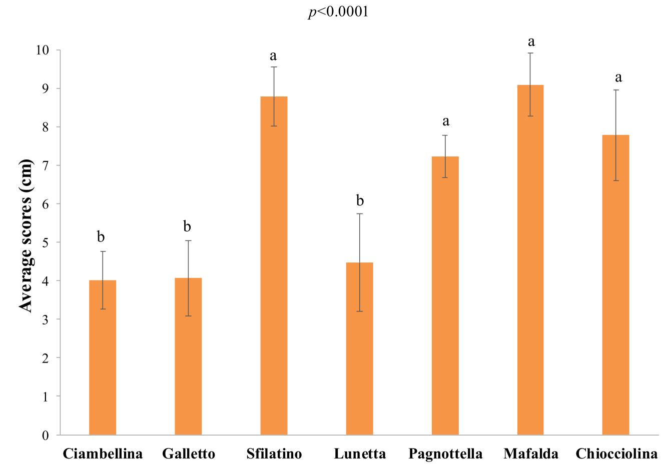

Fig. 4. Histogram representing consumers' preference towards the different bread shapes. Results indicate mean values ± S.D. (standard deviation). Different superscript letters indicate statistically significant differences.

photothermal aging. Although the total phenolic content is influenced by the almond variety, the solvent used for the extraction procedure, data analysis (Table 5) revealed that TPC decreases in all samples regardless of enrichment when photothermal stress is apply for several days. The contribution of the addition of the almond skin to the Mafalda bread is evident by evaluating the sample subjected to photothermal stress (day 0). In fact, here have been registered a TPC from 76.04 to 146.25 and 173.03 mg GAE/g extract for the CTR, 5-PAS and 10-PAS, respectively. The 10-PAS sample, even if subjected to 9 days of photothermal stress, has a significant TPC content for the type of product under investigation (111.02 mg GAE/g extract). The reduction of TPC is also observed in the almond skin with values from 50.45 to 46.73 mg GAE/g extract for sample subjected to photothermal stress day 0 and 9, respectively. Previously, Gaglio et al. (2023) evaluated the TPC in 5 and 10 % almond skin from "Tuono" variety enriched bread and found values of 0.73 and 0.88 mg GAE/g food matrix, respectively. Kahlaoui et al. (2022) observed significant variability in the total phenolic content (TPC) of almond skins. Notably, green almond skins exhibited higher TPC values compared to those obtained from mature hulls. Interestingly, bread enriched with powdered green almond skin displayed a TPC that was seven times higher than that of wheat bread used as a control.

Table 5
TPC in sample submitted to different period of photothermic degradation.

Sample	Day	TPC (mg GAE)/g extract)
Powdered Almond Skin (PAS)	0	50.45 ± 1.82^{a}
	3	50.21 ± 1.60^{a}
	6	48.54 ± 1.51^{b}
	9	46.73 ± 1.23^{c}
p value		0.001
Mafalda bread (CTR)	0	76.04 ± 1.82^{a}
	3	67.08 ± 1.50^{b}
	6	61.52 ± 1.21^{c}
	9	40.54 ± 1.08^{d}
p value		0.001
Mafalda bread (5-PAS)	0	146.25 ± 1.22^{a}
	3	139.76 ± 1.45^{b}
	6	134.82 ± 1.05^{c}
	9	102.51 ± 1.12^{d}
p value		0.001
Mafalda bread (10-PAS)	0	173.03 ± 2.40^{a}
	3	165.48 ± 2.52^{b}
	6	156.21 ± 2.67^{c}
	9	111.02 ± 2.23^{d}
p value		0.001

Data are expressed as means ± S.D. ($n = 3$). PAS: Powdered Almond Skin. Data within a column followed by different letters are significantly different.

3.6. Antioxidant potential of Mafalda bread photothermally aged

It is well known that when oxygen is metabolised, it creates unstable molecules called 'free radicals', which steal electrons from other molecules, causing damage to DNA and other cells. For this reason, it is it is very important to consume foods capable of counteracting the oxidative process (Lobo et al., 2010).

The radical scavenging potential of Mafalda bread subject to photothermal aging (CTR, 5-PAS, and 10-PAS) revealed that the DPPH radical is more sensible to the action of the extract than the ABTS one (Table 6). In fact, in this latter case the enrichment, even if by 10-PAS, did not determine a significant increase in scavenger activity (18.01 % vs 24.25 % at 200 μg/mL, respectively) (Table 6). A significant increase in DPPH radical scavenger activity was observed in 10-PAS sample, with IC_{50} from 555.72 to 103.29 μg/mL for enriched bread subjected to 0 and 9 days of photothermal stress, respectively. On the contrary in bread control the activity is not affected by the photothermal stress (Table 6).

Regarding the protection from lipid peroxidation a promising increase of bioactivity was observed in sample subjected to 9 days of photothermal stress with IC_{50} values of 19.78 and 23.89 μg/mL at 30 and 60 min of incubation, respectively (Table 6). In general, the data analysis showed a good protective power from lipid peroxidation of the extract obtained from enriched bread. Pearson's correlation coefficient highlights that phenols are not the only compounds responsible for the bioactivity of the extracts. Our data agreed with those reported by Gaglio et al. (2023) since it is demonstrated that almond skin enrichment improve the antioxidant capacity of the bread. In earlier research, Kahlaoui et al. (2022) found that bread enriched with 8 % powdered green Achaak hull exhibited a remarkable 7-fold increase in total phenolic content (TPC) and a 25-fold boost in antioxidant activity compared to control bread. Similarly, Pasqualone et al. (2020) observed a comparable effect in biscuits formulated with almond skin.

Table 6
Antioxidant activity of Mafalda bread and almond skin enriched bread submitted to photothermic degradation.

Sample	Day	% ABTS radical Inhibition at 200 mg/mL	DPPH (IC_{50} μg/mL)	β-Caroten bleaching test (IC_{50} μg/mL)	
				30 min^	60 min^
Mafalda bread (CTR)	0	18.01 ± 1.78^a	528.22 ± 11.86^a	96.81 ± 6.77^d	31.06 ± 2.63^a
	3	15.96 ± 1.22^a	675.50 ± 13.66^b	50.87 ± 5.72^c	47.18 ± 3.22^b
	6	10.00 ± 1.22^b	690.60 ± 13.95^b	14.61 ± 2.48^a	31.86 ± 2.94^a
	9	9.43 ± 1.22^b	564.47 ± 11.97^a	24.07 ± 2.00^b	30.19 ± 2.27^a
p value		0.001	0.001	0.001	0.001
Mafalda bread (5-PAS)	0	24.21 ± 1.88^a	463.08 ± 10.12^b	90.67 ± 5.90^c	44.72 ± 4.82^c
	3	19.57 ± 1.65^b	482.03 ± 10.37^c	51.66 ± 5.43^b	33.65 ± 3.50^b
	6	17.67 ± 1.60^b	486.90 ± 11.22^c	48.70 ± 4.77^b	25.13 ± 2.06^a
	9	8.80 ± 0.76^c	417.88 ± 10.02^a	29.42 ± 2.84^a	29.28 ± 2.79^b
p value		0.001	0.001	0.001	0.001
Mafalda bread (10-PAS)	0	24.47 ± 1.95^a	555.72 ± 12.41^c	95.92 ± 7.44^d	37.49 ± 3.97^d
	3	18.82 ± 1.63^b	611.88 ± 12.50^d	67.93 ± 4.21^c	45.14 ± 4.86^c
	6	13.26 ± 1.24^c	190.55 ± 4.23^b	36.28 ± 3.50^b	35.75 ± 3.94^b
	9	9.77 ± 0.94^d	103.29 ± 4.07^a	19.78 ± 2.80^a	23.89 ± 2.65^a
p value		0.001	0.001	0.001	0.001

Data are expressed as means ± S.D. (*n* = = 3).
^ time of incubation. PAS: Powdered Almond Skin. Data within a column followed by different letters are significantly different.

3.7. Evaluation of mold-free shelf life

The appearance of visible signs of mold development are depicted in Fig. 5. The first spot, as highlighted by the blue ovals on the bread surfaces, appeared at day 11 in CTR trial, while both breads produced with PAS addition, were characterized by the first mold spots at day 13.

As indicated by Ju et al. (2020), the day before the first evident sign of mold appearance on the bread slices was considered the shelf life for that specific bread trial. Thus, CTR bread presented a 10-day shelf life, while both PAS added breads showed a longer shelf life (12 days).

4. Conclusions

Almond skin, a by-product rich in polyphenols, has been successfully incorporated into bread production at an industrial level. Prior to implementation, modifications were made to the peeling plant to address hygiene concerns related to wet almond skin. Notably, doughs processed with PAS showed no presence of spore-forming aerobic bacteria, members of the Enterobacteriaceae family, or total coliforms, highlighting the efficiency of the almond processing plant upgrades. Industrial-scale bread production using PAS did not adversely affect the sourdough inoculum used as a starter. Although the final breads differed significantly from control breads, they received positive feedback from consumers. The Mafalda shape, a typical choice in central-west Sicily, emerged as the most preferred. Furthermore, the phenolic content and

Fig. 5. Visual appearance of fungal growth on sliced breads. Abbreviations: CTR, control breads; 5-PAS, experimental breads enriched with 5 % (*w/w*) of Powdered Almond Skin (PAS); 10-PAS, experimental breads enriched with 10 % (*w/w*) of PAS.

radical scavenging activity of PAS-enriched breads clearly demonstrate their potential as functional food for daily consumption.

Ethical statement

The present work did not involve human subjects for medical purposes.

CRediT authorship contribution statement

Enrico Viola: Writing – original draft, Methodology, Investigation. **Natale Badalamenti:** Writing – original draft, Methodology, Investigation. **Maurizio Bruno:** Formal analysis, Data curation. **Rosa Tundis:** Writing – original draft, Methodology, Investigation. **Monica Rosa Loizzo:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Giancarlo Moschetti:** Supervision. **Francesco Sottile:** Funding acquisition, Conceptualization. **Vincenzo Naselli:** Methodology, Investigation. **Nicola Francesca:** Validation, Project administration. **Luca Settanni:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Raimondo Gaglio:** Writing – review & editing, Writing – original draft, Software, Methodology, Data curation.

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.

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