Journal of Functional Foods 123 (2024) 106607

Contents lists available at ScienceDirect

Journal of Functional Foods

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

Exploring the functional potential of pea-based sourdough in traditional durum wheat *focaccia*: Role in enhancing bioactive compounds, *in vitro* antioxidant activity, *in vitro* digestibility and aroma

Francesca Vurro [a], Maria Santamaria [b], Carmine Summo [a], Antonella Pasqualone [a,*], Cristina M. Rosell [b,c]

[a] Department of Science of Soil, Plant and Food Science, University of Bari, 70126 Bari, Italy
[b] Institute of Agrochemistry and Food Technology, IATA-CSIC, Paterna, Spain
[c] Department of Food and Human Nutritional Science, University of Manitoba, Winnipeg, Canada

ARTICLE INFO

Keywords:
Flat bread
Fermentation
Legume flour
Antioxidants
In vitro digestion
Odorants

ABSTRACT

Legume-based sourdough is gaining momentum. This study aimed to compare the effectiveness of type I sourdough prepared with durum wheat semolina (S1), pea flour (S2) or 50:50 semolina/pea flour (S3) in improving the nutritional quality, antioxidant compounds, *in vitro* digestibility and aroma of traditional durum wheat *focaccia*. Six *focaccias* were prepared: three with 40 % of S1, S2 and S3, and three with the corresponding amount of unfermented flours. Pea sourdough increased the content of phenolic compounds (8.82 ± 0.12 mg GAE/g d.m. in *focaccia* with 40 % pea flour and 4.92 ± 0.41 mg GAE/g d.m. in unfermented semolina *focaccia*), and consequently increased the antioxidant activity. *Focaccias* with pea flour or pea sourdough were "source of protein" and "high fiber", according to UE Reg. 1924/2006. Pea sourdough slowed down starch *in vitro* digestibility while enhancing protein digestibility and leading to a more complex volatile profile, with increased content of aldehydes, alcohols and Maillard reaction compounds.

1. Introduction

Bread is a staple food with a rich history and a prominent cultural significance, especially when considering the gastronomic culture of Mediterranean populations. Leavening, the cornerstone of breadmaking, can be achieved using baking soda ("soda bread"), baker's yeast (composed mainly of *Saccharomyces cerevisiae*) or sourdough, a microbial consortium of bacteria and yeasts (Suchintita Das et al., 2023). Sourdough represents the oldest application of biotechnology in the production of cereal-based foods and is classified into three types, depending on how the process of fermentation starts and its degree of hydration. Type I is obtained by spontaneous fermentation of a dough made of flour and water in a ratio of about 2:1; in type II, fermentation of a more hydrated dough is driven by the addition of selected microorganisms; type III is the dry form of type II, obtained by spray-drying, drum-drying or freeze-drying (Chavan & Chavan, 2011). Over the years, sourdough, especially spontaneous type I, has gradually been replaced by baker's yeast to reduce fermentation times and overcome the difficulties associated with the daily back-slopping of sourdough. On the other hand, the use of sourdough is increasingly recommended due to health benefits related to protein and carbohydrate improved digestibility, reduction of antinutrients (*e.g.* phytates, tannins and enzyme inhibitors), increased antioxidant activity and enhanced aroma of the end product (Coda et al., 2017). In recent years there has been a revival of fermentation with sourdough, linked to the general rediscovery of the traditions of the past. This trend was particularly evident during the COVID-19 crisis, which led many consumers to make their own bread at home with sourdough, either by choice or because baker's yeast supplies were depleted (Easterbrook-Smith, 2021).

Focaccia is an Italian traditional oil-seasoned flatbread, commonly eaten as a street food throughout the country (Pasqualone et al., 2011). This flatbread is made by flattening a dough in a baking pan with fingertips, then fermenting, seasoning it generously with vegetable oil and various toppings, and finally baking (Vurro et al., 2022). In southern Italy, *focaccia* is traditionally prepared with durum wheat semolina (*Triticum turgidum* var. *durum*) (Pasqualone et al., 2019), a raw material that has recently proven to be a viable alternative to wheat flour in a time of climatic and socio-political changes (Mavroeidis et al., 2022).

* Corresponding author.
E-mail address: antonella.pasqualone@uniba.it (A. Pasqualone).

https://doi.org/10.1016/j.jff.2024.106607
Received 27 April 2024; Received in revised form 12 November 2024; Accepted 20 November 2024
Available online 26 November 2024
1756-4646/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

Focaccia is very appreciated for its sensory properties but is known to be rich in fat and poor in protein and fiber (Pasqualone et al., 2011, 2022).

The addition of legume flours, such as chickpea and pea flours (De Angelis et al., 2023; Pasqualone et al., 2019), has been proposed to improve the protein and fiber content of *focaccia*, as well as the content of bioactive compounds, known to have beneficial effects on human health (Padhi et al., 2017). However, to reduce antinutrients, legume flours should be fermented, producing a sourdough (Coda et al., 2017), as shown in recent studies that proposed using pea-based sourdough in conventional and gluten-free bread (Bourré et al., 2019; Drakula et al., 2024). These studies observed a reduction in bread volume, a less important parameter in a product such as *focaccia*, which is typically characterized by reduced thickness. On the other hand, sourdough fermentation achieved a decrease in pea odor, generally poorly accepted by consumers (Bourré et al., 2019; Drakula et al., 2024). In addition, peas, primarily appreciated for protein content, contain also antioxidant compounds, namely phenolics, able to effectively inhibit free radicals and to prevent oxidative reactions at cellular level. Therefore, the consumption of peas goes beyond a merely nutritional function, offering potential health benefits (Wang et al., 2022). Pea is the second legume cultivated in Italy after faba bean, with an increasing production in 2023 (ISTAT, 2024).

The use of sourdough from any type of legume flour is still unexplored in *focaccia*. Therefore, the aim of this study was to improve the nutritional features, *in vitro* antioxidant activity, *in vitro* digestibility and aroma of traditional Italian durum wheat *focaccia* by using pea-based sourdough.

2. Materials and methods

2.1. Materials

Durum wheat semolina (De Cecco, Fara S. Martino, Italy) (carbohydrates 68 g/100 g; protein 14 g/100 g; fat 1.5 g/100 g; fiber 2.9 g/100 g; ash 0.87 g/100 g), baker's yeast (Mulino Caputo, Naples, Italy), olive oil (Olearia De Santis, Bitonto, Italy) and sea salt (Com-Sal Srl, Pesaro, Italy) were purchased from local retailers. Pea flour (carbohydrates 56 g/100 g; protein 24 g/100 g; fat 1.1 g/100 g; fiber 8.8 g/100 g; ash 3.01 g/100 g) was kindly provided by Andriani Spa (Gravina in Puglia, Italy).

2.2. Preparation of sourdough

Three type I sourdoughs were prepared according to Eraslan et al. (2023), with few modifications, from: i) durum wheat semolina 100 % (S1); ii) pea flour 100 % (S2); iii) semolina and pea flour 50:50 (*w*/w) (S3). In detail, flour and tap water were mixed manually to obtain a homogeneous dough, with a dough yield (DY) = 200 (DY = dough weight/flour weight ×100). The dough was incubated in a sealed jar at 30 °C for 16 h (Memmert proofer, EN.CO., Spinea, Italy) to achieve a spontaneous fermentation. Then, 50 g of fermented dough were mixed with 50 g of semolina/pea/semolina-pea flour and 50 g of water, incubating again for 16 h at 30 °C, followed by 8 h storage at 4 °C. Flour and water addition and mixing, fermentation, and cold storage were repeated daily for 15 days (back-slopping). The resulting sourdoughs were freeze-dried (LyovaporTM L-200 Lyophilizer, Buchi, Switzerland), milled (ETA-Vercella, Turin, Italy), and sieved at a particle size of 212 μm (Giuliani Tecnologie, Turin, Italy). Freeze-dried, powdered sourdoughs were packed in plastic bags, and stored at 4 °C.

2.3. Microbial counts in the sourdough

For each sample, 10 g of freeze-dried sourdough was mixed with 9 mL of sterile peptone water (0.1 %) (*w/v*) (Scharlab Chemine S.A., Barcelona, Spain) and homogenized in the stomacher for 3 min. The method UNE-EN 15787, 2022 was used to determine *Lactobacillus spp.*, with some modifications. The serial dilutions were prepared and then plated on Man, Rogosa and Sharpe agar (MRS) (Scharlab Chemine S.A., Barcelona, Spain). The plates were incubated under microaerophilia conditions at 37 °C for 48 h. The yeasts and molds count (ISO 21527-2 (2008)) were incubated in dichloran glycerin selective agar (DG18 agar) (Scharlab Chemine S.A., Barcelona, Spain) at 25 °C for 5 days under aerobic conditions. The analyses were performed in triplicate.

2.4. Preparation of focaccia

Six types of *focaccia* were prepared (Table 1), according to the production process described by Vurro et al. (2022), with few modifications. Flour, yeast, freeze-dried sourdough and water were mixed with a spiral kneader (Bosh MFQ40304, München, Germany) for 6 min. Then, salt and oil (50 % of the total amount) were added, continuing to knead for 6 min. The dough, manually flattened (about 1.5 cm thick) and circularly shaped with a pastry ring (diameter of 10.8 cm) (Tescoma, Cazzago San Martino, Italy), was placed into metal pans previously greased with oil (30 % of the total amount) and left to rise for 90 min at 35 °C, RH = 33.5 % (Memmert proofer, EN.CO., Spinea, Italy). A piece of dough was sampled at the end of leavening for analyses. Finally, the remaining oil (20 % of the total amount) was evenly poured on the surface of *focaccia*, which was baked in an electric oven (Oem Ali Group, Bozzolo, Italy) at 200 °C for 25 min. Three different batches were made for each *focaccia* sample. The baked *focaccias* and the *focaccia* dough samples were freeze-dried (LyovaporTM L-200 Lyophilizer, Buchi, Switzerland), powdered, packed in plastic bags, and stored at 4 °C.

3. pH and total titratable acidity

The pH and total titratable acidity (TTA) were determined in sourdough and *focaccia*, as well as in *focaccia* dough sampled before baking. The pH was determined by a pHmeter with a food penetration probe (HANNA instruments, Woonsocket, RI, USA). The TTA was measured as described in the American Association of Cereal Chemists (AACC) method 02–31.01 (AACC International, 2010). The analyses were performed in triplicate.

3.1. Antinutritional factors

The antinutritional factors were determined in the flours, sourdough, and *focaccia*. Stachyose, raffinose and sucrose were determined as described by De Angelis et al. (2021). In detail, 10 mg of sample were vortexed for 5 min with 5 mL of deionized water (ELGA Purelab, High Wycombe, UK), then the supernatant was recovered, filtered through 0.22 μm cellulose acetate filters (Thermo Fisher Scientific, Monza, Italy), and analyzed by using a high-performance liquid chromatograph (Agilent Technologies, Santa Clara, USA) equipped with a 1260 Infinity Refractive Index Detector (Agilent Technologies, Santa Clara, USA). Conditions were isocratic, using deionized water (ELGA Purelab, High

Table 1
Formulation of *focaccia* samples (S1 = 100 % wheat sourdough; S2 = 100 % pea sourdough; S3 = 50:50 wheat-pea sourdough).

Ingredients (g)	Type of *focaccia*					
	With sourdough			Without sourdough		
	T1	T2	T3	T4	T5	T6
Durum wheat semolina	60	60	60	100	80	60
S1	40	0	0	0	0	0
S2	0	0	40	0	0	0
S3	0	40	0	0	0	0
Pea flour	0	0	0	0	20	40
Olive oil	10	10	10	10	10	10
Salt	2	2	2	2	2	2
Baker's yeast	1	1	1	1	1	1
Water	70	70	70	70	70	70

Wycombe, UK) as mobile phase, at a flow rate of 0.8 mL min^{-1}, through a 300 × 7.8 mm cation exchange column (Rezex RCM column, Ca^{2+}, 8 μm, Torrance, CA, USA) maintained at 80 °C. Identification was made by comparing the retention time with that of the corresponding standard (Merck KGaA, Darmstadt, Germany). For quantification, calibration curves were previously set up by preparing aqueous solutions of stachyose, raffinose and sucrose (Merck KGaA, Darmstadt, Germany) at concentrations between 0.005 and 1 g/L. The content of phytic acid was measured with the assay kit (Megazyme International, Bray, Ireland), following the manufacturer's procedure. Three replicated analyses were carried out.

3.2. Bioactive compounds and in vitro antioxidant activity of focaccia

The total carotenoid pigments were determined according to the AACC method 14–60.01 (AACC International, 2010), measuring the absorbance at 435.8 nm with a Cary 60 UV–Vis spectrophotometer (Agilent Technologies Inc., Santa Clara, CA, USA). The total carotenoid content was expressed as mg β-carotene/kg d.m. The phenolic compounds were extracted and quantified as described in Pasqualone et al. (2023), expressing the results as mg of gallic acid (GAE)/g d.m. The antioxidant activity *in vitro* was determined by 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazylcrylhydrazyl (DPPH) assays as reported by Vurro et al. (2022). The results were expressed as μmol Trolox equivalents (TE)/g d.m. Three replicates were carried out for all the analyses.

3.3. Nutritional composition of focaccia

The moisture content was determined by a moisture analyzer at 105 °C (Radwag Wagi Elektroniczne, Radom, Poland) according to the AACC method 44–01.01 (AACC International, 2010). The protein content (total nitrogen × 6.25) and the ashes were determined as described in the AACC methods 46–11.02 and 08–01.01, respectively (AACC International, 2010). The lipid fraction of *focaccia* was extracted and quantified as described by the AOAC 945.38 F (AOAC, 2006). The total dietary fiber was determined by the enzymatic–gravimetric procedure, according to the AOAC method 991.43 (AOAC, 2006). Total carbohydrates were calculated by difference, subtracting to 100 the sum of moisture, lipids, ashes and protein. Results were expressed as g/100 g of fresh matter. The energy value (kcal) was calculated considering the Atwater general conversion factors and the contribution of 2 kcal/g from the total dietary fiber, according to Annex XIV of Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October, 2011. Total starch was quantified with a commercial kit (Megazyme International, Bray, Ireland) following the manufacturer's procedure. Results were expressed as g/100 g d.m. The analyses were performed in triplicate.

3.4. In vitro protein digestibility of focaccia

In vitro protein digestibility (IVPD) was determined as described in Espinosa-Ramírez et al. (2018). An aliquot of sample containing 6.25 mg of protein was suspended in 1 mL of water and placed in a water bath at 37 °C. The pH was adjusted to 8.00. Trypsin solution (Trypsin from porcine pancreas, Sigma-Aldrich, St. Louis, MO, USA) was prepared at a concentration of 1.6 mg/mL, with an activity of 13,766 BAEE units/mg protein, adjusting the pH to 8.00. An aliquot of 0.1 mL of enzyme solution was added to the sample suspension and the drop of pH was recorded, starting from 5 s after the addition, at 1 min intervals for 10 min. IVPD was calculated according to the equation: $IVPD = 210.464 - 18.1x$, where x = pH after 10 min. Three replicates were carried out.

3.5. In vitro carbohydrate digestibility of focaccia

In vitro digestibility of carbohydrates was determined by measuring the release of glucose from the samples incubated first with porcine pancreatic α-amylase (Sigma-Aldrich, St. Louis, MO, USA) and then with amyloglucosidase (AMG) (Novozymes, Bagsværd, Denmark), followed by colorimetric reaction catalyzed by glucose oxidase–peroxidase (GOPOD) (Megazyme International, Bray, Ireland) as described by Santamaria et al. (2022) and absorbance measure at 510 nm with a microplate reader (Epoch, BioTek, Winooski, VT, USA). Starch was calculated as glucose (mg) × 0.9. Rapidly digestible starch (RDS) was assessed after 20 min of hydrolysis, slowly digestible starch (SDS) between 20 and 120 min, while resistant starch (RS) was the unhydrolyzed fraction after 24 h. The digestion kinetics was calculated according to the equation: $C = C\infty\left(1 - e^{-kt}\right)$ where C = percentage of starch hydrolyzed at t time, $C\infty$ = equilibrium concentration or maximum hydrolysis and k = kinetic constant. The analyses were performed in triplicate.

3.6. Volatile profile of sourdough and focaccia

The volatile compounds (VOCs) of freeze-dried sourdough and *focaccia* were evaluated by gas chromatography/mass spectrometry analysis (GC–MS), after an extraction phase with headspace solid-phase microextraction (HS-SPME), as reported in Vurro et al. (2022). VOCs were extracted by exposing for 50 min at 40 °C a 75 μm Carboxen/polydimethylsiloxane (CAR/PDMS) SPME fiber (Supelco, Bellefonte, PA, USA) in the headspace of a vial containing 0.5 g of sample, 4 mL of aqueous solution of NaCl (20 g/100 g) and 150 μL of 1-propanol. The fiber was then desorbed in the injection port of a 6850 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled with a 5975 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA), operating in splitless mode at 230 °C for 3.5 min. Separation of the VOCs was performed on a HP-Innowax (Agilent Technologies, Santa Clara, CA, USA) polar capillary column (60 m length × 0.25 mm i.d. × 0.25 μm film thickness) under the following conditions: injector temperature at 250 °C; helium carrier gas flow at 1.5 mL min^{-1}, and pressure at 30 kPa. The oven temperature was programmed to remain at 35 °C for 5 min, increase to 50 °C at 5 °C min^{-1}, hold for 5 min, then ramp to 210 °C at 5.5 °C min^{-1}, and finally hold at 210 °C for 5 min. The mass detector settings were as follows: interface temperature 230 °C, source temperature 230 °C, ionization energy 70 eV, and scan range 33–260 amu. The identification of VOCs was performed with the reference mass spectra of the National Institute of Standards and Technology (NIST) and Wiley libraries. Their quantification was carried out using 1-propanol as the internal standard. The concentrations of VOCs were expressed as μg/g of sample. The analyses were carried out in triplicate.

3.7. Statistical analysis

Statistical analysis was carried out by Minitab Statistical Software 21 (Minitab Inc., State College, PA, USA). The results were all expressed as mean ± standard deviation (SD) of replicates. The significant differences ($\alpha = 0.05$) were verified through the application of parametric one and two-way analysis of variance (ANOVA), followed by the Tukey HSD test. The two variables considered in the two-way analysis of variance were the inclusion of sourdough (S) and the inclusion of pea flour (P).

4. Results and discussion

4.1. Sourdough properties

Table 2 shows pH, TTA and microbial count of sourdoughs. After 15 days, the lowest pH value was reached in the wheat-only sourdough (S1), while the addition of 50 % pea flour (S3) increased the pH, reaching the maximum value of 4.15 in pea-only sourdough (S2). The highest ΔpH was observed in S1, while no significant differences were assessed between S2 and S3. The nutritional composition of pea and

Table 2
pH, total titratable acidity (TTA), and microbial counts (LAB, yeasts and molds) of sourdoughs (S1 = 100 % wheat sourdough; S2 = 100 % pea sourdough; S3 = 50:50 wheat-pea sourdough).

Sample	Initial pH	Final pH (after 15 d)	ΔpH	TTA (mL NaOH 0.1 M/ 10 g)	LAB (Log CFU/ g)	Yeasts and molds (Log CFU/g)
S1	5.75 ± 0.05^{b}	3.4 ± 0.00^{c}	2.35 ± 0.05^{a}	16.12 ± 0.88^{b}	6.34 ± 0.05^{b}	2.28 ± 0.09^{c}
S2	6.10 ± 0.00^{a}	4.15 ± 0.05^{a}	1.95 ± 0.05^{b}	22.93 ± 1.01^{a}	8.88 ± 0.06^{a}	5.30 ± 0.05^{a}
S3	5.85 ± 0.05^{b}	3.75 ± 0.05^{b}	2.1 ± 0.00^{b}	21.42 ± 0.52^{a}	5.03 ± 0.05^{c}	3.98 ± 0.05^{b}

Data are presented as means ± SD of replicates. Different letters in the same column indicate significant differences at $p \leq 0.05$.

wheat flours and the environmental microbiota were responsible for the differences observed (Millar et al., 2019), being the incubation conditions kept constant. The pH of sourdoughs was in line with the most common range of 3.5–4.3, reported in literature (Chavan & Chavan, 2011). The spontaneous microbiota fermented the carbohydrates and produced organic acids, influencing both pH and TTA of sourdoughs. TTA was higher in S2 and S3, suggesting that more organic acids were produced when pea flour was included in sourdough (Jekle et al., 2010), but without affecting the final pH. The discrepancy observed between pH and TTA was probably related to the buffering capacity of pea flour, due to its higher mineral content than wheat flour (Millar et al., 2019), in agreement with the findings of other authors in buckwheat, quinoa, and teff sourdoughs (Wolter et al., 2014).

LAB counts were higher than yeast and mold ones (Table 2), which suggests their better adaption to the fermenting conditions. Significant differences were found in the microbial composition of the tested sourdoughs. Both LAB and yeasts and molds were higher in the 100 % pea sourdough (S2). Lazo-Vélez et al. (2021) explained high levels of LAB and yeasts with high amounts of mono- and disaccharides, which contribute to their metabolism. However, those values decreased in S3, probably due to the coexistence of species through either mutualism or antagonism. The flour type influences the development of bacteria and yeast species, which affects the digestion of different carbohydrates (Chavan & Chavan, 2011). Furthermore, yeast and mold counts were lower in wheat containing sourdoughs (S1 and S3). It has been reported that wheat sourdough does not exceed 5 log CFU/g in fungal counts (yeasts and molds) (Sáez et al., 2018).

In legumes and whole cereals, fermentation is typically adopted to reduce the antinutritional compounds (Sharma, 2021). The latter belong to different classes of metabolites, among which phytates and oligosaccharides of the raffinose family (RFOs) are the main ones in legumes (Sharma, 2021). A significant reduction of phytates was observed in the three sourdoughs compared to the starting flours (Table 3), indicating that the acidic conditions enhanced the endogenous phytase activity of the flours, probably reinforced also by the enzymatic activity of microorganisms (Curiel et al., 2015). The phytates were reduced from 0.19 g/100 g d.m. in semolina to 0.04 g/100 g d.m. in S1 (100 % wheat sourdough). The initial concentration detected in semolina was lower than data reported by Millar et al. (2019) but in line with Hager et al. (2012). The concentration of phytates of pea flour, accounting for 0.82 g/100 g d.m. and intermediate to the values reported by Millar et al. (2019) and Pedrosa et al. (2020), lowered to 0.57 g/100 g d.m. in S2 (100 % pea sourdough). By reducing phytates, known to chelate calcium, iron, copper and zinc, sourdough could increase the availability of minerals.

Similarly, microbial enzymes, such as α-galactosidase, catalyzed the hydrolysis of RFOs (Curiel et al., 2015), which significantly decreased

Table 3
Antinutritional factors of flours and sourdoughs (S1 = 100 % wheat sourdough; S2 = 100 % pea sourdough; S3 = 50:50 wheat-pea sourdough).

Sample	Phytates g/ 100 g d. m.	Stachyose (mg/g d.m.)	Raffinose (mg/g d. m.)	Sucrose (mg/g d. m.)
Durum wheat semolina	0.19 ± 0.01^{a}	4.68 ± 0.36^{a}	2.35 ± 0.11^{a}	11.02 ± 0.29^{a}
S1	0.04 ± 0.00^{b}	0.84 ± 0.04^{b}	n.d.	0.57 ± 0.03^{b}
Pea flour	0.82 ± 0.01^{a}	57.27 ± 0.76 ^{a}	11.57 ± 1.27^{a}	31.56 ± 0.19^{a}
S2	0.57 ± 0.01^{b}	14.50 ± 4.93^{b}	0.32 ± 0.02^{b}	0.78 ± 0.02^{b}
Mix semolina-pea flour (50:50)	0.52 ± 0.05^{a}	26.95 ± 2.07^{a}	6.40 ± 0.21	26.14 ± 3.17^{a}
S3	0.11 ± 0.00^{b}	9.79 ± 2.13^{b}	n.d.	0.61 ± 0.02^{b}

Data are presented as means ± SD of replicates. Different letters in the same column indicate significant differences at $p \leq 0.05$. The comparison has been performed comparing the sourdough with the corresponding flour. n.d. = not detected.

with fermentation in all three sourdoughs considered, compared to the starting flours. The main gastrointestinal disorders associated with the consumption of legume-based foods are attributed to these carbohydrates. However, researchers have recognized and reassessed the prebiotic action exerted on the genera *Bifidobacteria* and *Lactobacillus* that populate the large intestine, which may provide human health benefits (Curiel et al., 2015).

4.2. *Focaccia* physic-chemical and nutritional properties

Both the variables "inclusion of sourdough" and "inclusion of pea flour" had a significant effect ($p \leq 0.05$) on the pH and TTA of the doughs and *focaccia* samples, confirmed by a significant interaction of the two variables (Table 4). The use of sourdough resulted in a significant decrease in pH and an increase in TTA of dough and *focaccia* (T1, T2 and T3 samples) compared to non-fermented flours (T4, T5 and T6 samples). Furthermore, T3 *focaccia*, containing 100 % pea sourdough, showed the highest TTA, in agreement with the results observed in the starting sourdoughs (Table 2). Among the *focaccia* samples without sourdough, T5 and T6, which contained pea flour, had higher TTA than T4, prepared without pea flour. This result could be due to the higher fiber content of pea flour. A study conducted by Al Khatib et al. (2020) showed a strong positive correlation between TTA and fiber content in pita bread.

The phenolic compounds significantly increased with the inclusion

Table 4
pH and total titratable acidity (TTA) of dough and *focaccia* samples. Codes T1-T6 correspond to 6 different formulations as reported in Table 1. P = Inclusion of pea flour; S = Inclusion of sourdough.

Formulation	pH		TTA (mL NaOH 0.1 M/ 10 g)	
	Dough	*Focaccia*	Dough	*Focaccia*
T1	4.49 ± 0.04^{e}	4.50 ± 0.00^{c}	7.15 ± 0.25^{b}	4.50 ± 0.50^{c}
T2	4.53 ± 0.03^{e}	4.54 ± 0.07^{c}	11.95 ± 1.25^{a}	7.60 ± 0.10^{b}
T3	4.73 ± 0.01^{d}	4.64 ± 0.00^{c}	13.18 ± 0.73^{a}	9.03 ± 0.48^{a}
T4	5.99 ± 0.01^{c}	6.29 ± 0.09^{b}	4.18 ± 0.18^{c}	1.23 ± 0.03^{e}
T5	6.17 ± 0.00^{b}	6.46 ± 0.12^{ab}	5.18 ± 0.18^{c}	2.73 ± 0.47^{d}
T6	6.29 ± 0.02^{a}	6.50 ± 0.04^{a}	5.60 ± 0.10^{bc}	2.85 ± 0.35^{d}
p-value (P*S)	$p \leq 0.05$	ns	$p \leq 0.05$	$p \leq 0.05$
p-value (S)	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
p-value (P)	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$

Data are presented as means ± SD of replicates. Different letters in the same column indicate significant differences at $p \leq 0.05$. ns = not significant.

of pea flour or pea sourdough in *focaccia,* and the interaction of the two variables was significant ($p \leq 0.05$) (Table 5). The observed contents of phenolic compounds in the samples with unfermented pea flour (T5 and T6) agreed with Davies-Hoes et al. (2017), who fortified bread with pea flour at different particle sizes. The acidification process and the microbial enzymatic activity, in fact, promoted the bioconversion of phenolic compounds, concentrated in the pea cotyledon (Padhi et al., 2017) and bound to the cell walls, into more available forms (Curiel et al., 2015; Eraslan et al., 2023). The increase in phenolic compounds observed with the inclusion of sourdough, compared to the non-fermented versions of *focaccias*, amounted to 32 %.

A significant effect ($p \leq 0.05$) of both "inclusion of sourdough" and "inclusion of pea flour" was observed for the content of carotenoids, with a significant effect of the interaction ($p \leq 0.05$) (Table 5). Their concentration, ranging from 4.42 to 26.39 mg β-carotene/kg d.m., decreased with the addition of sourdough, as observed when comparing T5 with T2 (−14 %) and T6 with T3 (−16 %). This reduction was likely ascribed to oxidation events related to the incorporation of oxygen and the production of hydrogen peroxide by microbial metabolism during sourdough fermentation (Antognoni et al., 2019). Carotenoids, characterized by pro-vitamin A and antioxidant activity, are the main pigments of durum wheat, and are responsible for the typical golden color of semolina, pasta and baked goods (Pasqualone et al., 2019). Likewise, together with tocopherols, carotenoids are the main lipophilic antioxidants of pea cotyledons (Padhi et al., 2017), with a content varying according to the varieties (10.1–40.21 mg β-carotene /kg) (Ashokkumar et al., 2015; Padhi et al., 2017). The *in vitro* antioxidant activity, evaluated by the DPPH and ABTS assays, highlighted a positive influence exerted by pea flour, both unfermented and fermented ($p \leq 0.05$). Therefore, this ingredient shows functional potential in terms of antioxidant properties, in addition to other health benefits reported in the literature, such as hepatoprotective, anti-hyperglycaemic and anti-tumour effects (Padhi et al., 2017).

Table 6 shows the proximate composition of the *focaccias.* Both "inclusion of sourdough" and "inclusion of pea" had a significant effect on the moisture and fiber content ($p \leq 0.05$), while for lipids and protein, only "inclusion of pea" had a significant effect ($p \leq 0.05$) and the interaction between the two variables was not significant. The moisture content varied from 22.28 to 28.22 g/100 g, with the lowest value in wheat-only *focaccia* prepared with sourdough (T1). The different fiber content (higher in pea flour) could corroborate the different capacities of the flours blends to retain water, influencing therefore the moisture content of *focaccia*. Lipids were in the range 8.12–8.84 g/100 g. This limited range of variation was due to the use of the same amount of olive oil in all the formulations and to the minimal contribution of lipids by the other ingredients used. The ash content was directly related to the minerals of the flours used. Indeed, the highest concentration of ash was observed in all the samples containing pea flour or pea sourdough (T2, T3, T5 and T6), which were richer in minerals than wheat flour, as also observed by Millar et al. (2019). All the samples with pea flour had a higher protein content than those containing only wheat flour (T1 and T4), and were able to provide more than 12 % of the energy value of the product. Therefore, the "source of protein" nutritional claim applied, according to Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December, 2006. Contrarily to protein, total carbohydrates were lower in all pea-fortified samples. The substitution of semolina with pea flour diluted the total starch content, considering the direct correlation with the chemical composition of the starting flours, while the fermentation did not interfere ($p \geq 0.05$). Similar results were found recently by Moreno-Araiza et al. (2023), when comparing pea fortified bread with bread prepared with wheat flour only. Also, the fiber content of *focaccias* containing pea flour and pea sourdough (T2, T3, T5 and T6) was markedly higher than 6 g/100 g, meeting the requirements for the "high fiber" nutritional claim (Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December, 2006). A significant ($p \leq 0.05$) effect of "inclusion of sourdough", "inclusion of pea" and their interaction was observed for the antinutritional compounds (namely raffinose family oligosaccharides and phytates) (Table 6), suggesting that the inclusion of legumes increases their concentration, but fermentation reduces it. Indeed, the concentration of antinutritional compounds was higher in T5 and T6, with pea flour, than in the wheat-only *focaccias*, but significantly decreased when pea was fermented into sourdough. Baik and Han (2012) and Coda et al. (2017) highlighted the ability of fermentation to reduce the antinutrients in bread enriched with chickpeas, lentils and faba beans.

Overall, the obtained results highlight that the nutritional profile of the fortified *focaccias* significantly improved by adding pea sourdough due to the increase in protein, fiber and bioactive compounds, without markedly raising the antinutrients. This improvement limits the typical negative nutritional features of *focaccia*, particularly its richness in fat and poverty in protein and fiber (Pasqualone et al., 2011, 2022). The addition of pea sourdough is therefore a good strategy to improve the nutritional features of this traditional product, with potential health benefits, linked to the reduction of the risk of chronic and inflammatory diseases (Rungruangmaitree & Jiraungkoorskul, 2017). Similar nutritional improvements have been previously observed in bakery products enriched with flours or sourdough prepared from legumes other than peas, such as black chickpea flour used in the formulation of *focaccia* (Pasqualone et al., 2019), or type I sourdough prepared from chickpea or chickpea-bean-lentil flour blends, used in breadmaking (Eraslan et al., 2023; Rizzello et al., 2014).

Table 5
Bioactive compounds and antioxidant activity of *focaccia* samples. Codes T1-T6 correspond to 6 different formulations as reported in Table 1. P = Inclusion of pea flour; S = Inclusion of sourdough.

Sample	Carotenoids (mg β-carotene/kg d.m.)	Phenolic compounds (mg GAE/g d.m.)	DPPH (μmol TE/g d.m.)	ABTS (μmol TE/g d.m)
T1	4.42 ± 0.34^{f}	7.15 ± 0.11^{b}	0.24 ± 0.03^{c}	0.02 ± 0.00^{d}
T2	13.14 ± 0.53^{d}	8.07 ± 0.20^{a}	0.52 ± 0.03^{b}	0.48 ± 0.05^{c}
T3	18.96 ± 0.31^{b}	8.82 ± 0.12^{a}	0.90 ± 0.05^{a}	1.14 ± 0.04^{b}
T4	8.13 ± 0.56^{e}	4.92 ± 0.41^{d}	0.35 ± 0.02^{c}	0.53 ± 0.04^{c}
T5	15.24 ± 0.52^{c}	5.33 ± 0.34^{d}	0.54 ± 0.05^{b}	1.00 ± 0.05^{b}
T6	26.39 ± 0.85^{a}	6.13 ± 0.58^{c}	1.01 ± 0.10^{a}	1.83 ± 0.19^{a}
*p-value (P*S)*	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
p-value (S)	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
p-value (P)	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$

GAE = gallic acid equivalents; DPPH = 2,2-diphenyl-1-picrylhydrazylcrylhydrazyl; ABTS = 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid. Data are presented as means ± SD of replicates. Different letters in the same column indicate significant differences at $p \leq 0.05$. ns = not significant.

4.3. *In vitro digestibility of proteins and carbohydrates*

Both the variables "inclusion of sourdough" and "inclusion of pea flour" had a significant effect ($p \leq 0.05$) on the *in vitro* digestibility of proteins and carbohydrates of *focaccia* samples (Table 7). *In vitro* protein digestibility (IVPD) was determined by hydrolysis with trypsin, recording the subsequent drop in pH (Coda et al., 2017; Espinosa-Ramírez et al., 2018). This analysis gives an indication of the potential behaviour of protein during the digestion process, related to the nutritional quality in terms of availability of amino acids (Coda et al., 2017; Espinosa-Ramírez et al., 2018). IVPD values above 78 % were observed

Table 6
Nutritional composition and antinutritional compounds of *focaccia* samples. Codes T1-T6 correspond to 6 different formulations as reported in Table 1. P = Inclusion of pea flour; S = Inclusion of sourdough.

Sample	Moisture (g/100 g)	Lipids (g/100 g)	Protein (g/100 g)	Carbohydrates (g/100 g)	Fiber (g/100 g)	Ash (g/100 g)	Energy value (kcal/100 g)	Total starch (g/100 g d.m.)	Stachyose (mg/g d.m.)	Raffinose (mg/g d.m.)	Sucrose (mg/g d.m.)	Phytates (g/100 g d.m.)
T1	22.28 ± 1.93^{c}	8.84 ± 0.18^{a}	9.08 ± 0.52^{c}	57.95 ± 2.22^{a}	4.87 ± 0.21^{c}	1.85 ± 0.04^{c}	335.64 ± 6.98^{a}	52.94 ± 4.38^{ab}	0.57 ± 0.01^{d}	0.1 ± 0.01^{d}	5.75 ± 0.56^{d}	0.13 ± 0.00^{d}
T2	27.51 ± 0.39^{ab}	8.17 ± 0.17^{ab}	10.64 ± 0.33^{b}	51.68 ± 0.38^{bc}	7.50 ± 0.36^{b}	2.01 ± 0.09^{bc}	307.80 ± 1.79^{cd}	47.07 ± 1.43^{bc}	4.59 ± 0.7^{c}	0.01 ± 0^{d}	6.68 ± 0.67^{d}	0.20 ± 0.01^{c}
T3	28.22 ± 0.26^{a}	8.12 ± 0.56^{b}	12.49 ± 0.04^{a}	48.89 ± 0.78^{c}	8.93 ± 0.25^{a}	2.29 ± 0.06^{a}	300.70 ± 2.36^{d}	40.72 ± 1.70^{cd}	8.87 ± 0.7^{b}	0.46 ± 0^{c}	5.22 ± 0.47^{d}	0.37 ± 0.01^{a}
T4	26.38 ± 0.27^{ab}	8.73 ± 0.07^{ab}	8.75 ± 0.40^{c}	54.56 ± 0.71^{b}	5.37 ± 0.60^{c}	1.59 ± 0.07^{d}	321.03 ± 0.92^{b}	57.74 ± 1.45^{ab}	3.22 ± 0.05^{cd}	0.01 ± 0^{d}	15.19 ± 0.4^{c}	0.15 ± 0.01^{d}
T5	25.74 ± 0.12^{b}	8.72 ± 0.01^{ab}	10.93 ± 0.86^{b}	52.67 ± 0.86^{b}	8.10 ± 0.40^{ab}	1.95 ± 0.09^{bc}	316.64 ± 1.08^{bc}	49.27 ± 6.40^{abc}	10.81 ± 1.58^{b}	2.14 ± 0.12^{b}	21.79 ± 0.33^{b}	0.29 ± 0.03^{b}
T6	27.43 ± 0.16^{ab}	8.40 ± 0.18^{ab}	13.31 ± 0.45^{a}	48.75 ± 0.26^{c}	9.07 ± 0.21^{a}	2.11 ± 0.01^{ab}	305.70 ± 0.51^{d}	36.76 ± 0.42^{d}	26.88 ± 1.52^{a}	7.8 ± 0.02^{a}	27.69 ± 2.85^{a}	0.39 ± 0.01^{a}
*p-value (P*S)*	$p \leq 0.05$	ns	ns	$p \leq 0.05$	ns	$p \leq 0.05$	$p \leq 0.05$	ns	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
p-value (S)	$p \leq 0.05$	ns	ns	ns	$p \leq 0.05$	$p \leq 0.05$	ns	ns	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
p-value (P)	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$

Data are presented as means ± SD of replicates. Different letters in the same column indicate significant differences at $p \leq 0.05$. ns not significant. * "Source of protein" according to Regulation (EC) No 1924/2006.

Table 7
In vitro protein digestibility (IVPD), content of different starch fractions (RDS = rapidly digestible starch; SDS = slowly digestible starch; RS = resistant starch), and kinetic parameters of starch hydrolysis ($C\infty$ = equilibrium concentration or maximum hydrolysis, k = kinetic constant) assessed in *focaccia* samples. Codes T1-T6 correspond to 6 different formulations as reported in Table 1. P = Inclusion of pea flour; S = Inclusion of sourdough.

Sample	IVPD (g/100 g protein)	RDS (g /100 g starch)	SDS (g /100 g starch)	RS (g /100 g starch)	$C\infty$	k
T1	78.61 ± 0.09^{de}	62.44 ± 3.26^{a}	25.53 ± 2.16^{d}	12.02 ± 1.1^{b}	91.82 ± 0.74^{a}	0.062 ± 0.005^{a}
T2	78.79 ± 0.27^{d}	53.42 ± 1.04^{b}	36.85 ± 1.06^{c}	9.73 ± 2.1^{b}	78 ± 0.54^{b}	0.044 ± 0.000^{b}
T3	81.32 ± 0.09^{a}	43.36 ± 0.03^{c}	43.70 ± 1.38^{a}	12.94 ± 1.42^{b}	72.61 ± 0.2^{c}	0.034 ± 0.001^{cd}
T4	79.96 ± 0.18^{b}	43.08 ± 3.43^{c}	44.69 ± 2.51^{a}	12.23 ± 0.92^{b}	79.36 ± 0^{b}	0.029 ± 0.000^{d}
T5	79.33 ± 0.09^{c}	46.95 ± 1.08^{c}	42.22 ± 0.97^{ab}	10.82 ± 2.06^{b}	68.02 ± 1.62^{d}	0.041 ± 0.004^{bc}
T6	78.15 ± 0.18^{e}	41.41 ± 2.61^{c}	39.35 ± 0.08^{bc}	19.23 ± 2.7^{a}	50.13 ± 2.29^{e}	0.035 ± 0.002^{cd}
*p-value (P*S)*	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
p-value (S)	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
p-value (P)	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$

Data are presented as means ± SD of replicates. Different letters in the same column indicate significant differences at $p \leq 0.05$. ns = not significant.

in all the *focaccia* samples (Table 7). The addition of unfermented pea flour (T5 and T6) resulted in lower IVPD compared to *focaccia* prepared with semolina alone (T4), probably due to the fiber and antinutrients contributed by the pea. The decrease of IVPD, indeed, progressed as the amount of pea flour increased. The fortification with sourdough prepared with pea flour alone (T3) overcame this drawback as it led to significantly higher IVPD than *focaccias* with the highest amount of unfermented pea flour (T6). This improvement was due to both the proteolytic activity of the LAB and the inactivation of the protease inhibitors occurred during sourdough fermentation (Coda et al., 2017; Espinosa-Ramírez et al., 2018). However, conflicting results were observed for the samples containing semolina and semolina-pea sourdough (T1 and T2), which presented an inferior IVPD with respect to the unfermented ones (T4 and T5). A similar situation was reported by other authors (Catzeddu et al., 2023), who explained it with either the possibility that higher hydrolysis has already occurred in these samples during the fermentation, prior the analysis, or that protein aggregated or complexed the starch, with a reduction of activity of the enzyme added during the analysis.

The *in vitro* digestibility of starch was evaluated by measuring the release of glucose during the incubation with hydrolytic enzymes. Overall, a decrease of RDS and an increase of SDS was observed in the *focaccia* samples containing unfermented pea flour or pea-based sourdough compared to samples prepared with unfermented semolina or semolina sourdough. In detail, sample T1, prepared with semolina sourdough, was characterized by the highest content of RDS and the lowest content of SDS (Table 7). The addition of increasing amounts of pea sourdough (samples T2 and T3) progressively and significantly lowered the content of RDS compared to T1, while the amount of SDS increased significantly. Among the samples prepared without sourdough, T6, prepared with the greatest addition of pea unfermented flour, showed the lowest amount of RDS and the highest amount of RS. The levels of RS in the other samples did not show significant differences among them. Accordingly with these data, the maximum hydrolysis ($C\infty$) and the kinetic constant (k) were the highest in *focaccia* prepared with semolina sourdough (T1), and significantly decreased when pea flour was added, especially unfermented (T6). Therefore, while *focaccia* prepared with semolina sourdough could have a greater impact on glycaemic levels, the addition of peas could slow down glucose release. The slower rate of starch degradation after the addition of pea flour or pea sourdough was probably related to the fiber and protein content of pea flour, which created a physical barrier limiting the activity of the enzymes (Lu et al., 2018), as well as to the effect of acidification, similarly inhibiting the activity of α-amylase and α-glucosides (Demirkesen-Bicak et al., 2021).

4.4. Volatile profile of dry sourdoughs and focaccias

The olfactory impact of foods contributes significantly to consumer acceptance and choice, requiring special attention in the development of new products, especially with legume flours, to which unpleasant odors are generally attributed (Trindler et al., 2022). On the other hand,

sourdough has a positive effect on the aroma of bakery products, which is influenced by the complexity of the microbiota, time and temperature of fermentation, and number of back-sloppings (Chavan & Chavan, 2011; Pétel et al., 2017).

In order to quali-quantitatively analyse the main volatile compounds of sourdoughs and *focaccias*, they were extracted by headspace solid-phase microextraction, then a gas-chromatographic analysis was carried out, coupled to mass spectrometry. The content of aldehydes differed among sourdoughs (Table 8), with higher concentrations in S2 and S3, both containing pea flour. The most abundant aldehyde was hexanal, correlated with the linolenic acid oxidation and with the fermentation process (Pétel et al., 2017; Trindler et al., 2022). This aldehyde is principally associated with beany, fatty, rancid and green notes, which are generally negatively considered (Trindler et al., 2022; Xiang et al., 2023). A similar trend was observed for nonanal, originated by the oxidation of oleic acid, as well as for heptanal and octanal, from oleic acid and/or linoleic acid, equally responsible for grassy and vegetal notes (Xiang et al., 2023). Only one ketone, the 6-methyl-5-heptene, was identified and quantified. It was present only in pea-containing sourdough, although in little amounts.

Lipid oxidation also generated alcohols, such as 1-hexanol and 2-heptanol, while the fermentation of carbohydrates produced ethyl alcohol (Xiang et al., 2023). Of particular relevance, in S2 and S3 (containing pea), were 1-octen-3-ol, 2-methyl-1-butanol, and 3-methyl-1-butanol (the latter quantitatively relevant), all typically detected in the volatile profile of peas (Trindler et al., 2022; Xiang et al., 2023). The first two compounds could derive from leucine and isoleucine, involved in the Ehrlich pathway in yeast cells (De Luca et al., 2021).

2-Pentylfuran, another compound associated with the typical pea odor (Trindler et al., 2022; Xiang et al., 2023), was significantly higher in S2 and S3 (2.34 and 1.67 μg/g respectively), than in S1.

The LAB fermentation produces abundant acetic acid, which is responsible for the sour aroma of bread prepared with sourdough (Pétel et al., 2017). The concentration of acetic acid was higher in the sourdough formulated with semolina only, than in the others. Numerous esters were also detected, mostly acetates and lactates. Esters arise from fermentation and can enhance the complexity of the volatile profile, contributing with several sensory notes. For example, ethyl lactate, higher in S1, is associated with caramel and butter notes, whereas octyl acetate, higher in S2 and S3, confers green and mushroom notes. Finally, ethyl acetate, one of the most abundant volatile compounds reported in sourdough, characterized by a fruity odor (Pétel et al., 2017), was more concentrated in S1. These differences were related to the raw material and wild microflora composition, inducing different metabolic pathways (Pétel et al., 2017).

Table 8 reports also the volatile compounds of *focaccia* samples. The volatile compounds of sourdough were generated mainly by enzymatic and microbial processes during fermentation, while thermal reactions caused the formation of new compounds in the baked *focaccia*, such as the Maillard reaction products. An overall comparative evaluation shows that the amounts of volatile compounds decreased significantly from sourdough to *focaccias*. Previous studies carried out on bread reported that this decrease is principally due to the evaporation during baking but also to the "dilution" of sourdough in the final product. For example, from sourdough to bread the majority of acids and esters, which are among the main fermentation compounds and are very volatile, tend to disappear (Pétel et al., 2017), as it was the case also in the examined *focaccias*. On the contrary, 2-methylbutanal and 3-methylbutanal increase from sourdough to bread due to the free amino acids provided by sourdough, similarly to the findings observed in *focaccia* samples.

The use of pea flour and pea sourdough had a significant ($p \leq 0.05$) impact also on the quali-quantitative profile of the volatile compounds of *focaccia*. Aldehydes were the predominant class, followed by alcohols. The concentration of hexanal, octanal, nonanal, 2-heptenal and 2-octenal was higher in the *focaccias* with pea flour and pea-based sourdough, in line with their recognized role in the perception of typical pea odor. 2,4-Heptadienal was detected only in T2 and T3 *focaccias*, in agreement with its presence in the volatile profile of the two pea-based sourdoughs used (S2 and S3). Lipid oxidation, fermentation and Maillard reaction produced benzaldehyde, with almond and sweet flavors (Pétel et al., 2017), significantly higher in the T1, T2 and T3 *focaccias,* containing sourdough. This compound is typical of the volatile profile of bread and baked goods, resulting influenced by the type of yeast, the amount added, and the temperature of fermentation (De Luca et al., 2021). The 2- and 3-methylbutanal, oxidized metabolites of 2- and 3-methylbutanol, were markedly higher when sourdough was used, with significantly lower concentration in the case of pea-based sourdough (T2 and T3). The content of these Strecker aldehydes, originated from leucine and isoleucine, presented a wide range of variation, between 0.82 and 15.49 μg/g for 2-methylbutanal and 1.67–57.67 μg/g for 3-methylbutanal.

Ethyl alcohol, coming from the fermentative process, largely evaporated during baking and ranged from 0.91 to 3.42 μg/g. 3-Methyl-1-butanol, known to play a role in pea odor perception, was more abundant in pea-containing *focaccias* than in those prepared with semolina only. It ranged between 1.72 and 4.15 μg/g. Similarly, the 1-octen-3-ol was higher when pea flour, and especially pea-based sourdough, were used.

A significant reduction of esters, attributable to the baking process, was observed by comparing the volatile profiles of sourdoughs and *focaccias*, as previously reported by other authors (Pétel et al., 2017). As far as acids are concerned, the only one detected was acetic acid, which was only present in the samples with sourdough (about 0.17–0.21 μg/g). Acetic acid, with sour notes, could positively enrich the aroma of the *focaccias,* representing an added value.

The Maillard reaction between sugars and reducing amino acids generated new compounds known to impact on the aroma of bread and bakery goods (Pétel et al., 2017). The sugar to amino acids ratio influences the intensity of the reaction and, therefore, the concentration of the end products of the reaction, such as furans and pyrazines (De Luca et al., 2021). The concentration of furans and pyrazines was generally higher in *focaccias* with sourdough and in those with pea flour, due to higher lysine content of pea flour, further enhanced by the proteolytic process occurred during sourdough fermentation, that released small peptides and free amino acids (Millar et al., 2019; Troadec et al., 2022).

In general, a positive effect of sourdough fermentation is recognized (Troadec et al., 2022), although the comparison among studies is limited by the difference in microbial composition and fermentation conditions, both strongly influencing the final odor properties. Overall, the use of pea sourdough in *focaccia* increased the levels of some typical odorants of legumes, but also led to higher concentrations of acetic acid, esters, and Maillard reaction compounds which could mask the legume-related ones, generally considered unpleasant. Therefore, the use of pea-sourdough in the production of baked goods should be encouraged.

5. Conclusions

Focaccia is a flatbread generally high in lipids and carbohydrates and relatively low in protein and fiber. This study showed that the addition of pea sourdough is effective in improving the nutritional properties of this flatbread. In fact, reformulated *focaccia* enriched with pea sourdough could be labeled as a "source of protein" and "rich in fiber." Furthermore, pea sourdough improved the characteristics of this traditional street food not only in terms of nutritional profile but also by expressing its functional potential. Indeed, the fermentation of pea contributed to the increase in total phenolic content, resulting in higher *in vitro* antioxidant activity. The antinutritional factors identified in pea flour, such as phytates and oligosaccharides of the raffinose family, were reduced by fermentation, thanks to the microbial activity and acidification occurring in the preparation of sourdough. In addition, a positive effect on the *in vitro* digestibility was observed. Pea sourdough slowed

Table 8
Volatile profile of sourdough samples (S1 = 100 % semolina sourdough; S2 = 100 % pea sourdough; S3 = 50:50 semolina-pea sourdough) and *focaccia* samples. Codes T1-T6 correspond to 6 different formulations as reported in Table 1.

Compounds (μg/g)	S1	S2	S3	T1	T2	T3	T4	T5	T6
Aldehydes									
2-Furancarboxaldheyde	–	–	–	2.03 ± 0.11^{a}	0.52 ± 0.02^{b}	0.57 ± 0.08^{b}	0.27 ± 0.04^{c}	0.54 ± 0.02^{b}	0.52 ± 0.05^{b}
2-Heptenal	–	–	–	0.33 ± 0.03^{d}	1.34 ± 0.05^{b}	2.67 ± 0.03^{a}	0.00 ± 0.00^{e}	0.90 ± 0.05^{c}	0.91 ± 0.04^{c}
2-Octenal	–	–	–	0.00 ± 0.00^{b}	0.22 ± 0.38^{b}	1.69 ± 0.19^{a}	0.00 ± 0.00^{b}	0.07 ± 0.01^{b}	0.10 ± 0.01^{b}
2,4-Heptadienal	0.00 ± 0.00^{c}	7.64 ± 0.53^{a}	3.45 ± 0.97^{b}	0.00 ± 0.00^{b}	0.08 ± 0.02^{a}	0.10 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
2,4-Hexadienal	0.20 ± 0.00^{c}	3.68 ± 0.28^{a}	2.50 ± 0.10^{b}	–	–	–	–	–	–
2-Methylbutanal	–	–	–	15.49 ± 1.54^{a}	7.46 ± 0.59^{b}	3.17 ± 0.31^{c}	0.82 ± 0.10^{e}	1.66 ± 0.01^{d}	1.16 ± 0.02^{d}
3-Methylbutanal	–	–	–	57.67 ± 4.33^{a}	26.43 ± 2.16^{b}	10.36 ± 0.19^{c}	1.67 ± 0.14^{e}	3.09 ± 0.26^{d}	2.47 ± 0.18^{d}
Benzaldehyde	–	–	–	3.67 ± 0.33^{a}	2.88 ± 0.15^{b}	3.56 ± 0.25^{a}	0.63 ± 0.08^{d}	1.23 ± 0.20^{c}	1.51 ± 0.06^{c}
Heptanal	2.02 ± 0.17^{c}	7.84 ± 0.38^{b}	8.83 ± 0.06^{a}	–	–	–	–	–	–
Hexanal	24.73 ± 1.75^{c}	47.82 ± 0.65^{a}	28.2 ± 0.02^{b}	4.65 ± 0.56^{d}	14.13 ± 0.78^{b}	17.69 ± 1.08^{a}	1.40 ± 0.11^{e}	4.84 ± 0.28^{d}	6.93 ± 0.41^{c}
Hexenal	1.08 ± 0.05^{c}	4.01 ± 0.05^{a}	1.95 ± 0.12^{b}	–	–	–	–	–	–
Nonanal	8.38 ± 0.95^{b}	10.37 ± 0.20^{a}	10.16 ± 0.29^{a}	1.84 ± 0.30^{a}	1.57 ± 0.03^{a}	1.76 ± 0.09^{a}	0.22 ± 0.02^{d}	0.33 ± 0.01^{c}	0.53 ± 0.01^{b}
Octanal	0.81 ± 0.04^{c}	1.31 ± 0.01^{a}	1.18 ± 0.03^{b}	1.54 ± 0.04^{b}	1.51 ± 0.11^{b}	2.82 ± 0.12^{a}	0.25 ± 0.01^{e}	0.49 ± 0.05^{d}	0.78 ± 0.04^{c}
Alcohols									
1-Heptanol	–	–	–	0.27 ± 0.07^{c}	0.78 ± 0.05^{b}	1.46 ± 0.05^{a}	0.24 ± 0.02^{c}	0.78 ± 0.12^{b}	0.96 ± 0.03^{b}
2-Heptanol	1.36 ± 0.08^{c}	2.77 ± 0.16^{a}	2.34 ± 0.01^{b}	–	–	–	–	–	–
1-Hexanol	16.02 ± 0.18^{c}	26.23 ± 0.86^{b}	31.46 ± 0.88^{a}	–	–	–	–	–	–
1-Octen-3-ol	2.64 ± 1.29^{c}	11.15 ± 1.46^{a}	5.30 ± 0.73^{b}	0.22 ± 0.12^{cd}	0.80 ± 0.04^{ab}	1.11 ± 0.26^{a}	0.06 ± 0.01^{d}	0.23 ± 0.03^{cd}	0.51 ± 0.07^{bc}
2-Ethyl-1-hexanol	2.97 ± 0.08^{a}	2.69 ± 0.20^{a}	3.20 ± 0.31^{a}	0.00 ± 0.00^{b}	0.21 ± 0.01^{a}	0.00 ± 0.00^{b}	0.21 ± 0.02^{a}	0.19 ± 0.03^{a}	0.19 ± 0.01^{a}
2-Methyl-1-butanol	0.00 ± 0.00^{c}	3.90 ± 0.10^{a}	0.47 ± 0.03^{b}	–	–	–	–	–	–
3-Methyl-1-butanol	72.56 ± 1.25^{b}	102.31 ± 0.76^{a}	100.56 ± 7.39^{a}	2.31 ± 0.14^{c}	2.74 ± 0.08^{b}	4.15 ± 0.12^{a}	1.72 ± 0.02^{e}	2.00 ± 0.15^{de}	2.09 ± 0.06^{cd}
Ethyl alcohol	11.52 ± 0.75^{b}	15.00 ± 1.00^{a}	13.89 ± 0.07^{a}	0.91 ± 0.08^{e}	2.00 ± 0.08^{bc}	3.42 ± 0.11^{a}	1.52 ± 0.05^{d}	1.89 ± 0.13^{c}	2.30 ± 0.22^{b}
Nonanol	–	–	–	0.11 ± 0.01^{b}	0.72 ± 0.02^{a}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Esters									
1-Butanol-3-methyl acetate	18.75 ± 1.02^{a}	7.79 ± 0.50^{b}	0.00 ± 0.00^{c}	–	–	–	–	–	–
Decanoic acid ethyl ester	2.13 ± 0.03^{a}	1.90 ± 0.08^{b}	1.54 ± 0.01^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.52 ± 0.09^{a}	0.11 ± 0.04^{b}	0.12 ± 0.00^{c}
Ethyl acetate	66.61 ± 2.13^{a}	7.59 ± 0.73^{c}	30.87 ± 0.96^{b}	–	–	–	–	–	–
Ethyl heptanoate	0.00 ± 0.00^{c}	0.62 ± 0.07^{b}	2.30 ± 0.26^{a}	–	–	–	–	–	–
Ethyl lactate	15.89 ± 0.19^{a}	2.11 ± 0.16^{c}	11.67 ± 0.75^{b}	0.45 ± 0.01^{a}	0.40 ± 0.02^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Hexanoic acid ethyl ester	1.67 ± 0.03^{b}	4.90 ± 0.22^{a}	1.13 ± 0.08^{b}	–	–	–	–	–	–
Octanoic acid ethyl ester	1.52 ± 0.06^{b}	0.00 ± 0.00^{c}	8.47 ± 0.31^{a}	–	–	–	–	–	–
Octyl acetate	0.00 ± 0.00^{c}	8.42 ± 0.18^{a}	4.20 ± 0.07^{b}	–	–	–	–	–	–
Acids									
Acetic acid	37.58 ± 2.65^{a}	26.63 ± 0.91^{b}	27.11 ± 0.72^{b}	3.18 ± 0.03^{a}	3.17 ± 0.05^{a}	3.21 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Ketones									
6-Methyl-5-heptene	0.00 ± 0.00^{c}	1.20 ± 0.10^{a}	0.97 ± 0.07^{b}	–	–	–	–	–	–
Furans									
2-Furanmethanol	–	–	–	0.16 ± 0.03^{d}	0.23 ± 0.02^{c}	0.25 ± 0.02^{b}	0.18 ± 0.00^{c}	0.31 ± 0.04^{a}	0.25 ± 0.01^{b}
2-Pentylfuran	1.59 ± 0.05^{c}	2.34 ± 0.03^{a}	1.67 ± 0.05^{b}	0.35 ± 0.04^{a}	0.18 ± 0.01^{c}	0.26 ± 0.02^{b}	0.07 ± 0.00^{d}	0.09 ± 0.01^{d}	0.10 ± 0.01^{d}
Pyrazines									
Ethyl-pyrazine	–	–	–	0.11 ± 0.00^{c}	0.13 ± 0.00^{b}	0.15 ± 0.00^{a}	0.07 ± 0.01^{e}	0.07 ± 0.01^{e}	0.09 ± 0.00^{d}
Methyl-pyrazine	–	–	–	0.27 ± 0.01^{c}	0.31 ± 0.01^{b}	0.42 ± 0.01^{a}	0.12 ± 0.01^{e}	0.12 ± 0.01^{e}	0.17 ± 0.03^{d}

Data are presented as means ± SD of replicates. The statistical analysis was performed by comparing the three sourdough and the six *focaccia* samples separately. Different letters in the same row indicate significant differences at $p \leq 0.05$.

down the starch digestibility, while enhancing the digestibility of protein. Finally, pea sourdough led to a more complex volatile profile, by increasing the content of aldehydes, alcohols and Maillard reaction compounds, which positively reflect on the aroma of *focaccia*.

These findings show that the use of pea sourdough is a simple and effective way to improve the quality of bakery products while modulating digestibility, and suggest promoting the use of pea sourdough on a larger scale.This simple reformulation is a response to evolving consumer needs for traditional, nutritionally balanced foods prepared using minimally processed and locally available ingredients. Reformulated bakery products, such as *focaccia*, with pea sourdough, could prompt an increase in the consumption of legumes, as suggested by WHO, fitting well into the direction of a protein transition to more sustainable sources. Further research is ongoing regarding the effects on the consumer acceptability and shelf life of *focaccia*, and to explore the benefits of antifungal activity and potential anti-staling effects of pea sourdough.

Funding

This paper was supported by the PRIMA program under grant agreement No. 2031, project Flat Bread of Mediterranean area: Innovation and Emerging Process and Technology (Flat Bread Mine). The PRIMA program is an Art.185 initiative supported and funded under Horizon 2020, the European Union's Framework Program for Research and Innovation. The results and content found in this paper reflect only the authors' view. The PRIMA Foundation is not responsible for any use that may be made of the information this paper contains.

Ethics statement

- The study did not involve humans or animals
- Neither the manuscript nor any parts of its content are currently under consideration or published in another journal.
- All authors have approved the manuscript and agree with its submission.

CRediT authorship contribution statement

Francesca Vurro: Writing – original draft, Investigation, Formal analysis, Data curation. **Maria Santamaria:** Writing – original draft, Investigation, Formal analysis, Data curation. **Carmine Summo:** Writing – review & editing, Formal analysis. **Antonella Pasqualone:** Writing – review & editing, Project administration, Formal analysis. **Cristina M. Rosell:** Writing – review & editing, Project administration, Formal analysis, Conceptualization.

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.

Data availability

Data will be made available on request.

References

AACC International. (2010). Approved methods of analysis. In *American association of cereal chemists* (11th ed.) St. Paul, MN, USA.

Al Khatib, A., Hussein, H. H., Joumaa, Z., Farhat, M., & Tlais, S. (2020). Assessment of fiber-enriched pita bread in the Lebanese market: A positive correlation between dry crude fiber content and TTA. *International Journal of Environment, Agriculture and Biotechnology, 5*(5), 1351–1356. https://doi.org/10.22161/ijeab.55.22

Antognoni, F., Mandrioli, R., Potente, G., Taneyo Saa, D. L., & Gianotti, A. (2019). Changes in carotenoids, phenolic acids and antioxidant capacity in bread wheat doughs fermented with different lactic acid bacteria strains. *Food Chemistry, 292*, 211–216. https://doi.org/10.1016/j.foodchem.2019.04.061

AOAC. (2006). Official methods of analysis of AOAC. In *Official methods of analysis* (18th ed.) Arlington, WA, USA.

Ashokkumar, K., Diapari, M., Jha, A. B., Tar'an, B., Arganosa, G., & Warkentin, T. D. (2015). Genetic diversity of nutritionally important carotenoids in 94 pea and 121 chickpea accessions. *Journal of Food Composition and Analysis, 43*, 49–60.

Baik, B. K., & Han, I. H. (2012). Cooking, roasting, and fermentation of chickpeas, lentils, peas, and soybeans for fortification of leavened bread. *Cereal Chemistry, 89*(6), 269–275. https://doi.org/10.1094/CCHEM-04-12-0047-R

Bourré, L., McMillin, K., Borsuk, Y., Boyd, L., Lagassé, S., Sopiwnyk, E., Jones, S., Dyck, A., & Malcolmson, L. (2019). Effect of adding fermented split yellow pea flour as a partial replacement of wheat flour in bread. *Legume Science, 1*(1), Article e2. https://doi.org/10.1002/leg3.2

Catzeddu, P., Fois, S., Tolu, V., Sanna, M., Braca, A., Vitangeli, I., Anedda, R., & Roggio, T. (2023). Quality evaluation of fresh pasta fortified with sourdough containing wheat germ and wholemeal semolina. *Foods, 12*(14), 2641. https://doi.org/10.3390/foods12142641

Chavan, R. S., & Chavan, S. R. (2011). Sourdough technology-a traditional way for wholesome foods: A review. *Comprehensive Reviews in Food Science and Food Safety, 10*, 169–182. https://doi.org/10.1111/j.1541-4337.2011.00148.x

Coda, R., Varis, J., Verni, M., Rizzello, C. G., & Katina, K. (2017). Improvement of the protein quality of wheat bread through faba bean sourdough addition. *LWT, 82*, 296–302. https://doi.org/10.1016/j.lwt.2017.04.062

Curiel, J. A., Coda, R., Centomani, I., Summo, C., Gobbetti, M., & Rizzello, C. G. (2015). Exploitation of the nutritional and functional characteristics of traditional Italian legumes: The potential of sourdough fermentation. *International Journal of Food Microbiology, 196*, 51–61. https://doi.org/10.1016/j.ijfoodmicro.2014.11.032

Davies-Hoes, L. D., Scanlon, M. G., Girgih, A. T., & Aluko, R. E. (2017). Effect of pea flours with different particle sizes on antioxidant activity in pan breads. *Cereal Chemistry, 94*(5), 866–872.

De Angelis, D., Pasqualone, A., Allegretta, I., Porfido, C., Terzano, R., Squeo, G., & Summo, C. (2021). Antinutritional factors, mineral composition and functional properties of dry fractionated flours as influenced by the type of pulse. *Heliyon, 7*(2), Article e06177. https://doi.org/10.1016/j.heliyon.2021.e06177

De Angelis, D., Vurro, F., Santamaria, M., Garzon, R., Rosell, C. M., Summo, C., & Pasqualone, A. (2023). Effect of dry-fractionated pea protein on the physicochemical properties and the nutritional features of gluten-free focaccia flat bread. *LWT, 182*, Article 114873. https://doi.org/10.1016/j.lwt.2023.114873

De Luca, L., Aiello, A., Pizzolongo, F., Blaiotta, G., Aponte, M., & Romano, R. (2021). Volatile organic compounds in breads prepared with different sourdoughs. *Applied Sciences, 11*(3), 1330. https://doi.org/10.3390/app11031330

Demirkesen-Bicak, H., Arici, M., Yaman, M., Karasu, S., & Sagdic, O. (2021). Effect of different fermentation condition on estimated glycemic index, in vitro starch digestibility, and textural and sensory properties of sourdough bread. *Foods, 10*(3), 514. https://doi.org/10.3390/foods10030514

Drakula, S., Novotni, D., Čukelj Mustač, N., Voučko, B., Krpan, M., Vahčić, N., Hruškar, M., & Ćurić, D. (2024). Volatile profile and sensory properties of gluten-free bread with yellow pea flour and sourdough. *European Food Research and Technology, 250*, 1–16. https://doi.org/10.1007/s00217-023-04439-y

Easterbrook-Smith, G. (2021). By bread alone: Baking as leisure, performance, sustenance, during the Covid-19 crisis. *Leisure Sciences, 43*, 36–42. https://doi.org/10.1080/01490400.2020.1773980

Eraslan, H., Wehbeh, J., & Ermis, E. (2023). Effect of sourdough prepared with the combination of chickpea and carob on bread properties. *International Journal of Gastronomy and Food Science, 32*, Article 100753. https://doi.org/10.1016/j.ijgfs.2023.100753

Espinosa-Ramírez, J., Garzon, R., Serna-Saldivar, S. O., & Rosell, C. M. (2018). Functional and nutritional replacement of gluten in gluten-free yeast-leavened breads by using β-conglycinin concentrate extracted from soybean flour. *Food Hydrocolloids, 84*, 353–360. https://doi.org/10.1016/j.foodhyd.2018.06.021

European Parliament and Council. Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December. (2006). On nutrition and health claims made on foods (2006). *Official Journal of the European Union, L404*, 9–25.

European Parliament and Council. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October. (2011). On the provision of food information to consumers, amending regulations (EC) no 1924/2006 and (EC) no 1925/2006 of the European Parliament and of the council, and repealing commission directive 87/250/EEC, council directive 90/496/EEC, commission directive 1999/10/EC, directive 2000/13/EC of the European Parliament and of the council, commission directives 2002/67/EC and 2008/5/EC and commission regulation (EC) no 608/2004. *Official Journal of the European Union, L304*, 18–63.

Hager, A. S., Wolter, A., Jacob, F., Zannini, E., & Arendt, E. K. (2012). Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours. *Journal of Cereal Science, 56*(2), 239–247.

ISO 21527-2. (2008). *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds*. Geneva, Switzerland: ISO.

ISTAT. (2024). Cereali, legumi, radici, bulbi e tuberi. http://dati.istat.it/Index.aspx?QueryId=33702 Accessed April 27, 2024.

Jekle, M., Houben, A., Mitzscherling, M., & Becker, T. (2010). Effects of selected lactic acid bacteria on the characteristics of amaranth sourdough. *Journal of the Science of Food and Agriculture, 90*(13), 2326–2332. https://doi.org/10.1002/jsfa.4091

Lazo-Vélez, M. A., Garzon, R., Guardado-Félix, D., Serna-Saldivar, S. O., & Rosell, C. M. (2021). Selenized chickpea sourdoughs for the enrichment of breads. *LWT, 150*, Article 112082. https://doi.org/10.1016/j.lwt.2021.112082

Lu, Z. H., Donner, E., & Liu, Q. (2018). Effect of roasted pea flour/starch and encapsulated pea starch incorporation on the in vitro starch digestibility of pea

breads. *Food Chemistry, 245*, 71–78. https://doi.org/10.1016/j.foodchem.2017.10.037

Mavroeidis, A., Roussis, I., & Kakabouki, I. (2022). The role of alternative crops in an upcoming global food crisis: A concise review. *Foods, 11*(22), 3584.

Millar, K. A., Gallagher, E., Burke, R., McCarthy, S., & Barry-Ryan, C. (2019). Proximate composition and anti-nutritional factors of fava-bean (*Vicia faba*), green-pea and yellow-pea (*Pisum sativum*) flour. *Journal of Food Composition and Analysis, 82*, Article 103233. https://doi.org/10.1016/j.jfca.2019.103233

Moreno-Araiza, O., Boukid, F., Suo, X., Wang, S., & Vittadini, E. (2023). Pretreated green pea flour as wheat flour substitutes in composite bread making. *Foods, 12*(12), 2284.

Padhi, E. M. T., Liu, R., Hernandez, M., Tsao, R., & Ramdath, D. D. (2017). Total polyphenol content, carotenoid, tocopherol and fatty acid composition of commonly consumed Canadian pulses and their contribution to antioxidant activity. *Journal of Functional Foods, 38*, 602–611. https://doi.org/10.1016/j.jff.2016.11.006

Pasqualone, A., De Angelis, D., Squeo, G., Difonzo, G., Caponio, F., & Summo, C. (2019). The effect of the addition of Apulian black chickpea flour on the nutritional and qualitative properties of durum wheat-based bakery products. *Foods, 8*(10), 504. https://doi.org/10.3390/foods8100504

Pasqualone, A., Delcuratolo, D., & Gomes, T. (2011). Focaccia Italian flat fatty bread. In *Flour and breads and their fortification in health and disease prevention* (pp. 47–58). Elsevier Inc.. https://doi.org/10.1016/B978-0-12-380886-8.10005-4

Pasqualone, A., Vurro, F., Summo, C., Abd-El-Khalek, M. H., Al-Dmoor, H. H., Grgic, T., et al. (2022). The large and diverse family of Mediterranean flat breads: A database. *Foods, 11*, 2326. https://doi.org/10.3390/foods11152326

Pasqualone, A., Vurro, F., Wolgamuth, E., Yusuf, S., Squeo, G., De Angelis, D., & Summo, C. (2023). Physical-chemical and nutritional characterization of Somali *laxoox* flatbread and comparison with Yemeni *lahoh* flatbread. *Foods, 12*, 3050. https://doi.org/10.3390/foods12163050

Pedrosa, M. M., Varela, A., Domínguez-Timón, F., Tovar, C. A., Moreno, H. M., Borderías, A. J., & Díaz, M. T. (2020). Comparison of bioactive compounds content and techno-functional properties of pea and bean flours and their protein isolates. *Plant Foods for Human Nutrition, 75*, 642–650.

Pétel, C., Onno, B., & Prost, C. (2017). Sourdough volatile compounds and their contribution to bread: A review. In *, 59. Trends in food science and technology* (pp. 105–123). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2016.10.015.

Rizzello, C. G., Calasso, M., Campanella, D., De Angelis, M., & Gobbetti, M. (2014). Use of sourdough fermentation and mixture of wheat, chickpea, lentil and bean flours for enhancing the nutritional, texture and sensory characteristics of white bread. *International Journal of Food Microbiology, 180*, 78–87. https://doi.org/10.1016/j.ijfoodmicro.2014.04.005

Rungruangmaitree, R., & Jiraungkoorskul, W. (2017). Pea, *Pisum sativum*, and its anticancer activity. *Pharmacognosy Reviews, 11*(21), 39.

Sáez, G. D., Saavedra, L., Hebert, E. M., & Zárate, G. (2018). Identification and biotechnological characterization of lactic acid bacteria isolated from chickpea sourdough in northwestern Argentina. *LWT, 93*, 249–256. https://doi.org/10.1016/j.lwt.2018.03.040

Santamaria, M., Montes, L., Garzon, R., Moreira, R., & Rosell, C. M. (2022). Unraveling the impact of viscosity and starch type on the in vitro starch digestibility of different gels. *Food & Function, 13*(14), 7582–7590. https://doi.org/10.1039/D2FO00697A

Sharma, A. (2021). A review on traditional technology and safety challenges with regard to antinutrients in legume foods. *Journal of Food Science and Technology, 58*, 2863–2883. Springer https://doi.org/10.1007/s13197-020-04883-8.

Suchintita Das, R., Tiwari, B. K., & Garcia-Vaquero, M. (2023). The fundamentals of bread making: The science of bread. In *Traditional European breads: An illustrative compendium of ancestral knowledge and cultural heritage* (pp. 1–40). Cham: Springer International Publishing.

Trindler, C., Annika Kopf-Bolanz, K., & Denkel, C. (2022). Aroma of peas, its constituents and reduction strategies. Effects from breeding to processing. *Food Chemistry, 376*, Article 131892. https://doi.org/10.1016/j.foodchem.2021.131892

Troadec, R., Nestora, S., Niquet-Léridon, C., Marier, D., Jacolot, P., Sarron, E., … Jouquand, C. (2022). Effect of leavening agent on Maillard reaction and the bifidogenic effect of traditional French bread. *Food Chemistry, 393*, Article 133387. https://doi.org/10.1016/j.foodchem.2022.133387

UNE-EN 15787. (2022). *Methods of sampling and analysis - detection and enumeration of Lactobacillus spp. used as feed additive*. Madrid, Spain: UNE.

Vurro, F., Summo, C., Squeo, G., Caponio, F., & Pasqualone, A. (2022). The use of durum wheat oil in the preparation of focaccia: Effects on the oxidative stability and physical and sensorial properties. *Foods, 11*(17), 2679. https://doi.org/10.3390/foods11172679

Wang, J., Kadyan, S., Ukhanov, V., Cheng, J., Nagpal, R., & Cui, L. (2022). Recent advances in the health benefits of pea protein (*Pisum sativum*): Bioactive peptides and the interaction with the gut microbiome. *Current Opinion in Food Science, 48*, Article 100944. https://doi.org/10.1016/j.cofs.2022.100944

Wolter, A., Hager, A. S., Zannini, E., Galle, S., Gänzle, M. G., Waters, D. M., & Arendt, E. K. (2014). Evaluation of exopolysaccharide producing *Weissella cibaria* MG1 strain for the production of sourdough from various flours. *Food Microbiology, 37*, 44–50. https://doi.org/10.1016/j.fm.2013.06.009

Xiang, L., Zhu, W., Jiang, B., Chen, J., Zhou, L., & Zhong, F. (2023). Volatile compounds analysis and biodegradation strategy of beany flavor in pea protein. *Food Chemistry, 402*, Article 134275. https://doi.org/10.1016/j.foodchem.2022.134275