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Development and Characterization of a Sunflower-Seed Press-Cake-Based Fermented Food Prototype

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Abstract

Growing environmental and food security concerns have increased interest in circular strategies to valorize agri-food by-products. Sunflower-seed press cake (SSPC), a protein-rich residue from oil extraction, is largely underutilized despite its high nutritional and functional value. This study aimed to develop a fermented plant-based food prototype (PBFP) from SSPC using *Lactococcus lactis* B12 and *Penicillium camemberti*, evaluating microbiological safety, chemical characteristics, and sensory acceptability. A blend containing 40% SSPC and 60% water was autoclaved, inoculated, and ripened for 4 weeks under controlled temperatures. Microbial counts, pH evolution, free amino acids, biogenic amines, volatile organic compounds (VOCs), cyclopiazonic acid (CPA) content, and sensory attributes were evaluated using cultural techniques, HPLC, HS-SPME/GC-MS, LC–ESI–MS/MS (QTRAP 4000), and sensory evaluation. *L. lactis* efficiently acidified the matrix (pH ≈ 4.5–4.9), ensuring microbial food safety, with high LAB counts (~10^9 CFU/g) and absence of pathogens (*Listeria monocytogenes* and *Salmonella* spp.) and hygienic markers < 2 log CFU/g (*B. cereus*, *E. coli*, and *Enterobacteriaceae*). Free amino acids decreased during fermentation, and no histamine or tyramine was detected. VOC analysis revealed diacetyl, acetoin, 2,3-butanediol, and 1-octen-3-ol, contributing to mild dairy-like notes. CPA was detected at 0.48 ng/g, well below levels reported in cheeses. Sensory evaluation showed no significant differences in overall intensity between inoculated and control blends, although qualitative descriptors indicated subtle changes in aroma and texture. These results demonstrate the feasibility of safely producing a fermented plant-based prototype from SSPC. Future studies should explore longer ripening times, additional microbial consortia, and strategies to enhance texture and aroma complexity.

Keywords: biogenic amines; by-products valorization; cyclopiazonic acid; *Lactococcus lactis*; plant-based food; *Penicillium camemberti*; sunflower-seed press cake; volatile organic compounds

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1. Introduction

In recent years, growing concerns about environmental sustainability, resource efficiency, and global food security have intensified scientific research on strategies capable of

promoting circularity within agri-food systems. Although the European Union is currently revising the targets set out in the European Circular Economy Action Plan introduced in 2015 [1], reducing food losses across the supply chain remains a major challenge due to technological limitations, consumer behavior, and policy fragmentation [2]. As a consequence, a substantial proportion of biomass generated along the food chain remains underutilized, despite the fact that many side streams and by-products retain significant nutritional and functional value. Among these, protein-rich residues are a compelling resource, as they offer considerable potential for upcycling into new, high-value food products or ingredients.

Within this context, vegetable oil production represents an important sector of the European agro-industrial system, with the sunflower crop (*Helianthus annuus* L.) forming the backbone of both food and feed supply chains, with an annual production of 9.5 M tons [3]. It has been estimated that up to 36% of the obtained mass ends up as waste streams, including nutrient-dense press cakes, which are traditionally destined for animal feed, energy generation or disposal in landfills [4–6]. Sunflower-seed press cake (SSPC) is the main solid residue obtained after mechanical oil extraction and can be considered an underexploited material for human nutrition due to its high protein content (30–55%), with good digestibility [7–9] and a favorable amino acid (AA) profile, as well as considerable fiber content (13–32%) [10]. SSPC also contains bioactive compounds, such as phenolics (1–4%) and tocopherols [11,12]. In addition to its nutritional attributes, the structural properties of this material, shaped by the interplay of cell wall components, fiber fractions, and residual lipids, confer functional characteristics that may be harnessed in food, feed, or biomaterial applications [13].

Among the strategies proposed for the valorization of agro-industrial by-products, fermentation is an ancient and affordable approach that can promote product safety, extend shelf life, enhance flavors, and produce volatile organic compounds (VOCs). In particular, solid-state fermentation is characterized by lower energy consumption and minimal wastewater generation compared to conventional methods [14]. Lactic acid bacteria (LAB) and the mold *Penicillium camemberti* are well known for their effectiveness in transforming both milk- and plant-based food substrates. LAB play a crucial role because they produce lactic acid and antimicrobial metabolites, thereby contributing to product safety [15]. Among them, *Lactococcus lactis* has shown promising performance in plant-based fermentations, imparting yogurt-like aromatic notes, as evidenced in recent studies [16]; meanwhile, *P. camemberti* is used for its ability to influence key sensory qualities in soft-ripened cheeses, such as texture, smell, and flavor [17,18], although its behavior in plant-based substrates remains less explored.

Fermentation of protein-rich by-products raises specific safety concerns, including the formation of biogenic amines (BAs) and mycotoxins, which must be carefully evaluated. LAB are considered among the main microbial groups associated with BA production in fermented foods, particularly under low pH conditions, as BA accumulation represents a cellular defense mechanism to withstand acid stress [19]. The European Food Safety Authority [20] notes that histamine and tyramine are the most toxicologically significant, although current data are insufficient to define quantitative limits for fermented foods or other categories. Indeed, these two biogenic amines are both known to cause severe symptomatology characterized by gastrointestinal, skin, respiratory, neurological, and cardiovascular disorders [19]. All other amines, such as cadaverine, putrescine, spermidine, and spermine, are managed through guidance values, good manufacturing practices, and product-specific recommendations. However, although there is extensive literature on the presence of biogenic amines in animal-based foods such as cheese and cured meats, information on plant-based products is more limited [21,22]. On the other hand, *P. camemberti*

is associated with the production of the mycotoxin cyclopiazonic acid (CPA), depending on the strain, substrate, and growth conditions [23]. Recent studies have investigated CPA production by *P. camemberti* in some plant-based matrices [24]; however, its behavior in protein-rich sunflower-seed press cake has not yet been assessed, making safety evaluation essential.

The aim of this work was to valorize SSPC by developing a fermented plant-based food prototype (PBFP). The product was obtained through sequential fermentation with *L. lactis* B12 and *P. camemberti* strains isolated from commercial dairy products. The work focused on evaluating the microbiological safety and chemical characteristics of the fermented product, including volatile organic compounds (VOCs), free amino acids, biogenic amines, and mycotoxins, together with an exploratory sensory assessment.

2. Materials and Methods

2.1. Preparation of the Plant-Based Food Prototype (PBFP)

The SSPC was provided by Schalk Mühle GmbH & CoKG (Kalsdorf bei Ilz, Austria). It was obtained by de-oiling and grinding the kernels of organic dehulled sunflower seeds. The chemical composition (%, *w*/*w*) of SSPC, as reported by the manufacturer, was: 7.2 of fat, 4.1 of carbohydrates, 23.0 of fiber, 53.0 of protein, and traces of minerals. The mixture used to produce the PBFP consisted of 40% SSPC and 60% demineralized water (final dry matter of the blend 34.9%, *w*/*w*). Before blending, calcium citrate tetrahydrate (ThermoFisher Scientific, Waltham, MA, USA) was added to the demineralized water at 16.6 g/kg to achieve a final calcium content of 0.4% (*w*/*w*) in PBFP. The calcium levels were selected to meet approximately 50% of the daily reference intake, as indicated in Regulation (EU) No. 1169/2011 [25] on the provision of food information to consumers. The blend was homogenized in a planetary mixer (model CNUM5ST, Bosch, Gerlingen, Germany) at speed 2 (medium–low) for 10 min, followed by speed 3 (medium–high) for another 10 min. The mixture was placed in special containers (Scharlab, Barcelona, Spain), autoclaved at 121 °C for 15 min, and then promptly cooled to room temperature.

The material was inoculated under aseptic conditions with an aliquot of fresh cultures of *L. lactis* B12 and spores of *P. camemberti* to obtain a starting concentration of 6.0 log CFU/g and 5.0 log CFU/g, respectively. The control sample corresponded to the non-inoculated blend. After stirring, approximately 100 g aliquots of the inoculated blend were transferred into sterile perforated polyethylene wheels, 8 cm in diameter and 8 cm high (Tecnolatte, Lodi, Italy), and incubated at 30 °C for 48 h. This step ("warm" phase) aimed to promote acidification upon lactococcal growth to ensure product safety and enhance flavor. Subsequently, the samples were transferred to chambers at controlled temperatures (5, 15 and 25 °C) with relative humidity > 90% for a ripening period of two or four weeks. The flow chart of PBFP production is presented in Figure 1.

2.2. Microbial Strains

The strains used in this work were *L. lactis* B12, isolated from a commercial kefir [26], and *P. camemberti* spores derived from a commercial, lyophilized starter culture for bloomy rind cheeses (Tecnolatte).

A concentrated cell suspensions of *L. lactis* B12 were prepared by inoculating 100 µL of the glycerol stock culture into 20 mL of MRS broth and incubating at 30 °C for 24 h. The resulting fresh culture was centrifuged at 8000× *g* (Rotina 380 R, Hettich, Tuttlingen, Germany), washed and resuspended in tryptone (1 g/L, pH 6.5) salt (NaCl 8 g/L) broth, and stored at 4 °C for a maximum of 10 days.

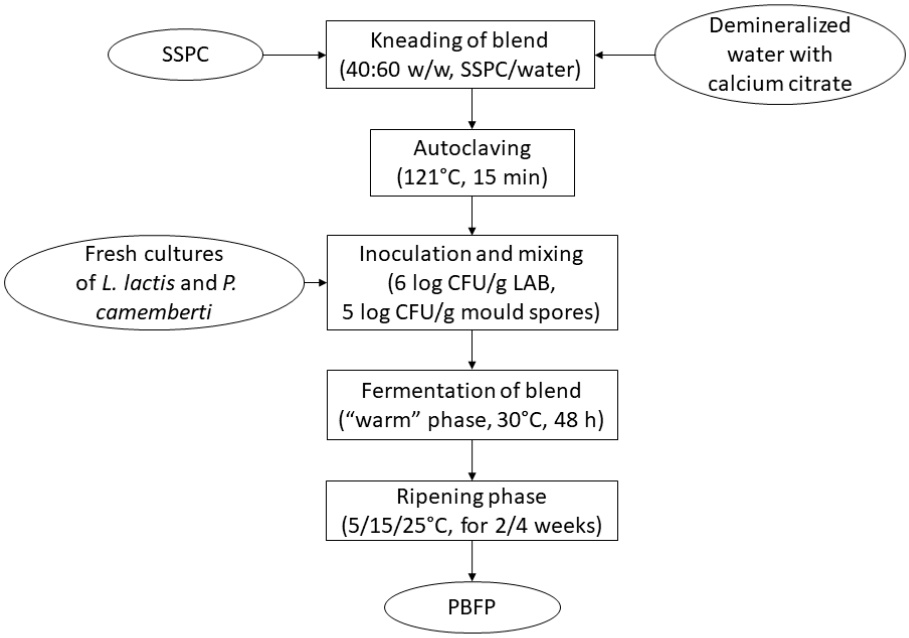

Figure 1. Flow chart of plant-based food prototype (PBFP) production from sunflower press cake (SSPC).

Regarding the isolation and identification of the mold strain, after decimal dilutions of the lyophilized starter culture sample in sterile peptone water (peptone 10 g/L, pH 6.8), 100 µL aliquots were spread onto potato dextrose agar plates (Sigma Aldrich, St. Louis, MO, USA) and incubated at 25 °C for five days. Consecutive streaking of a single colony on the same medium and incubation in the same conditions were carried out to obtain an isolated culture of the mold. Then the fungal DNA was extracted from liquid cultures grown in potato dextrose broth (Sigma Aldrich) at 25 °C for 48 h in agitation (120 rpm), and the mold was identified as *P. camemberti* by sequencing of the ITS region, following the protocol reported by Vigentini et al. (2016) [27]. Concentrated spore suspension of *P. camemberti* was obtained as follows: fresh colonies were cultivated on PDA by incubating at 25 °C for five days; 5 mL of sterile peptone water containing 0.1% Tween 80 (Sigma Aldrich) was added to each Petri dish, and the superficial mycelium was disrupted using a sterile spatula. Then, the suspension was recovered, shaken and filtered through a double layer of sterile gauze to separate the spores from the hyphae. The enumeration of the spores was done by microscopical observation (Nexscope, NE700 series, Ningbo Yongxin Optics Co., Ningbo, China) using a Thoma counting chamber at 400× magnification. Depending on the required concentration, the suspension was centrifuged at 8000× *g* (Rotina 380 R), resuspended in peptone water and stored at 4 °C for a maximum of 10 days.

2.3. Microbial Analysis of the Samples

Approximately 10 g of SSPC/water blend or PBFP sample was aseptically collected into sterile plastic bags containing approximately 90 mL of tryptone salt broth. After homogenization using a Stomacher® 400 apparatus (Seward, Worthing, UK) for 2 min, decimal dilutions were performed in the same solution. Counts of LAB, yeasts and molds, *Escherichia coli* and Enterobacteriaceae, and presumptive *Bacillus cereus* were carried out using the plate count technique. Particularly, lactic acid bacteria were enumerated on MRS agar (Scharlab) after incubation in anaerobiosis (GasPack system, Merck, Darmstadt, Germany) at 30 °C for 48 h; yeasts and molds were cultivated on yeast extract glucose chloramphenicol agar (Merck) at 25 °C for 72 h; *Escherichia coli* and *Enterobacteriaceae* were determined in tryptone bile x-glucuronide agar medium (ThermoFisher Scientific) and

incubated at 30 °C for 48 h; colonies of presumptive *Bacillus cereus* were enumerated on polymyxin pyruvate egg yolk mannitol bromothymol blue agar (Merck) after incubation at 30 °C for 48 h [28].

The potential presence of bacterial pathogens was evaluated in a 25 g sample. Regarding *Salmonella*, the pre-enrichment step was done in buffered peptone water (Merck), with incubation at 37 °C for 24 h; then appropriate aliquots of the pre-enriched culture were transferred to Rappaport–Vassiliadis broth (Scharlab), which was incubated at 42 °C for 24 h and Muller–Kauffmann tetrathionate broth (Scharlab), which was incubated at 37 °C for 24 h. The enriched cultures were respectively streaked onto selective agar plates, such as brilliant green agar (Scharlab) and xylose lysine desoxycholate agar (Merck), and incubated at 37 °C for 24 h. Suspected typical colonies based on characteristic morphology were selected to undergo biochemical or serological tests for confirmation [28]. As regards *Listeria monocytogenes,* a primary enrichment was carried out in half Fraser broth (Scharlab) and incubated at about 30 °C for 24 h. Then, a 1 mL aliquot of the pre-enriched culture was transferred to Fraser broth (Scharlab) and incubated at 37 °C for 24 h. The enriched culture was streaked onto selective agar plates, such as polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol agar (Scharlab) and agar Listeria Ottaviani Agosti (Scharlab), and incubated at 37 °C for 24 h. Suspected typical colonies based on characteristic morphology were selected to undergo biochemical or serological tests for confirmation [28].

2.4. Chemical Analysis of the Samples

2.4.1. pH Measurement

The pH value of the PBFP before, during, and at the end of fermentation was monitored using a pH meter (Jenway, model 3510, ThermoFisher Scientific). Approximately 5 g of material was taken in sterile conditions and diluted with 5 mL of demineralized water to allow pH measurement in a liquid state.

2.4.2. HPLC Analysis of Free Amino Acids (AA), Histamine, and Tyramine

A total of 2.5 g of SSPC/water blend or final PBFP was solubilized in 40 mL of citrate buffer (pH 2.2), stirred for 15 min and thoroughly ground by Ultra-Turrax® for 5 min. The suspension was filtered through paper. A total of 10 mL of filtrate was added to 10 mL of 7.5% sulfosalicylic acid and made up to 25 mL with citrate buffer. After paper filtration, the extract solution was diluted 1:10 with water and filtered through a 0.22 µm PVDF filter (Millipore, Burlington, MA, USA). HPLC analysis of primary free AA and biogenic amines was carried out after o-phthaldialdehyde (OPA) pre-column derivatization. The ethanolic OPA reagent was prepared by dissolving 0.250 g of OPA in 1.5 mL of ethanol mixed with 4–5 mL of 0.4 M borate buffer (pH 10.5). After sonication (5 min) to dissolve the OPA reagent, a final volume of 10 mL was made up using borate buffer solution. The filtered free AA extract supplemented with borate buffer (1:5, v/v) was submitted to in-line pre-column OPA derivatization in the ratio 2:1 (v/v) for 5 min at 20 °C and immediately injected into the HPLC. The OPA AA derivatives were determined using a Waters 2695 HPLC module system (Waters, Milford, MA, USA) equipped with a Waters 2475 fluorescence detector. OPA-derivatized amino acids were separated with a Nova-Pak® C18 (5 µm; 3.9 × 150 mm) and pre-column (3.9 × 20 mm; Waters) using a binary gradient. Mobile phase A consisted of 1% tetrahydrofuran, 8% methanol (MeOH), and 91% 10 mM citrate buffer (pH 7.3) ($v/v/v$). Mobile phase B was 80% MeOH and 20% 10 mM citrate buffer (pH 7.3) (v/v). The gradient program (at a flow rate of 1 mL/min) was 0–6 min, 0% B; 6–17 min, 0–14% B; 17–30 min,14–20% B; 30–38 min, 20–30% B; 38–50 min, 30–40% B; and 50–78 min, 40–80% B. After the separation run, the column was rinsed and conditioned for 8 min. The column temperature was 35 °C. The OPA derivatives were detected by monitoring fluorescence

(350 nm excitation and 440 nm emission wavelengths). Analyses were carried out in triplicate and quantified through the external standard method (0.1–20 mg/L). Proline was determined after pre-column derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) solution (1 mg FMOC-Cl in 10 mL of acetonitrile). The filtered free AA extract was in-line derivatized for 5 min at 20 °C after addition of borate buffer and FMOC solution in the ratio 1:5:3 (*v*/*v*/*v*) and injected into the HPLC. The FMOC derivatives were detected by monitoring fluorescence (266 nm excitation and 305 nm emission wavelengths). Data acquisition and processing were performed by Empower 3 software (Waters). The LOD/LOQ values, calculated using the signal-to-noise approach, were 0.8/2.6 µg/kg of the blend containing 40% SSPC and 60% water and 0.3/1.0 µg/kg for histamine and tyramine, respectively.

2.4.3. Determination of Volatile Organic Compounds (VOCs) by Headspace Solid-Phase Microextraction Coupled with Gas Chromatography-Mass Spectrometry (HS-SPME/GC-MS)

Two g of SSPC/water blend or final PBFP were put in 20 mL vials sealed with polypropylene screw-on caps and PTFE/silicone septa (Supelco, Bellefonte, PA, USA). Vials were heated for 10 min at 45 °C to equilibrate the system, and the VOCs were extracted from the vial headspace for 40 min using a divinylbenzene/carboxen/polydimethylsiloxane 50/30 µm, 2 cm long SPME fiber (Supelco). Thermal desorption of VOCs was carried out by keeping the fiber in the split/split-less injector of GC at 270 °C for 10 min. The VOCs were analyzed using a GC model 6890 (Agilent Technologies, Barcelona, Spain) coupled with an MS model 5973 (Agilent). Peak separation was achieved using a 60 m length Zebron ZB-WAX Plus column (0.25 mm internal diameter × 0.25 µm film thickness (Phenomenex, Torrance, CA, USA)), as previously described by Battelli et al. [29], with modifications.

To improve peak resolution, the split/splitless injector was provided with a 0.75 mm i.d. inlet liner and operated in split mode with a split ratio of 7:1. The following chromatographic conditions were used: oven temperature was held at 45 °C for 10 min, programmed to 150 °C at a rate of 5 °C/min, then to 222 °C at a rate of 12 °C/min. Helium was used as a carrier gas at a flow rate of 1.2 mL/min. The MS parameters were as follows: interface, 220 °C; source, 230 °C; quadrupole, 150 °C. The MS used the electron impact (EI) mode (70 eV), and the mass range m/z 33–300 was scanned. All analyses were carried out in triplicate. The identification of VOCs was performed by comparison with the mass spectra of the Wiley 7n-1 MS library on Agilent MSD ChemStation software (Version: MSD ChemStation F.01.03.2357).

For each VOC, the normalized peak area of the corresponding quantifier ion in the mass spectrum was considered. Statistical testing for differences in VOCs data (area of the chromatographic peak) was not applicable, as the study had an exploratory aim, focusing on monitoring VOCs formed after warm and ripening phases and identifying VOCs peculiar to PBFP.

2.4.4. Determination of Cyclopiazonic Acid (CPA)

Ultrapure water, acetonitrile (ACN), MeOH, chloroform, n-heptane, and 2-isopropanol were of analytical grade and purchased from Merck. Formic acid (98–100%) and ammonium acetate (≥98%) were purchased from Merck. The reference material CPA (0.1 mg/mL in ACN) was purchased from LGC (Wesel, Germany), and internal standard (IS) flufenamic acid (10 mg) was obtained from Merck. Reference material and IS stock solutions were prepared at the final concentration of 10 µg/mL through an appropriate dilution with MeOH. All solutions were stored in the dark at −20 °C. Working solutions were prepared in MeOH from stock solutions (10 ng/mL and 100 ng/mL for CPA and 100 ng/mL for IS) and used for the preparation of a calibration curve (0, 0.10, 0.25, 0.50, 1.0, 2.50, 5.0 ng/g)

in non-inoculated PBFP (500 µL). Prior to extraction, 1 g of each PBFP sample (including n = 1 test, n = 1 non-inoculated, and n = 8 control samples) was homogenized with 1 mL of deionized water. An aliquot of 500 µL was supplemented with 10 µL of IS (100 ng/mL), 250 µL of formic acid (26 M), and 4 mL of extraction solvent mixture (chloroform/n-heptane/2-isopropanol; 50/33/17, *v*/*v*/*v*). After agitation for 10 min and centrifugation at 3000× *g* for 10 min, the organic layer was separated and dried under a stream of nitrogen. The residue was reconstituted in 50 µL MeOH, and an aliquot (2 µL) was injected into a liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) system. The LC–MS/MS analyses were performed on a Dionex UltiMate 3000 HPLC system (ThermoFisher) interfaced with a QTRAP 4000 triple quadrupole mass spectrometer (Sciex, Darmstadt, Germany) operating in electrospray ionization (ESI) mode. Chromatographic separation was achieved on a Kinetex Biphenyl 100 Å column (100 mm length × 2.1 mm i.d, 1.7 µm particle size) at 50 °C using 2 mM of ammonium acetate in water (mobile phase A) and MeOH (mobile phase B). The 16 min linear gradient was the following: 0.0–2.0 min (10% B), 2.0–4.0 min (increase to 40% B), 4.0–6.0 min (40% B), 6.0–8.0 (increase to 100% B), 8.0–12.0 (100% B), 12.0–12.1 min (decrease to 10% B), and 12.1–16.0 min (10% B). The flow rate was 0.13 mL/min. Detection was performed in negative ion mode using multiple reaction monitoring (MRM) (Table 1). The MS parameters were set as follows: entrance potential −10 eV, curtain gas 50 psi, ion spray voltage −4.500 eV, ion source temperature 500 °C, and ion source gases 1 and 2 at 50 psi. Data acquisition and processing were performed using Analyst®1.6.1 and MultiQuant®2.1.1 software (Sciex), respectively. Sensitivity was expressed in terms of limit of detection (LOD) and limit of quantification (LOQ). The LOQ was determined as the lowest concentration with values for precision and accuracy within ±20% and a signal-to-noise (S/N) ratio of the peak areas ≥10. The LOD is the lowest concentration with an S/N of the peak areas ≥3.

Table 1. Mass spectrometry parameters used for CPA analysis.

Q1	Q3	ID	DP (eV)	CE (eV)
280.0	176.0	IS-Flufenamic Acid-1	−80.0	−44.0
280.0	236.0	IS-Flufenamic Acid-2	−80.0	−25.0
335.0	154.0	Cyclopiazonic Acid-1	−58.0	−40.0
335.0	140.0	Cyclopiazonic Acid-2	−58.0	−35.0
335.0	180.0	Cyclopiazonic Acid-3	−58.0	−35.0

2.5. Sensory Evaluation of Samples

An exploratory descriptive sensory analysis of the non-inoculated (control) and the inoculated blend, fermented for 1 month (PBFP), was conducted by two different panels with experience in sensory analysis. Panel A consisted of 8 females and 3 males, and Panel B of 9 females and 4 males, both across a range of ages (25–65 years) and nationalities [Italian (17), Iranian (3), Brazilian (1), Georgian (1), Indian (1) and Spanish (1)]. Each participant was a volunteer recruited from a group of the University of Milan workers and provided their informed consent prior to participation. Sensory evaluation was performed in compliance with the ISO 13299:2016 standard [30] and following the IFST Guidelines for Ethical and Professional Practices for the Sensory Analysis of Foods (2025) [31]. Participants were instructed not to ingest the product during the evaluation. Ethical Committee approval was waived, as the study involved voluntary adult participants, did not include product ingestion, did not collect personal or sensitive data, and was conducted only after microbiological analyses had confirmed product safety.

The experimental design included two separate sessions, one for the control sample and one for the inoculated sample. One panel analyzed the first sample and the other panel the second, representing two independent groups. Approximately 10 g of the sample was served at room temperature on a transparent plate, together with a fork. Participants were also instructed to drink water and consume unsalted crackers during the evaluation to cleanse the palate, as well as to spit out the tasted product. The analyses covered four sensory attributes, previously evaluated in other sensorial analyses by each member of the panels: appearance, odor, taste, and texture. Data were collected using a visual analog scale (VAS) from 0 cm (extremely dislike) to 10 cm (extremely like), with the midpoint (5 cm) serving as a central reference. Participants were asked to mark an x on the scale and had the possibility to add qualitative comments for each attribute.

2.6. Statistical Analysis

Microbial counts (log CFU/g) were obtained from three independent replicates for each experimental condition. Data were analyzed using a two-way analysis of variance (ANOVA), considering ripening time (1 and 2 weeks) and temperature (5, 15, and 25 °C) as fixed factors, including their interaction. When appropriate, post hoc (Tukey's test) comparisons were performed. Statistical significance was set at $p < 0.05$.

Amino acid data were analyzed using a parametric approach. Differences between control and inoculated samples for each amino acid were assessed using Welch's t-test for independent samples, which does not assume equal variances between groups. Given the limited number of biological replicates, results were interpreted with caution.

Sensory data were analyzed using a non-parametric method due to the independence of the panel groups, the limited number of panelists, and the exploratory character of the sensory analysis. Differences between control and inoculated samples for each sensory attribute (appearance, odor, taste, and texture) were assessed using the Mann–Whitney U test for independent samples. Statistical significance was set at $p < 0.05$.

R software (Version: 2026.01.2+418) was used to perform the elaboration of the data and the statistical analysis.

3. Results and Discussion

3.1. Assessment of the Fermentation Behavior and Microbiological Stability of the SSPC/Water Blend

Considering the SSPC-to-water ratio and the SSPC composition provided by the supplier, the composition (% *w*/*w*) of the blend was: 2.9 fat, 1.6 carbohydrates, 9.2 fiber, and 21.2 protein. The SSPC/water blend was autoclaved before inoculation of microbial strains, since the proliferation of potentially dangerous spore-forming bacteria was observed in SSPC in previous works [26,32]. *L. lactis* B12 was chosen for inoculation since this strain has been shown to rapidly acidify similar material [9,16]. Consequently, a target pH below 4.8 was established for the proposed new food product to ensure optimal acidification and microbial safety. Furthermore, calcium citrate was added as a potential carbon source for *L. lactis*, which may contribute to the formation of acetoin and diacetyl [18]. Spores of *P. camemberti* were inoculated to promote the formation of aroma compounds and mycelium on the surface, which gives the product its shape to resemble the characteristics of bloomy-rind cheeses.

The samples were subjected to an initial step called the 'warm' phase at 30 °C for 48 h to promote the growth of lactic acid bacteria (LAB) and the acidification of the matrix. The samples were then incubated at 5 °C, 15 °C, or 25 °C in the dark for two weeks (the ripening phase). The three temperatures were tested based on the selection criteria of reducing and maintaining a safe pH, as well as forming mycelium over the total surface

area of the food prototype. Additionally, *B. cereus* counts were performed to verify the absence of proliferation by these spore-formers. Figure 2 shows the pH trend during both the warm and the ripening phases. The microbial counts of the samples are reported in Table 2.

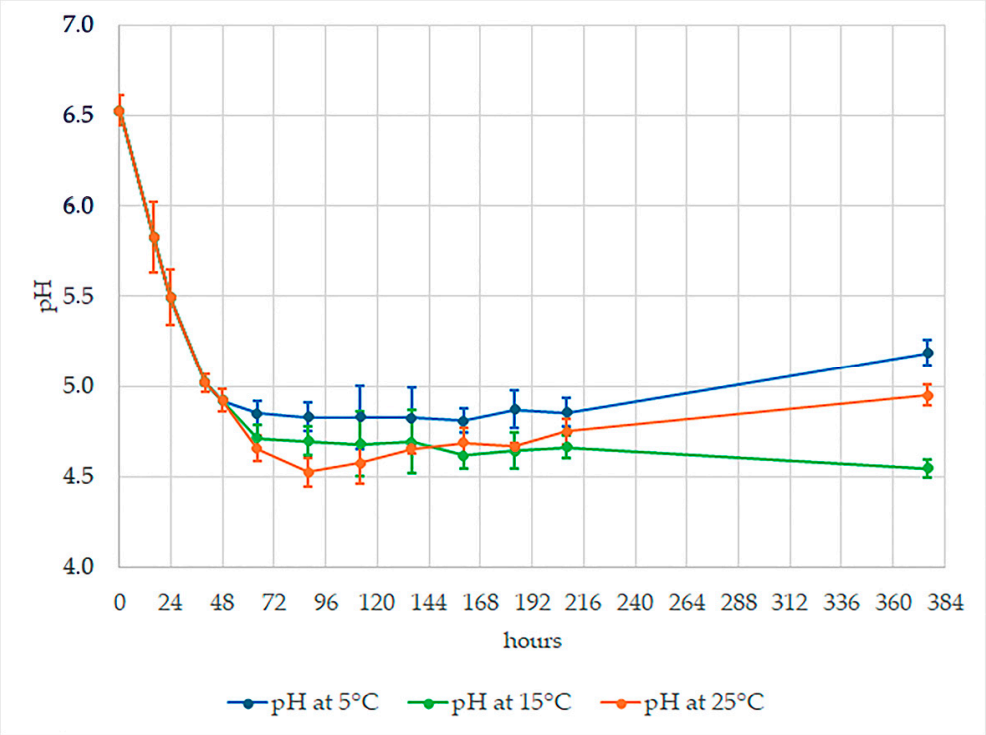

Figure 2. pH trends in samples during warm and ripening phases of SSPC/water blend at different temperatures. Data represent the average of three replicates; bars show the standard deviation.

Table 2. Plate counts of the samples during the "warm" and the ripening phases at different temperatures. Data are $\log_{10}$ CFU/g mean of three replicates ± standard deviation.

Microbial Group	After 48 h at 30 °C	After 1 Week at			After 2 Weeks at		
		5 °C	15 °C	25 °C	5 °C	15 °C	25 °C
LAB	8.92 ± 0.25	9.39 ± 0.02	9.42 ± 0.04	9.46 ± 0.01	9.11 ± 0.63	9.26 ± 0.24	9.38 ± 0.12
Molds	4.68 ± 0.37	4.71 ± 0.51	5.86 ± 0.20	5.57 ± 0.77	4.39 ± 0.91	5.26 ± 0.73	5.36 ± 0.62
Presumptive *B. cereus*	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00

During the warm phase, the pH decreased to 4.9, and the LAB counts reached values of about 10^9 CFU/g, confirming the ability of the *L. lactis* B12 strain to grow optimally on this substrate (Figure 2). Then, at 15 °C, the pH remained consistently below 4.7 throughout the two-week ripening period, suggesting that this temperature supported LAB activity and viability. In contrast, at the other temperatures, the pH started to rise after one week and exceeded 5.1 by the end of the incubation for the samples maintained at 5 °C. This may be due to the slowdown of LAB metabolism in competition with the metabolic activities of the molds, combined with the consumption of lactic acid by *P. camemberti*, as normally occurs on bloomy-rind cheeses [18].

Mold counts exhibited the highest values after one week at all the tested temperatures, followed by a decrease in the second week. At 5 °C, the fungal concentration remained fairly

stable at around 5×10^4 CFU/g, whereas at 15 °C and 25 °C it rose over the two weeks. Visible mycelium appeared on the surfaces of the samples incubated at the highest temperatures after the first week, but the shapes were completely covered only at 15 °C after two weeks of incubation. Microbial counts were also analyzed using two-way ANOVA, considering ripening time and temperature as main factors. No significant differences were found ($p > 0.05$) for LAB and mold counts. Comparable results were obtained in a study on a vegan camembert analog based on flaxseed oil cake, which was fermented using a mixed strain culture of LAB, *P. camemberti*, and *Geotrichum candidum* [33]. Throughout the ripening period at 12 °C, mold counts remained around 10^5 CFU/g, while after approximately two weeks, a development of white surface mycelium was observed, accompanied by a slight increase in pH, likely due to fungal metabolic activities. No presumptive *B. cereus* colonies were observed in all samples, which demonstrates that the preventive heat treatment and the rapid acidification of the material were effective. Considering these results, the temperature of 15 °C was chosen for the subsequent experiment, which followed the same protocol but with the ripening phase extended to one month.

3.2. pH Evolution and Microbiological Characteristics of SSPC/Water Blend and PBFP

Microbiological analyses were performed on samples before and after fermentation in order to assess their safety. Figure 3 shows the pH trend during both the warm and the ripening phases and some images of the product at different times.

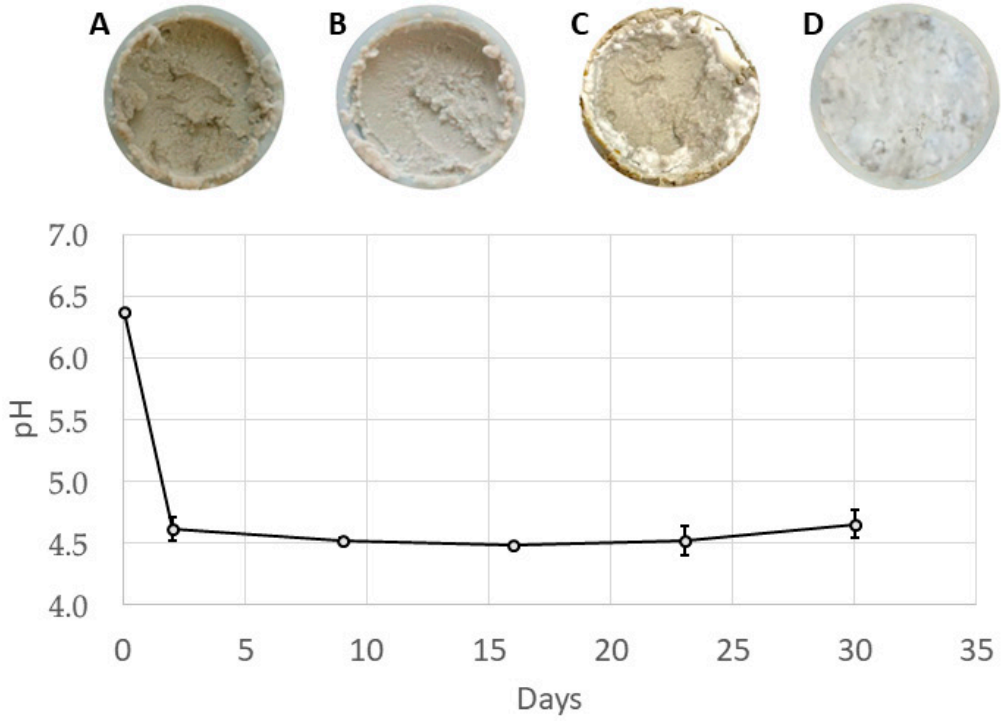

Figure 3. Monitoring of the pH values and images of the SSPC/water blend and PBFB during "warm" and ripening phases. pH data represent the average of three replicates; bars show the standard deviation. A = sample at the starting time; B = sample after the "warm phase"; C = sample after a week; and D = sample after two weeks of ripening.

During incubation at 30 °C, the pH dropped to 4.6, likely causing the material to lighten due to the effect of phenolic compounds on its color. In the following ripening phase at 15 °C, the value remained stable at around 4.5, with a slight rise after one month.

B. cereus, Enterobacteriaceae, and *E. coli* were not detected in any sample (<2 log CFU/g), indicating that good hygienic practices were maintained throughout production and storage. Neither *Listeria monocytogenes* nor *Salmonella* spp. were detected in 25 g. LAB reached

a very high count (9.6 $\log_{10}$ CFU/g) during the warm phase, and it declined to 8.7 $\log_{10}$ CFU/g after one month of incubation, likely due to the acidified environment and depletion of available nutrients. These results clearly confirm the role of *L. lactis* B12 in promoting acidification, thereby contributing to the microbial safety of the product. Despite being visibly present on the sample surfaces as white mycelium from day 10 onward, *P. camemberti* counts remained stable at approximately 5 $\log_{10}$ CFU/mL until the end of the maturation.

Various oilseed cakes have been valorized through fermentation with *Lactiplantibacillus plantarum* strains. Several studies reported that pH values below 4.5 can be reached within a few hours in oilseed by-products, even with different formulations, while improving nutritional value and reducing antinutritional factors [34–36].

3.3. Chemical Characterization of SSPC/Water Blend and PBFP

3.3.1. Determination of Free Amino Acids (AAs), Histamine, and Tyramine

The SSPC is a rich source of proteins (mainly albumins and globulins) with levels ranging from 30% to 55%, depending on defatting degree and processing method [8,37,38]. In the present work, free AAs were determined in the starting SSPC/water blend and in the finished PBFP after 30 days of ripening. The average total amount recovered was 979 ± 21 mg/100 g in the unfermented SSPC/water blend, while in the fermented PBFP it decreased to 707 ± 16 mg/100 g (Table 3). This decrease affected all free AAs to an almost equal extent, and it could be related to their consumption by fungi and bacteria. The presence of gamma-aminobutyric acid was also revealed. This non-proteinogenic AA derives from the enzymatic decarboxylation of L-glutamic acid controlled by certain microorganisms, commonly with LAB [39]. In order to evaluate whether the AA quantities in the two samples were statistically different, the Welch's t-test was performed. It indicated significantly lower concentrations of aspartic acid, glutamic acid, asparagine, arginine, methionine, valine, tryptophan, isoleucine, leucine, and lysine in the inoculated samples compared to the control ($p < 0.05$). Total amino acid content was also significantly reduced ($p = 0.0006$). However, due to the limited number of biological replicates, these results should be interpreted with caution.

The free AA content of SSPC has scarcely been investigated, and, to the best of our knowledge, only Pöri et al. (2023) [40] have evaluated the free AA content in fermented sunflower press cake. The authors analyzed a cold-pressed SSPC, which was defatted by supercritical carbon dioxide extraction and blended with water at a 30:70 (% *w*/*w*) ratio. The mixture was then fermented (37 °C/24 h) with *Lactobacillus helveticus* FAM1213 at an inoculum of 6–7 log CFU/g. These authors found the free AA content to increase from about 200 mg/100 g DM in the unfermented blend to approximately 600 mg/100 g DM in the fermented counterpart. As found in our work, aspartic and glutamic acid largely prevailed over other free AAs in the fermented blend, accounting for 31–32% of the total. This behavior also overlapped with data in the literature reporting a 2–6-fold increase after fermentation of legume cake, depending on the microorganism (*Lactobacillus*, *Bacillus*, *Saccharomyces*, mixed cultures) and experimental conditions (time, temperature, pH) adopted [41,42]. Overall, the total amount of free AA was negligible compared to that releasable during gastrointestinal digestion of SSPC. Indeed, in our previous work [9], we demonstrated the strong breakdown and high digestibility of SSPC proteins during in vitro static gastrointestinal digestion, which resulted in the release of grams of free AA per 100 g.

Finally, given that LAB are the main biogenic amine producers in fermented food, histamine and tyramine were analyzed in the final product to assess its safety.

Table 3. Free AA content (mg/100 g, ±SD) of the starting SSPC/water blend (SSPC) and in the plant-based fermented prototype (PBFP) after 30 d of ripening (GABA: gamma-aminobutyric acid). Data are means ± SD, n = 3. Statistical significance was assessed using the Welch's *t*-test. Different letters within the same row indicate significant differences ($p < 0.05$).

Amino Acid	SSPC/Water Blend	PBFP
Aspartic acid	111 ^{a} ± 7	74 ^{b} ± 4
Glutamic acid	236 ^{a} ± 9	178 ^{b} ± 1
Asparagine	4 ^{a} ± 1	1 ^{b} ± 1
Serine	23 ± 4	19 ± 1
Glutamine	37 ± 5	29 ± 3
Histidine	29 ± 4	23 ± 3
Glycine	45 ± 4	37 ± 5
Threonine	46 ± 5	37 ± 2
Arginine	57 ^{a} ± 5	30 ^{b} ± 2
Alanine	67 ± 5	57 ± 4
GABA	18 ± 2	14 ± 2
Tyrosine	15 ± 1	14 ± 3
Methionine	25 ^{a} ± 2	15 ^{b} ± 1
Valine	42 ^{a} ± 3	31 ^{b} ± 4
Tryptophan	52 ^{a} ± 4	21 ^{b} ± 2
Phenylalanine	40 ± 3	33 ± 2
Isoleucine	35 ^{a} ± 2	27 ^{b} ± 1
Leucine	54 ^{a} ± 4	38 ^{b} ± 2
Lysine	15 ^{a} ± 2	9 ^{b} ± 1
Proline	24 ± 1	20 ± 3
Total	979 ^{a} ± 21	707 ^{b} ± 16

Although there is extensive literature on the presence of biogenic amines in animal-based foods such as cheese and cured meats, information on plant-based products is more limited [21,43]. Jastrzębska et al. (2023) [22] investigated the concentrations of biogenic amines (BA) in fermented vegetable juices. While significant differences and some discrepancies were observed among the samples of different products, the highest levels of BA were found to be histamine and tyramine. In our experiments, histamine and tyramine were not detected in either SSPC/water blend or PBFP samples. This may be due to the fact that many *L. lactis* strains lack the genes encoding histamine and tyramine decarboxylases (HDC and TDC, respectively), and the absence of these genes is a criterion for selecting dairy starter cultures [44,45].

3.3.2. VOCs Analysis of SSPC/Water Blend and PBFP

The VOCs profile was evaluated in both SSPC/water blend and in PBFP after one month of ripening. Overall, 66 VOCs were detected (Table 4), belonging to several chemical classes, including ketones, alcohols, esters, sulfur compounds, fatty acids, aldehydes, terpenes, furans, and diazines. For each VOC, the normalized peak area of the corresponding quantifier ion in the mass spectrum was considered. For brevity, only VOCs attributable to the activities of *L. lactis* and *P. camemberti* and those specific to PBFP are discussed below.

Table 4. Semi-quantification of VOCs in the starting SSPC/water blend and in the plant-based fermented prototype (PBFP) after 1-month ripening. For each VOC, the normalized peak area of the corresponding quantifier ion in the mass spectrum was considered. The relative abundance of each VOC is indicated as follows: +++ for peak areas above 8 log units, ++ for peak areas above 7 log units, and + for peak areas ranging from 5 to 6 log units.

Volatile Organic Compounds	SSPC/Water Blend	PBFP
Acetone	+	++
Ethyl acetate	+	+
2-Butanone	+	
Ethanol	+	++
2,3-Butanedione (diacetyl)		++
Pentanal	+	
α-pinene	++	++
Camphene	+	+
Hexanal	+	+
1-Propanol, 2-methyl-	+	+
β-Pinene	++	+
1-Butanol, 3-methyl-, acetate		+
3-Thujen-2-ol	+	
2-Heptanone	+	+
1-Butanol, 2-methyl-	+	+
Furan, 2-pentyl-	+	+
1-Pentanol	+	+
p-Cymene	+	+
2-Octanone	+	+
3-Hydroxybutanone (acetoin)	+	+++
2-Heptanol		+
Pyrazine, 2,5-dimethyl-	+	+
2-Propanol, 1-butoxy-	+	
1-Hexanol	++	++
Dimethyl trisulfide	+	
2-Nonanone		+
Nonanal		+
Pyrazine, 2-methyl-5-(1-methylethyl)-		+
Ethanol, 2-butoxy-	+	+
Pyrazine, trimethyl-		+
2-Octanol	+	+
3,3-Dimethylcyclohexylidene (acetaldehyde)	+	+
Benzene, (2-methyl-1-propenyl)-		+
p-Cymenene	+	
β-Cymene	+	

Table 4. *Cont.*

Volatile Organic Compounds	SSPC/Water Blend	PBFP
1-Octen-3-ol	+	+
Acetic acid	++	++
Formic acid, heptyl ester		+
Camphenone, 6-	+	
Pyrazine, tetramethyl-		+
1-Anthracenamine		+
Benzaldehyde	+	+
Propanoic acid	+	
1-Octanol	+	+
Propanoic acid, 2-methyl-	+	
2,3-Butanediol	+	+++
Pinocarvone	+	
Terpinen-4-ol	+	
Butanoic acid	+	
(E)-Pinanone	+	
1-Nonanol	+	
(E)-Pinocarveol	+	+
Butanoic acid, 2-methyl-	+	++
trans-Verbenol	+	+
D-Verbenone	+	+
Methoxyphenyl-oxime	+	+
(−) Myrtenol	+	+
Carveol	+	
Hexanoic acid	+	
Phenol, 2-methoxy-		++
Benzyl alcohol		++
Phenylethyl Alcohol	+	+
Phenol		+
Octanoic acid	+	
Nonanoic acid	+	

Diacetyl, acetoin, and 2,3-butanediol were the most abundant VOCs of PBFP. Indeed, *L. lactis* metabolizes citrate to produce these aroma-active compounds, which are associated with buttery flavor [46]. Diacetyl can be reduced to acetoin, which in turn can be reduced to 2,3-butanediol. Additional VOCs may originate from fungal activity. For instance, 3-methyl-1-butanol likely derives from leucine degradation through the action of *P. camemberti* [47]. It can also promote the formation of pyrazines, although their production may be inhibited in the presence of *L. lactis* [48]. This may explain the presence of pyrazines in both the SSPC blend and PBFP. The alcohol 1-octen-3-ol, which confers a typical mushroom-like aroma, derives from linoleic and linolenic acids via lipoxygenase and hydroperoxide lyases produced by molds [17,47,48]. This compound was detected in both the SSPC blend and PBFP, suggesting that mechanisms other than lipid oxidation can contribute to its formation.

Benzaldehyde is an aromatic compound with floral, bitter almond, and fruity notes. Its metabolic pathway is not fully elucidated, but it may originate from the transformation of phenylalanine or oxidative pathways involving cinnamic acid [47]. *P. camemberti* has been identified as a potential catalyst in this transformation [18,48]. The presence of 2-methoxy-phenol (guaiacol) in PBFP may mainly originate from the natural lignin of sunflower, particularly the hulls, which are rich in guaiacyl units [49]. Moreover, SSPC may contain a high amount of phenolics (1–4% dry matter), mostly as esters of caffeic and synapic acids [49]. Enzymes from fungi can break down lignin and release bound phenolic acids, especially ferulic acid, which can then be converted into guaiacol [50]. Fermentation may increase guaiacol formation by lowering pH, which favors phenolic transformations. 2-Methyl-butanoic acid (isovaleric-type branched acid) prevailed in PBFP, and it likely originated from microbial metabolism of free AA during fermentation [51]. Different LAB can deaminate and decarboxylate isoleucine to form 2-methylbutanal, which is then oxidized to 2-methylbutanoic acid [52]. These VOCs are often associated with cheesy, sweaty, or rancid odors when present at high levels [46].

Terpenes were also detected among the VOCs, especially in the SSPC blend. Although sunflower seeds contain terpenes, the derived protein powder does not generally include important amounts of these compounds [37]. Indeed, the methods used to process sunflower seeds into protein powder generally do not imply isolating or concentrating terpenes. However, further investigation will be necessary to understand the presence of certain terpenes, which were primarily detected in the control sample.

3.3.3. Cyclopiazonic Acid (CPA) Analysis of SSPC/Water Blend and PBFP

In this work, the LC-MS/MS method allowed the identification and quantification of CPA within a calibration range of 0.1–5 ng/mg of PBFP. The resulting LOD and LOQ values were 0.02 and 0.05 ng/g, respectively. The concentration of CPA in the inoculated sample was 0.48 ng/g, whereas in the control sample it was below the LOD. The levels found are at least two orders of magnitude lower than those typically found in cheeses with *P. camemberti* grown on the rind.

The production of cyclopiazonic acid (CPA) by *P. camemberti* has long been recognized [53]. In the dairy industry, this mold has been used for over two centuries to make typical surface mold-ripened cheeses in northern France, some of which benefit from a Protected Designation of Origin, such as Camembert de Normandie or Brie de Meaux [54]. In 1979, Le Bars [53] showed that twenty examined strains isolated from cheese samples were all capable of synthesizing this toxin, and the amounts varied significantly depending on the strain, temperature and incubation time. CPA was primarily detected in the rind of cheeses with concentrations ranging from 50 to 1500 ng/g, but not in the "paste" (the inner part of cheese). A recent study of soft-ripened and blue cheeses marketed in the USA [55], using immunoassay-based methods, reported CPA in 45.6% of soft-ripened and 24.4% of blue cheeses, with median concentrations in positive samples of 48.5 ng/g and 30 ng/g, respectively. In the same study, much higher values were also observed, with maximum concentrations of 3820 ng/g in a Brie sample, highlighting wide variability linked to processing conditions and fungal strains employed. CPA is also found in traditional cereal-based fermented products like Ogi, a spontaneously fermented maize gruel [56]. Interestingly, the fermentation process, specifically the steeping phase involving lactic acid bacteria, has been shown to significantly reduce CPA concentrations originally present in the raw grains.

In a recent study, Ollinger et al. [24] examined the mycotoxin production of *P. camemberti* in starch agar medium and in samples of lactose- and lactose-free Camembert cheese, as well as in a vegan alternative made from a mixture of poppy seeds and hazelnuts. The

results suggested that the production of CPA depended on the available carbon source and that the highest concentration was found on starch agar, while the Camembert and vegan cheese samples were free of mycotoxins.

By applying genome analyses, Ropars et al. [57] demonstrated that *Penicillium* species used in cheese-making evolved through successive domestication events driven by human selection, resulting in genetically distinct fungi that are adapted to cheese production. Indeed, the 'white' mold of *P. camemberti* originates from 'grey-green' *P. biforme*, itself traced back to blue-green ancestor *P. fuscoglaucum*. Moreover, the two current domesticated varieties, *P. camemberti* var. *camemberti* and *P. camemberti* var. *caseifulvum*, exhibit enhanced growth and reduced toxin production, as well as characteristics that promote the ripening of specific cheeses.

From a toxicological perspective, CPA is a selective inhibitor of the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase, with major effects on muscles, liver, and spleen, and is classified as a neurotoxin [58]. Currently, maximum residue levels have not been specifically established for CPA in food or feed within major food safety frameworks, such as the European Union's regulations and the United States' legislation. Compared with more hazardous mycotoxins, CPA has received less regulatory focus, partly due to limited data on its effects on human health and exposure levels.

3.4. Sensory Evaluation of PBFP

The exploratory sensory evaluation was conducted as explained in paragraph 2.4. In two different sessions, one panel evaluated the control sample, while the other evaluated the inoculated blend. Each participant evaluated sample appearance, odor, taste, and texture using a 10 cm visual analog scale (VAS). Qualitative descriptors could also be added for each attribute.

The Mann–Whitney U test for independent groups revealed no statistically significant differences between the control and the inoculated blends for any of the four sensory attributes ($p > 0.05$), indicating that fermentation did not significantly affect the perceived intensity of the attributes. As shown in Figure 4, the median values of the non-inoculated and inoculated blends were comparable for each attribute, with moderate differences in perception variability, suggesting an overall evaluation around the midpoint of the scale. Median values ranged from 4.5 to 6.0 for all attributes, further confirming an overall evaluation around the midpoint of the scale (Table 5).

The similarity in sensory intensities between the control and the inoculated blend may reflect intrinsic limitations of the vegetable matrix in supporting extensive aroma development during fermentation. Unlike dairy matrices, which are well known for providing a suitable nutrient composition for the development of a wide range of flavors through fermentation, plant-based substrates such as sunflower press cake still represent a challenge for the development of pronounced sensory attributes. In dairy products such as Camembert cheese, the production of aromatic compounds is associated with the activity of a large microbial consortium (e.g., *L. lactis*, *P. camemberti*, *Geotrichum candidum*, *Debaryomyces hansenii*, and *Leuconostoc mesenteroides*), which together contribute to the complexity of the sensory profile [18]. The release of hydrolytic enzymes as a consequence of microbial autolysis during the ripening phase is also a fundamental aspect in the development of aromatic compounds [59]. In the present study, LAB and mold plate counts at the end of the maturation phase were still high, suggesting that the fermentation period may not have been sufficient to induce extensive microbial autolysis.

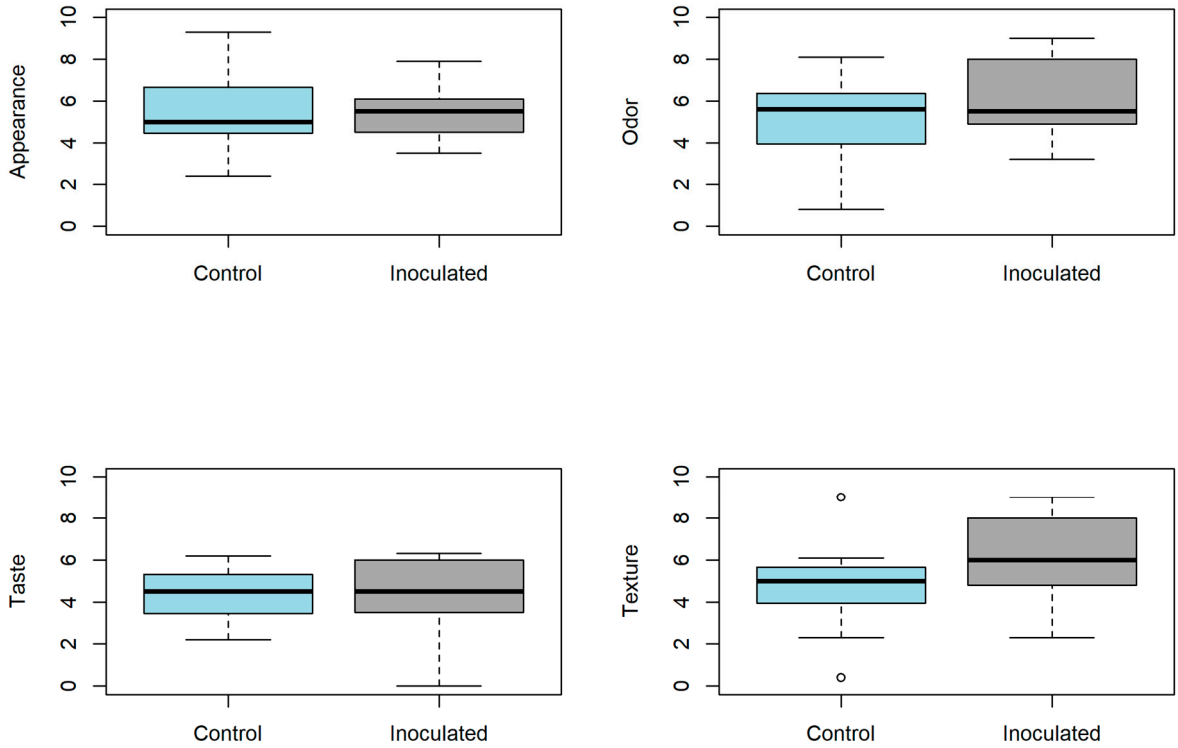

Figure 4. Distribution of scores obtained from the sensory evaluation on the pleasantness scale for the different attributes of the fermented (PBFP) and control samples.

Table 5. Comparison of the scores obtained by sensory evaluation for the different attributes of the fermented (PBFP) and the control samples with the Mann–Whitney U test.

Attribute	Sample	Median Value	IQR	*p*-Value
Appearance	Control	5.0	2.20	0.8617
Appearance	PBFP	5.5	1.60	
Odor	Control	5.6	2.40	0.6636
Odor	PBFP	5.5	3.10	
Taste	Control	4.5	1.85	0.8615
Taste	PBFP	4.5	2.50	
Texture	Control	5.0	1.70	0.1917
Texture	PBFP	6.0	3.20	

Despite the absence of significant differences in sensory intensity, fermentation affected the qualitative sensory profile of the inoculated blend. Panelists described both samples as beige/olive in color, with visible differences mainly related to the presence or absence of mold mycelium. However, the odor of the inoculated blend exhibited more acidic, fermented, and dairy-like notes (e.g., yogurt/kefir) compared to the control, which was mainly described by nutty, toasted, and vegetable notes. Taste perception remained comparable in intensity and qualitative descriptors, although a slightly higher acidity and mild mold-related notes were reported for the inoculated blend. Regarding texture, panelists perceived the inoculated blend as slightly creamier and softer than the control, suggesting a microbial effect on the matrix structure.

The presence of yogurt- and kefir-like notes in the odor of the inoculated blend could be related to the detection of diacetyl, acetoin, and 2,3-butanediol among the main volatile organic compounds, as these metabolites are typical contributors to flavor in fermented dairy products [60]. Zuljan et al. (2014) [61] demonstrated that the metabolic

pathway leading to the production of these compounds in *L. lactis* is activated under acidic conditions. Indeed, pyruvate metabolism, a precursor of diacetyl and acetoin, contributes to the maintenance of intracellular pH homeostasis. In our previous study [16], CATA sensory analysis of a sunflower press cake and sweet whey powder blend showed that the sample fermented with *L. lactis* was characterized by a milky odor and taste, as well as an acidic taste.

4. Conclusions

In this work, a plant-based fermented prototype (PBFP) was developed using a blend of sunflower-seed press cake (SSPC) and water, inoculated with *Lactococcus lactis* and *Penicillium camemberti* strains. The study assessed the fermentability, microbiological safety, chemical composition, volatile organic compounds, cyclopiazonic acid content, and sensory characteristics of the PBFP.

The results demonstrated that the SSPC/water blend was highly fermentable. *L. lactis* B12 efficiently acidified the matrix during the warm phase, reaching pH values below 4.6 and high LAB counts (~10^9 CFU/g), while *P. camemberti* successfully developed visible mycelium on the surface without producing detectable levels of cyclopiazonic acid. Microbiological analyses confirmed the absence of pathogens such as *Listeria monocytogenes*, *Salmonella spp.*, or process hygiene criteria like *B. cereus*, *Enterobacteriaceae*, and *E. coli*, supporting the safety of the PBFP. It is reiterated that lowering the pH within a food matrix is critical for shaping microbial communities, as it restricts the growth of spoilage contaminants and pathogens, making fermentation the oldest and most energy-efficient strategy for food preservation [62].

Chemical characterization revealed a decrease in total free amino acids during fermentation, likely due to microbial consumption, with no detectable histamine or tyramine, indicating a low risk of biogenic amine accumulation. VOCs analysis identified key aroma compounds, including diacetyl, acetoin, 2,3-butanediol, and 1-octen-3-ol, which contributed to subtle dairy-like and fermented notes. Sensory evaluation showed that fermentation did not significantly affect the overall intensity of appearance, odor, taste, or texture, although qualitative descriptors indicated mild microbial effects on aroma and creaminess.

Despite these promising findings, several limitations should be acknowledged. The fermentation period may not have been sufficient to induce extensive microbial autolysis and full aroma development. The plant-based matrix may inherently limit flavor complexity compared to dairy substrates, and the sensory panel was relatively small.

Future studies should explore extended ripening periods, the inclusion of additional microbial consortia, and the optimization of substrate composition to enhance flavor complexity and textural properties.

Overall, this study provides a proof-of-concept for the safe and controlled fermentation of SSPC, highlighting its potential as a sustainable protein source for the development of novel plant-based fermented foods.

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Abbreviations

The following abbreviations are used in this manuscript:

CPA	Cyclopiazonic Acid
PBFP	Plant-Based Food Prototype
QTRAP	Quadrupole Linear Ion Trap
SSPC	Sunflower-Seed Press Cake
VOCs	Volatile Organic Compounds

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