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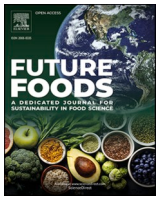

Tailored bioprocessing of brewers' spent grain for the development of upcycled plant-based spoonable snacks

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ABSTRACT

Reintegration of brewers' spent grain (BSG) into the food system remains a challenge. In this study, BSG underwent enzyme hydrolysis with Ondea Pro, followed by fermentation with *Lactiplantibacillus plantarum* POM1 or *Pediococcus claussenii* DSM 14800. Both bacterial strains exhausted C6 sugars and lowered the pH to below 4. This bioprocessing approach increased the total polyphenol, antioxidant capacity, and free amino acid content in a strain-dependent manner. Enzyme hydrolysis contributed to an increase in low molecular weight dietary fibre content, while fermentation reduced the abundance of volatile organic compounds with off-flavours such as malty, grassy and pungent, and increased fruity, citrus, sour and sweet aroma compounds. Unprocessed (control) and bioprocessed BSG were then used as ingredients for semi-solid -spoonable snack prototypes with or without strawberry purée. A consumer sensory study involving 119 untrained participants showed higher liking for unprocessed samples, likely due to the bitter taste of the bioprocessed samples. However, adding purée increased the liking in all cases, while only fermentation could enhance the presumably desired aroma notes and reduce the presumably undesired cereal aroma and flavour. These findings suggested that tailored bioprocessing, informed by sensory data, could support the development of functional upcycled food with a cleaner ingredients label.

1. Introduction

Consumer demand for functional non-dairy beverages has been rising in the past decade (Plamada et al., 2023). These plant-based dairy substitutes can be broadly divided into two categories. i) dairy alternatives, where plant-based ingredients act as a replacement carrier of nutrients and probiotics and ii) dairy analogues, where plant-based materials are transformed to recreate the flavour, texture and appearance that attempt to mimic their dairy counterparts (Pua et al., 2022). Among these, plant-based snacks, sometimes called "gurts", are snacks generally made by fermenting aqueous extracts from different raw materials (e.g. legumes, oil seeds, cereals or pseudocereals). These extracts have an appearance and consistency similar to cow's milk resulting from their breakdown and homogenisation (Grasso et al., 2020). Developing plant-based dairy substitutes utilising plant ingredients is challenging due to adverse flavour, texture, stability and consumer acceptance (Pua et al., 2022). In this scenario, using cereal side streams, such as brewers' spent grain (BSG), becomes even more challenging as it introduces technological, safety, and sensory issues (Henja et al., 2023).

In Europe, BSG, the most abundant by-product of brewing, is disposed of as low-value animal feed (up to 70 %), in landfills (up to 20

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%) and in biogas (up to 10 %) (Bianco et al., 2020). BSG can be considered microbiologically stable at the point of production and within acceptable limits for food use (2 –3 log CFU/g), but it is prone to rapid change as the high sugar and moisture content leads to microbial spoilage if not properly stored (Robertson et al., 2010). Furthermore, off-flavour and coarse mouthfeel are also problematic. However, BSG has enormous potential as protein, lignin, and lipids account for 19–30 %, 12–28 % and 10 % w/w, respectively and essential amino acids represent ~30 % of the total BSG protein, making it a source of high-quality protein (Fărcaş et al., 2017). BSG is rich in phenolics, particularly hydroxycinnamic acids (ferulic, *p*-coumaric, and caffeic acids) (Ikram et al., 2017), while fibre accounts for 30–50 % of w/w (Lynch et al., 2016; Nyhan et al., 2023). BSG also contains flavonoids ranging from 8.80 to 50.38 mg quercetin equivalent /100 g DW (Socaci et al., 2018). These characteristics make BSG attractive for upcycling into value-added ingredients rich in protein and fibre with a lower environmental impact than comparable plant-based ingredients (Nyhan et al., 2023). Utilising BSG to create plant-based snacks would be within the concept of upcycled food, and a few products do currently exist on the market. These range from common products such as English muffins, mini naan /flatbread, bread, pasta, crackers, crisps and flours (https://upcycledfoods.com) to meat substitute burgers (https://www.migros.ch/en) and vegan barley milk (https://taketwo.mur.io).

The reintegration of BSG into conventional food supply chains remains an outstanding research gap (Piercy et al., 2023). BSG has mainly been studied as an ingredient in solid food products, including bread, breadsticks, muffins, cookies, pasta, and pizza dough (Amoriello et al., 2020; Cermeño et al., 2021; Heredia-Sandoval et al., 2020; Schettino et al., 2021). Use in beverages or yoghurt-like products is more limited but does include a probiotic beverage containing viable *Bacillus subtilis* WX-17 from the fermented liquid fraction of BSG (Tan et al., 2020), a yoghurt-like prototype developed by fermenting the liquid fraction of fresh BSG (20 %) and commercial soymilk with the sensory characteristics of dairy yoghurt (Madsen et al., 2021). In addition, adding dried BSG powder to dairy yoghurt conferred prebiotic effects and reduced syneresis (Battistini et al., 2023; Naibaho et al., 2022). More recently, this range was extended to confectionary, sausage, burgers and mayonnaise (Naibaho et al., 2024). Thus, despite many promising applications of BSG in solid foods, very few studies have systematically investigated its integration into liquid or semi-solid products. Notably, none have utilised the whole BSG as the main ingredient and the effects on sensory attributes remain insufficiently understood.

Off-flavours in plant dairy counterparts can be reduced through roasting, adjusting pH, using deodorizing chemicals, or treating with enzymes, and flavours can also be improved by fermentation, such as with lactic acid bacteria (Wang et al., 2023). In the dairy counterparts, improving sensory liking by adding flavourings such as fruit puree is common (Keating and White, 1990), such as for sheep milk yoghurt (Garofalo et al., 2024). This approach has also been shown to be effective in plant-based prototypes such as with bananas (in soy yoghurt) (Oyeniyi et al., 2014), vanilla and coconut flavours (Cardello et al., 2024; Jaeger et al., 2023), where flavours are reported as one of the drivers of liking in plant-based yoghurt.

Fermentation is a versatile route to upcycle agrifood side streams such as BSG, thereby enhancing its nutritional ingredient functionality and technological quality (Martínez-Villaluenga and Peñas, 2023). Bioprocessing with enzymes and fermentation has been shown to be a feasible approach to modify complex substrates like BSG (Verni et al., 2020). Enzymatic treatments hydrolysed BSG fibres into fermentable sugars and proteins into free amino nitrogen (Moirangthem et al., 2024). This modified BSG into a substrate suitable for fermentation with desirable acidification and microbial growth profile. Such bioprocessing can also increase phenolic content by releasing bound phenolics and antioxidant potential. In particular, as reviewed by Anumudu et al., lactic acid fermentation by species such as *Lactiplantibacillus plantarum* not only converted hydrolysed sugars into lactic acid—rapidly lowering the pH and inhibiting spoilage organisms—but also promoted the synthesis of exopolysaccharides that improve product texture and mouthfeel (Anumudu et al., 2024).

The aim of this study was to investigate the nutritional and technological properties of bioprocessed BSG as an ingredient for a spoonable snack. In our previous study, we established the pretreatment and hydrolysis conditions with the enzyme cocktail Ondea Pro to support the fermentation of BSG with LAB L. *plantarum* POM1 (Moirangthem et al., 2024). In this study, we used the same enzymatic treatment as in our previous study and assessed the impact of bioprocessing with L. *plantarum* POM1 and *P. claussenii* DSM 14800. Along with a non-bioprocessed control, the two fermented BSG ingredients were then used to produce spoonable snack prototypes.

The novelty of this research is in the comprehensive, multiparametric approach to understanding the role of processing in the development of semi-solid products using whole BSG as the main ingredient. The effects of BSG bioprocessing on biochemical and bioactive parameters (such as organic acids, volatile organic compounds, free sugars and amino acids, polyphenols and antioxidant assays) as well as the sensory attributes of the resulting snack prototypes were investigated. These sensory attributes were finally evaluated through a consumer sensory study exploring liking and attributes of taste and aroma, along with the role of berry flavouring.

2. Materials and methods

2.1. Brewers' spent grain (BSG)

BSG was kindly provided by Sinebrychoff (Kerava, Finland). Fresh warm BSG with a moisture content of 71.5 ± 0.4 (% biomass) was collected after a final mash temperature of 80 °C within hours of its generation. The BSG consisted of hammer-milled pilsner malt and dehusked unmalted barley. BSG was cooled to 4 °C and wet milled twice using a Microcut MC15 with a K04 blade (approx. mesh size 0.5 mm) (Stephan Machinery GmbH, Hameln, Germany) without added water. The paste was stored in resealable plastic bags at −20 °C for further analysis.

2.2. BSG bioprocessing

Bioprocessing was conducted based on a modified version of the method reported earlier (Moirangthem et al., 2024). Briefly, wet-milled BSG was mixed with water and enzyme solution in a 1:12 ratio (dry weight /volume) and hydrolysed in a shaking incubator at 50 °C for 4 h at 300 rpm. Ondea Pro, a commercial brewing enzyme cocktail comprising cellulase, xylanase, α-amylase, protease, lipases and pullulanase (Novozyme, Denmark), was used to treat BSG at 5X the normal brewing dosage recommended by the manufacturer (1X = 2000 ppm). The mixture was cooled to 30 °C, and fermentation was carried out separately with i) L. *plantarum* POM1 (referred to as POM1) previously isolated from tomato (Di Cagno et al., 2009), and ii) available at the research collection of the Department of Food and Nutrition at the University of Helsinki, Finland and *P. claussenii* DSM 14800 purchased from DSMZ German Collection of Microorganisms and Cell Cultures GmbH, Germany (referred to as DSM), isolated from spoiled beer (Dobson et al., 2002), The strains were stored at −80 °C in 20 % glycerol and routinely cultivated in MRS broth at 30 °C for 24 h for fermentation. After 24 h, the cells were centrifuged (10,000 × *g* for 10 min at 4 °C), the pellets washed twice in 50 mM sterile phosphate buffer (4 °C, pH 7.0), resuspended in an equal volume of sterile Milli-Q water and used for the inoculation of enzyme hydrolysed BSG, targeting an initial cell density of 6.0 Log CFU /g. Following this, simultaneous saccharification and fermentation were conducted at 30 °C for 24 h, without shaking.

Samples were taken at 0 h (**T0**), 4 h (**T4** Enzymatic hydrolysis), and the end of 24-hour fermentation, calculated from the end of 4 h of hydrolysis (**T28 P** Fermented with POM1 and **T28 D** Fermented with DSM

14800). Aliquots for all the analyses were boiled and frozen immediately with the following exceptions: fresh aliquots immediately after sampling were assessed for microbial quality; aliquots for organic acid analysis were frozen immediately without boiling; and samples for polyphenol analysis were freeze-dried.

2.3. Moisture, protein, and dietary fibres

Moisture content was measured using the AACC method 44−15.02 (AACC, 2010). Ash content was measured using the AACC method 08–02 (AACC, 1999). Dietary fibres (high & low molecular weight) were estimated enzymatic-gravimetrically according to the AOAC 2017.16 method (AOAC, 2019). Total crude protein content was estimated using Leco 828 Series Carbon/ Nitrogen Analyzer with Cornerstone Brand Software (CN828) according to the manufacturer's instructions for analysing plant tissues. Protein content was calculated by multiplying the nitrogen concentration by 5.83 (Specific or Jones factor for barley).

2.4. Carbohydrate analysis

Monosaccharides were quantified using a WatersTM Acquity Ultra-High-Performance Liquid Chromatography system fitted with an Acquity BEH Amide 1.7 μm (2.1 × 100 mm) column and Acquity evaporative light scattering detector as described previously (Koirala et al., 2022). Glucose, fructose, arabinose, mannose, xylose and galactose (MERCK, Darmstadt, Germany) were used as standards at concentrations of 0.25, 0.20, 0.15, 0.10 and 0.05 mg/mL and 2-Deoxy-d-galactose (Sigma-Aldrich, UK) was used as the internal standard at 0.15 mg/mL.

For sample preparation, aliquots of the mixture were taken at different time points and boiled for 5 min to inactivate enzymes and microbes. After cooling and centrifugation (12,000x *g*, 10 min), the supernatant was filtered through a 0.2 μm syringe filter.

2.5. Organic acid analysis

Lactic and acetic acid levels were determined on a Waters high-performance liquid chromatography system fitted with an Aminex HPX-87H column (300 × 7.8 mm; Bio-Rad, Hercules, CA, USA) and Waters 2487 Dual λ Absorbance Detector 35 (operating at 210 nm) as described previously (Koirala et al., 2022; Xu et al., 2017) with lactic acid (Sigma-Aldrich) and acetic acid (Merck) as standards. An isocratic run with sulphuric acid (10 mM) at 0.6 mL /min for 25 min was used to elute lactic and acetic acids.

Sample extraction was conducted as described previously (Verni et al., 2022) using 5 % perchloric acid. The extracts were centrifuged (10,000× *g*, 15 min, 24 °C) and filtered through 0.45 μm Acrodisc® PTFE Syringe Filters before injection.

2.6. Bacterial enumeration, pH and total titratable acidity (TTA)

The microbial quality was assessed using selective media for presumptive LAB (lactobacilli MRS Agar), yeast and molds (malt extract agar), *Enterobacteriaceae* (violet red bile glucose agar), *Bacillus cereus* (PEMBA agar) and total mesophilic bacteria (plate count agar) as described previously (Koirala et al., 2022). The presence of *Escherichia coli* and *Salmonella* in control and bioprocessed samples was assessed according to ISO 16649–2 culture technique (ISO, 2001) and RT PCR (BACGene Salmonella spp.) (Quansah and Chen, 2021), respectively.

The pH of fermented BSG was measured using a pH Meter (Model 340, Mettler-Toledo, UK) and TTA using a manual titrator (Mettler Toledo DL53, Uster, Switzerland) with a modified AACC method 02–31.01, as described previously (Koirala et al., 2022; Xie et al., 2018). TTA was expressed as the 0.1 M NaOH (mL) volume used during titration.

2.7. Free amino acid (FAA) analysis

Amino acids were quantified using a Jeol JLC-500/V amino acid analyser (Jeol (UK) Ltd., Garden City, Herts, UK) fitted with a Jeol Na+ high-performance cation exchange column on samples deproteinised with 24 % (w/v) tri-chloroacetic acid (TCA) (Mounier et al., 2007). Norleucine at 125 nm /ml was used as the internal standard.

2.8. Bioactive compound analysis

For sample preparation, 0.5 g of freeze-dried sample was mixed overnight with 5 mL 80 % methanol at 1000 rpm at ~ 25 °C (room temperature) overnight. The mixture was centrifuged (3000x g, 10 min, 4 °C) and filtered through 0.45 μm Acrodisc® PTFE Syringe Filters before injection.

2.8.1. Bioactivity assays

Total polyphenol analysis (TPC) was estimated spectrophotometrically at 735 nm using the Folin–Ciocalteu's (FC) phenol method (Singleton et al., 1999) with gallic acid solutions as standards and expressed as mg gallic acid equivalents (GAE)/g DM. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay was estimated at 515 nm using DPPH stock solution in methanol (Goupy et al., 1999) with Trolox as standard and expressed as mg Trolox equivalent (TE)/g DM. While ferric reducing antioxidant power (FRAP) assay was estimated at 593 nm using FRAP reagent (Stratil et al., 2006) with Trolox solutions as standard and expressed as mg Trolox equivalent (TE)/g DM.

2.8.2. Quantification of individual phenolic compounds using UPLC-MS/MS

Polyphenols were separated using an Ultra-high performance liquid chromatography coupled to a tandem quadrupole mass spectrometer (UPLC-TQD, Waters Corp., Milford, MA, USA) on an Acquity UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm) with run conditions as described earlier (Gangopadhyay et al., 2016). While compounds were quantified using the multiple reaction monitoring (MRM) method by analysing at least two MRM transitions per compound (Birsan et al., 2019). Standards were ferulic acid (0.6 to 2.9 μg/mL), *p*-coumaric acid (0.01 to 0.2 μg/mL) and protocatechuic acid (0.1 to 0.4 μg/mL). Concentrations of the phenolic compounds were expressed as μg/100 g DM.

2.9. Volatile organic compounds

Head space solid phase microextraction (HS-SPME) analysis - Sample preparation:

Four g of sample with 100 μL of the internal standard at 5 ppm (4-methyl-2-pentanol and 2-methyl-3-heptanone) was added to a 20 ml screw-capped amber SPME vial and equilibrated to 40 °C for 10 min with pulsed agitation of 5 s at 500 rpm. Sample introduction was accomplished using a Gerstel MPS Autosampler (Element Cambridge, Cambridge, UK).

Gas chromatography mass spectrometry (GCMS) method

A single 50/30 mm CarboxenTM/divinylbenzene/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Agilent Technologies Ltd, Cork, Ireland) was used. The SPME fibre was exposed to the headspace above the samples for 20 min at a depth of 1 cm at 40 °C. The fibre was retracted, injected into the GC inlet, and desorbed for 2 min at 250 °C. Injections were made on a Shimadzu 2010 Plus GC (Shimadzu Europa GmbH, Duisburg, Germany) with a DB-624 UI (60 m x 0.32 mm x 1.8μm) column (Agilent Technologies Ltd, Ireland) using a split/split less injector with a 1/10 split. A Merlin micro seal was used as the septum. The temperature of the column oven was set at 40 °C, held for 5 min, increased at 5 °C/min to 230 °C, held for 15 min and then increased at 15 °C/min to 260 °C, and held for 5 min, yielding at total GC run time of 65 min. The carrier gas, helium, was held at a constant flow of 1.2ml/min. The detector was a Shimadzu TQ8030 mass spectrometer running

in single quad mode. The ion source temperature was 220 °C, and the interface temperature was 260 °C. The MS mode was electronic ionization (70v) with the mass range scanned between 35 and 250 amu. Compounds were identified using mass spectra comparisons to the NIST 2014 mass spectral library, a commercial flavour and fragrance library (FFNSC 2, Shimadzu Corporation, Japan) and an in-house library created using authentic compounds with target and qualifier ions and linear retention indices for each compound using Kovats index (van Den Dool and Kratz, 1963). Retention indices were matched against peer-reviewed publications where possible to confirm compound identification (Corral et al., 2015; Gianelli et al., 2011; Olivares et al., 2011) and against authentic standards. Spectral deconvolution was also performed to verify the identification of compounds using AMDIS. Batch processing of samples was carried out using MetaMS (Wehrens et al., 2014). MetaMS is an open-source pipeline for GC–MS-based untargeted metabolomics. An auto-tune of the GCMS was carried out before the analysis to ensure optimal GCMS performance. External standards: 1-butanol, dimethyl disulphide, butyl acetate and cyclohexanone at 10 ppm were run at the start and end of the sample set, and abundances were compared to known amounts to ensure that both the SPME extraction and MS detection were performing within specifications. Results were expressed as abundance values, equivalent to peak areas.

2.10. Sample preparation for consumer sensory study

Six prototypes were prepared at a scale of ~ 2 L, as shown in Table 1. Specifically, control samples 1 and 2 are unprocessed BSG. The control (non-bioprocessed) sample was exposed to the same conditions (50 °C for 4 h at 300 rpm followed by 30 °C for 24 h) but without enzymes and microbes. Samples 3–6 are bioprocessed as described in 2.2 and fermented with L. *plantarum* POM1 (samples 3 and 4) and *P. claussenii* DSM 14800 (samples 5 and 6). In addition, Samples 1, 3 and 5 have strawberry puree added, while samples 2, 4 and 6 do not. The strawberry puree was selected based on pilot testing from the various commercially available fruit purees. According to a study by Laaksonen et al., strawberry was the second most liked (and the second most frequently used) and overall popular flavour in the Nordics (Laaksonen et al., 2016).

All samples were pasteurised at 90 °C for 15 min. After cooling, the samples were blended with the respective recipe ingredients in a Designer 725 (Blendtec, USA) using the 'Whole Juice' function. The samples were chilled to 4 °C overnight. Sugar, specifically sucrose (Dan Sukker, Finland) (used at 3.8 % w/w), commercial citrus pectin (used at 1.0 % w/w) and rapeseed oil (Keiju, Finland) (used at 2.6 % w/w) were added to all samples. In contrast purée (100 % strawberry purée from Bonne Juomat Oy, Finland) (used at 10.2 % w/w), was added only to samples 1, 3 and 5. After adding the recipe ingredients, the total volume was ~2.4 L for each prototype. The recipe ingredients and concentrations were selected based on preliminary internal sensory screening trials (data not shown).

2.11. Sensory study

A consumer sensory study with untrained participants was conducted over 2 days at the Sensory Laboratory (ISO 8589) at the University of Helsinki. The samples were served in randomised orders, and data was collected using Fizz Acquisition sensory analysis software, version 2.51 (Biosystèmes, France). Participants used the nine-point hedonic scale to rate the acceptance of the prototypes based on aroma, taste (flavour), and overall sensory experience (Scale labels: **1**- Dislike extremely, **2**- Dislike very much, **3**- Dislike moderately, **4**- Dislike slightly, **5**- Neither like nor dislike, **6**- Like slightly, **7**- Like moderately, **8**- Like very much, **9**- Like extremely). In addition, seven aroma and seven flavour properties were evaluated using a Check-All-That-Apply (CATA) task. The response options in the CATA task for aroma were Fruity, Berry, Sour, Yoghurt, Mushroom, Cereal, and Others (free text). Correspondingly, the flavour response options in the CATA task were Sour, Bitter, Sweet, Umami, Dryness, Cereal, and Others (free text). These CATA properties were chosen based on an internal panel evaluation that highlighted the key characteristics of the prototypes. Demographic information collected included year of birth, gender, and frequency of plant-based yoghurt consumption. The questionnaires were provided in Finnish and English. The prototypes were served to the participants at 4 °C. Tap water, and corn snacks were provided to cleanse the palate between samples.

The study protocol followed the ethical guidelines of the sensory laboratory of the Department of Food and Nutrition, University of Helsinki, approved by the University of Helsinki Ethical Review Board in the Humanities and Social and Behavioural Sciences (Statement 46/2016). Participants were informed before the study that the prototypes were made from BSG. However, they were not informed about the health or environmental benefits, nor were they told about the addition of strawberry purée. Written informed consent was obtained from each participant. After completing the study, participants received a food gift as a reward.

2.12. Statistical analysis

Statistical analyses, including ANOVA and Tukey HSD post hoc tests, were performed using IBM SPSS Statistics (IBM Corp., Version 29.0.2.0.). Principal component analysis of the free amino acids was conducted using SIMCA 17.0.2 (Sartorius) after transforming the variables with centre (ctr) scaling. While for VOC, batch processing of samples, statistical analysis and plotting were carried out using R Studio (MetaMS) (Wehrens et al., 2014). Plots were created using ggplot2 and pheatmap. The hierarchical clustering heatmap was based on each sample's abundance of VOCs. PCA was used to find associations of VOC with the samples.

3. Results and discussion

3.1. BSG bioprocessing, acidification and carbohydrates profile

The native wet-milled BSG (T0) harboured low microbial density with ≤3 for LAB and ≤2 log CFU/g total aerobic bacteria. In addition, *Salmonella* spp., *E. coli, B. cereus, Enterobacteriaceae*, yeasts and molds were not detected at any of the time points. This further supports previous findings showing that no known human pathogens commonly survive in BSG after the brewing process (Bianco et al., 2020) and

Table 1
Ingredients added to BSG to produce the six prototypes for the consumer study.

		Bioprocessing		Recipe ingredients			
No.	Sample	Enzyme	Microbe	Purée	Sugar	Pectin	Rapeseed oil
1	Control with purée **(C P)**			✔	✔	✔	✔
2	Control no purée **(C NP)**				✔	✔	✔
3	POM1 with purée **(P)**	✔	✔	✔	✔	✔	✔
4	POM1 no purée **(P NP)**	✔	✔		✔	✔	✔
5	DSM 14800 with purée **(D P)**	✔	✔	✔	✔	✔	✔
6	DSM 14800 no purée **(D NP)**	✔	✔		✔	✔	✔

highlights the safe handling of the ingredients during the processing.

Preliminarily, six strains belonging *to Levilactobacillus, Pediococcus, Leuconostoc* and *Lactobacillus* genera were screened as starters for BSG fermentation. Among these, *Lactobacillus plantarum* POM1 and *Pediococcus claussenii* DSM 14800 were selected based on the kinetics of acidification (pH 4.5 in 24 h) during growth in enzymatically hydrolysed BSG (data not shown). *Lactobacilli* and *pediococci* are also known for their thermotolerance, the capacity to use several substrates and tolerance to low pH or high alcohol concentrations (Bosma et al., 2017). After 24 h of fermentation, the LAB count reached ca. 9.4 and 8.8 log CFU/g in T28 P and T28 D, respectively - Fig. 1.a.

The pH values ranged from 5.8 to 3.7 and 4.0, and total titratable acidity (TTA) ranged from 1.05 mL to 6.50 and 5.50 mL of 0.1 M NaOH for T0, T28 P, and T28D, respectively. These TTA values are lower than those of commercial dairy yoghurts (~10.8 to 13.8 mL NaOH), but they are at the higher spectrum of reported commercial oat-based yoghurts (1.2 to 7.8 mL NaOH) (Greis et al., 2022) and plant-based yoghurts (1.2 to 7.8 mL NaOH) (Grasso et al., 2020). In this study, the lactic acid at the end of bioprocessing ranged from ~ 12 mg/g to 15 mg/g for T28 D and T28 P, respectively. These values are similar to dairy yoghurt values (e.g. 11.1 mg/g) and higher than soy, coconut, cashew and hemp yoghurts (4.3, 3.6, 2.8 and 1.0 mg/g, respectively) (Grasso et al., 2020). This might be attributed to the higher release of carbon sources resulting from the enzymatic treatment before fermentation (Fig. 1d). Low levels of acetic acid, 0.6 in T28 D and 3 mg/g in T28 P, were observed, probably due to the facultative heterofermentative metabolism of L. *plantarum* (Gobbetti, 1998). Both the fermented BSG reached a pH of 4.6 or lower, which is the updated FDA requirement for dairy yoghurt (FDA, 2023). This could be due to the difference in availability of fermentable sugars, microbial metabolic characteristics, and the influence of the specific substrate. Soluble carbohydrates have been shown to impact the growth and acidification of LAB, in soy milk, which needed the addition of glucose and fructose (Angeles and Marth, 1971).

3.2. Carbohydrates: monosaccharides and dietary fibre

The native BSG (T0) contained only glucose, fructose and mannose–Fig. 1d The enzymatic hydrolysis for 4 h (T4) further increased the amount of these three monosaccharides and additionally released pentoses (xylose) by hydrolysis of BSG fibres. At the end of the 24-hour fermentations, there was complete consumption of glucose, mannose and fructose. Both LAB showed a preferential complete consumption of hexoses, while pentoses remained. Between T4 and T28, arabinose, a hexose, was released (due to the continued but suboptimal activity of the enzymes). Only in the case of fermentation with L. *plantarum* POM1, all the released arabinose was consumed. This is because *P. claussenii* has been reported to be able to ferment glucose, fructose and mannose but not xylose and arabinose (BacDive, 2024). L. *plantarum* has been reported to be able to ferment glucose, fructose and arabinose, while its ability to ferment xylose depends on the strain (Ahmad et al., 2018; Saulnier et al., 2007; Zhang and Vadlani, 2015). In our previous study, bioprocessing of BSG with the enzyme cocktail Ondea Pro and the LAB L.

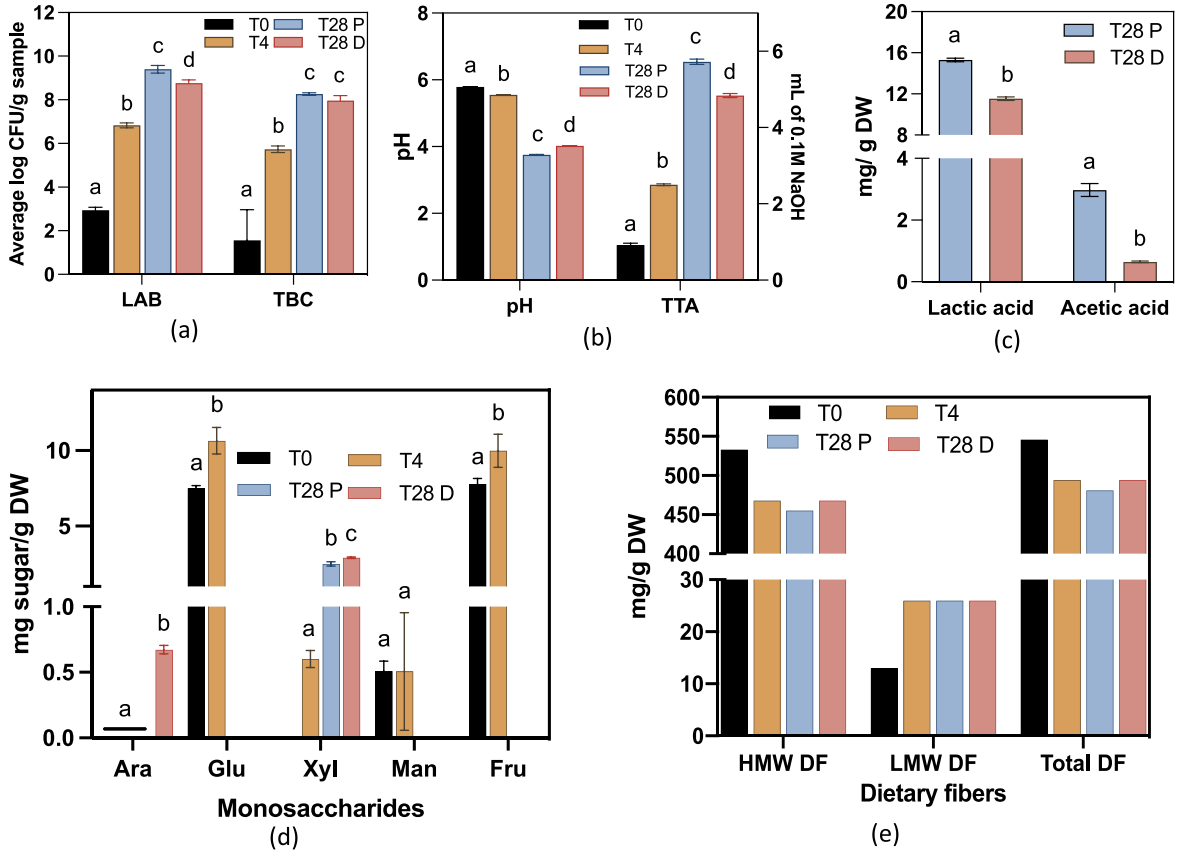

Fig. 1. Microbial quality and acidification. a) Cell count of lactic acid bacteria (LAB) and total aerobic bacteria count (TBC), b) pH and total titratable acidity (TTA), c) Organic acids. *Enterobacteriaceae, Bacillus cereus*, and yeasts and molds, *Salmonella* and *Escherichia coli* were not detected in any of the samples. Carbohydrate composition changes during bioprocessing. d) Monosaccharides, e) Dietary fibres, $n = 1$. a – d Values are expressed as means ±SD, $n = 3$. Bar means with different letters are statistically significant ($P < 0.05$).

Ara = arabinose, **Glu** = glucose, **Xyl** = xylose, **Man** = mannose, **Fru** = fructose. **HMW DF** = High molecular weight dietary fibres (**IDF** + **SDFP**). **IDF** = insoluble DF such as cellulose, lignin, and insoluble pentosans. **SDFP** = soluble high molecular weight DF, such as soluble pentosans, soluble pectin, and cereal β- glucan. **LMW DF** = Low molecular weight dietary fibres. **SDFS** = Low molecular weight soluble DF or Low molecular weight DF = ~ non-digestible oligosaccharides such as fructose oligosaccharides, galactose oligosaccharides and resistant maltodextrin.

plantarum POM1, the strain also consumed all the monosaccharides except xylose (Moirangthem et al., 2024). A similar observation was reported in the fermentation of BSG-pressed liquid, where glucose and maltose were preferentially consumed by L. *plantarum* (NFICC 27) compared to raffinose and maltodextrin (Shetty et al., 2023).

In this study, the native BSG had a total dietary fibre content of ~ 55 DW (T0-control), most of which were present as insoluble fibres -Fig. 1e. Bioprocessing (T28 D and T28 P) resulted in a decrease in high molecular weight dietary fibres (HMW DF) and total dietary fibre but a 2-fold increase in the low molecular weight dietary fibres (LMW DF). These observations could be mainly due to the enzymatic treatment with Ondea pro, as the values after fermentations (T28 p and T28 D) are similar to those after enzymatic hydrolysis (T4). As previously observed, the xylooligosaccharides and maltoligosaccharides profiles after hydrolysis and after fermentation of BSG with POM1 were similar (Moirangthem et al., 2024). Additionally, BSG oligosaccharide profiles via hydrolysed depend on the enzyme used (Sajib et al., 2018). These LMW DF, the non-digestible oligosaccharides, could act as potential prebiotics (Niittynen et al., 2007; Verni et al., 2020)

3.3. Free amino acids (FAA) profile during bioprocessing

The protein content of the native BSG was 25.1 ± 0.3 % DM. Overall, bioprocessing, especially via fermentation, resulted in a ca. 12–20-fold increase in all essential amino acids (except lysine, for which a 3-fold decrease was observed) and 3–6-fold increase in all non-essential amino acids – Table 2. The increase in 8 out of 9 essential amino acids is a positive consequence of bioprocessing. This is because amino acids in BSG are mostly restricted to the protein and lignocellulose structure and are, therefore, difficult to release (Lao et al., 2020). Such increase in

Table 2
Free amino acid content (FAA, mg/100 g DW).

	Essential Free Amino Acids				
	Taste*	**T0**	**T4**	**T28 P**	**T28 D**
Isoleucine	Bitter	1.5 ± 0.1^{a}	3.6 ± 0.5^{a}	17.4 ± 1.4^{b}	36.5 ± 2.5^{c}
Valine	Bitter-sweet	3.2 ± 0.2^{a}	9.1 ± 1.1^{a}	28.9 ± 2.2^{b}	55.4 ± 1.5^{c}
Leucine	Bitter	3.4 ± 0.3^{a}	7.4 ± 1.0^{a}	45.6 ± 3.5^{b}	82.3 ± 3.0^{c}
Lysine	Bitter-Salty-Sweet	2.0 ± 0.1^{a}	1.6 ± 0.2^{a,b}	0.6 ± 0.0^{c}	0.9 ± 0.0^{b,c}
Threonine	Sweet-Bitter	2.3 ± 0.1^{a}	2.6 ± 0.2^{a,b}	3.7 ± 0.3^{b}	15.3 ± 0.2^{c}
Tryptophan	Bitter	0^{a}	0^{a}	11.4 ± 0.5^{b}	16.3 ± 1.2^{c}
Methionine	Bitter	0.4 ± 0.0^{a,b}	0^{a}	10.1 ± 0.7^{b,c}	18.8 ± 3.4^{c}
Phenylalanine	Bitter	3.5 ± 0.2^{a}	10.3 ± 1.3^{a}	51.2 ± 2.9^{b}	77.3 ± 0.2^{c}
Histidine	Bitter	3.5 ± 0.7^{a}	57.3 ± 5.6^{b}	66.4 ± 4.7^{b}	95.4 ± 2.1^{c}
Total essential FAA		**19.7 ± 2.3**	**91.8 ± 14.2**	**235.0 ± 23.2**	**398.1 ± 16.8**
	Non-essential Free Amino Acids				
Serine	Sweet	1.8 ± 0.2^{a}	2.0 ± 0.2^{a}	5.6 ± 0.4^{b}	3.8 ± 0.0^{c}
Aspartic acid	Bitter-Salty-Sour	1.5 ± 0.0^{a}	1.3 ± 0.1^{a}	2.6 ± 0.1^{b}	4.3 ± 0.3^{c}
Cystine	Sulphurous	1.8 ± 0.0^{a}	5.9 ± 0.3^{b}	5.1 ± 0.2^{b}	5.9 ± 0.1^{b}
Glycine	Sweet	0.8 ± 0.1^{a}	1.2 ± 0.2^{a}	6.3 ± 0.6^{b}	10.8 ± 0.7^{c}
Tyrosine	Bitter	1.8 ± 0.0^{a}	1.6 ± 0.3^{a}	5.4 ± 0.4^{b}	17.8 ± 0.2^{c}
Proline	Sweet	7.8 ± 1.7^{a}	8.9 ± 1.0^{a}	6.5 ± 0.8^{a}	18.2 ± 1.8^{b}
Arginine	Bitter	3.6 ± 0.2^{a}	4.2 ± 0.5^{a}	19.8 ± 1.7^{b}	20.9 ± 0.4^{b}
Alanine	Sweet	2.0 ± 0.1^{a}	2.1 ± 0.3^{a}	7.2 ± 0.6^{b}	30.6 ± 0.7^{c}
Glutamic acid	Bitter-Salty-Sour	1.3 ± 0.0^{a}	2.1 ± 0.3^{a}	6.6 ± 0.6^{b}	37.4 ± 0.3^{c}
Total non-essential FAA		**22.4 ± 2.6**	**29.3 ± 4.6**	**65.1 ± 7.8**	**149.7 ± 1.3**
	Others (amino acid derivatives)				
Methionine Sulfone	-	0^{a}	0^{a}	1.0 ± 0.0^{a}	4.3 ± 0.4^{b}
Taurine	-	0^{a}	2.1± 2.9^{a}	13.1 ± 7.7^{a}	9.7 ± 0.6^{a}
Cysteic acid	-	1.3 ± 0.1^{a}	61.9 ± 7.7^{b}	54.4 ± 4.6^{b}	72.4 ± 1.1^{b}
GABA	-	1.5 ± 0.1^{a}	6.2 ± 0.7^{a,b}	24.1 ± 2.2^{c}	9.2 ± 0.4^{b}

GABA- Gamma-aminobutyric acid. Average ± SD, $n = 2$. * (Delompré et al., 2019; Schiffman et al., 1981). The heat map is based on global scores. Mean values with different letters in each row are statistically significant ($P < 0.05$).
Low → High.

FAA after LAB fermentation has been linked to increased protein digestibility and improved nutritional value of plant-based products (Gobbetti et al., 2020). More importantly, bioprocessing released two essential amino acids, tryptophan (11.4 and 16.3 mg/100 g, for bioprocessing with POM1 and DSM 14800, respectively) and methionine (10.1 and 18.8 mg/100 g, for bioprocessing with POM1 and DSM 14800, respectively), which were lacking or negligible (0.0 or 0.4 mg/100gm tryptophan and methionine, respectively) in the T0 (control sample), increasing the nutritional value of the product. In this study, the most abundant amino acids were proline (T0) and histidine (for both T28 P and T28D).

Generally, in the early growth stage, microbes consume amino acids for growth and produce more amino acids in the later stage (Zeng et al., 2022). In this study, the amino acid profile was strain-dependent, showing that the T28 D samples were exceptionally high (ca. 5-fold) in threonine, alanine, and glutamic acid compared to T28 P samples. While the T28 P sample was higher in γ-aminobutyric acid (GABA) compared to T28 D and T0 (24.2 vs. 9.2 vs. 1.5 mg/100 g DW). The difference in profile between the two fermented BSG is relevant for the sensory aspect, as amino acids could influence the flavour profile of the food product due to their sour, sweet, and bitter attributes (Ardö, 2006) – Table 2. The differences are more clearly highlighted in the scores and bi-plots (**Fig. S1 a, b**), where all groups (T0, T4, T28P and T28 D) are distinctly separated. The differentiating factor for T0 was Lysine, while for T28 P, it was GABA.

GABA is a functional non-protein amino acid neurotransmitter in the mammalian central nervous system (Sarasa et al., 2020). This translates to two possible effects on food products: anti-hypertension and anti-depression (Diana et al., 2014). The biosynthesis of GABA from glutamic acid is considered a resistance mechanism to regulate the internal pH in an acidic environment (Sarasa et al., 2020). All these align with the present study as T28 P has higher GABA levels (24.0 and 9.2 mg/100 g DW) but much lower glutamic acid (6.6, 37.4 mg/100 g DW), lower pH (3.8 vs 4) and higher organic acid (18.3 vs 12.2 mg/g DW) and TTA value (6.5 vs 5.5 mL) compared to T28 D. Similar species-dependent GABA content has been reported on LAB fermentation of BSG ranging from 7 to 9 compared to 3.7 mg/g for raw BSG (Zeng et al., 2022).

Hence, the FAA observed here depended on the substrate and the bioprocessing conditions (enzymes and microbes), and it has both nutritional and flavour repercussions. In this study, the starter culture played a more significant role than enzymes alone in releasing FAA.

3.4. Phenolic content and antioxidant activity

In this research, the native BSG (T0, control) had a total phenolic content (TPC) of ~ 1 mg GAE/g DW – Fig.2a, where the most abundant polyphenol was procyanidin B (PC), followed by catechin (C) at 402 and 369 μg/100 g DW, respectively. In BSG, the free or unbound phenolic acids constitute only <0.5 mg/g of BSG (McCarthy et al., 2013), while most are bound with cell wall components and cannot be released in the small intestine as humans lack esterase enzymes (Pérez-Jiménez et al., 2013). Thus, increasing the bioavailability of bound phenolic acids through bioprocessing could aid in achieving the potential health benefits of phenolic acids. The TPC increased to ~ 2 mg GAE/g DW after 4 h of enzyme hydrolysis (T4) and to ~ 3 mg GAE/g DW after fermentation

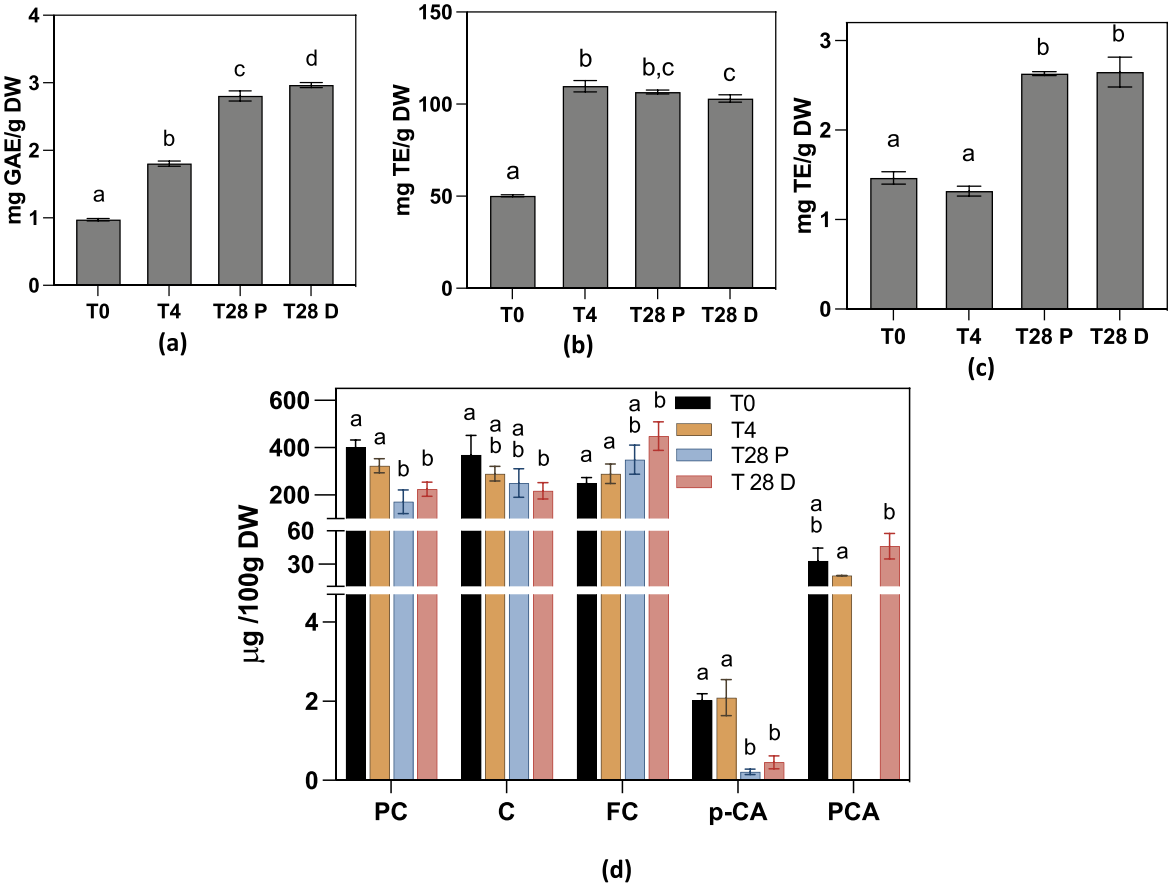

Fig. 2. Polyphenols and antioxidant activities of 80 % MeOH extracts. **a)** Total phenolic content, **b)** DPPH free radical scavenging activity, **c)** Ferric reducing antioxidant power, **d)** Quantified polyphenols. **T0**: 0 h (Control), **T4**: 4 h enzyme hydrolysed, **T28 P**: fermented with POM1 for 24 h, **T28 D**: fermented with DSM 14800 for 24 h. **PC**: Procyanidin B. **C**: Catechin. **FC**: Ferulic acid. ***p*-CA**: *p*-Coumaric acid. **PCA**: Protocatechuic acid. Expressed as mean ± SD, $n = 3$. Bar means with different letters are statistically significant ($P < 0.05$).

(T28 P & D). The most abundant polyphenol for both the bioprocessed samples was ferulic acid (FC)- 349 and 449 μg/100 g DW for T28 P and T28 D, respectively. In an earlier study, the TPC of enzyme hydrolysed and POM 1 fermented BSG was 3.8 mg GAE/g DW with a ferulic acid content of 145 μg/100 g DW (Moirangthem et al., 2024). This difference is presumed to be due to BSG batch variation, as ferulic acid was still the most abundant polyphenol in both studies.

Overall, bioprocessing significantly increased the antioxidant potential, ca. 2-fold –Fig. 2b–c. Fermentation of BSG has been reported to increase the antioxidant activity (DPPH) by 2.08-fold and total phenolics by 3.6 times compared to unfermented (Tan et al., 2020). BSG enzymatic hydrolysis (at enzyme optimum 50 °C) followed by fermentation at microbe optimum (30 °C) positively influenced the antioxidant properties (Verni et al., 2020). The increase in antioxidant capacity could be linked to the microbial activity that releases cell wall-bound polyphenols, bioactive peptides and amino acids (Abeynayake et al., 2022; Bamdad et al., 2017; De Montijo-Prieto et al., 2023; Verni et al., 2020, 2019). In this study, hydrophobic amino acid content was significantly increased during bioprocessing – Table 2.

Moreover, although the TPC and antioxidant activities of both bioprocessed samples were similar, T28 P had lower polyphenol content (μg/100 g DW) compared to T28 D– 0 vs 46 (Protocatechuic acid), 0.2 vs 0.45 (*p*-Coumaric acid), 171 vs 224 (Procyanidin B) and 349 vs 449 (Ferulic acid), respectively, showing the microbial influence on the polyphenol profile.

3.5. Volatile organic compounds (VOCs)

In total, 45 volatile compounds were identified in these samples: acids, alcohols, aldehydes, alkanes, benzenes, esters, ether, furans, ketones, sulphur compounds, terpenes and chlorinated compounds – Table 3. Principal Component (PC) Analysis Plots in Fig 3a–b highlight the volatile distribution between the samples. The total variance is 62.5 %, with the PC-1 axis accounting for 39.6 % of the difference, while the PC-2 axis accounting for 22.9 %. The T0 and T4 samples are located closely on the negative side of PC-1 and near the PC-2 axis. This indicates that the volatile profile did not change much over the first 4 h of enzymatic treatment. Both the T28 D and T28 P samples were located on the positive side of PC1 but distinctly separated from each other on the negative and positive sides of the PC-2 axis. This clearly highlights changes in the volatile profile from the control and 4-hour sample, but also that using different starter cultures generated independent volatile metabolites.

The T0 sample contained 39 VOCs, the most abundant of which on a percentage basis were: ethyl acetate (35.5 %), hexanal (15.3 %), heptane (9.9 %), 3-methyl butanal (7.4 %), 2-pentylfuran (5.4 %) and 2-methyl-3-butenal (2.7 %), with no other VOC at an abundance of >2.5 %. In contrast, the T28 P sample contained 44 VOCs with an approximate 55 % increase in the total abundance of all VOC compared to T0. The T28 P sample consisted mainly of the same VOC as T0, except the abundance of heptane (3.5 %), hexanal (2.9 %) and 3-methylbutanal (2.5 %) decreased, while the abundance of ethyl acetate (61 %) and 2-pentylfuran (3.7 %) increased, and acetic acid (10.4 %) was generated. Ethyl acetate is derived from the esterification of ethanol and acetic acid, both of which are derived from carbohydrate metabolism. It is also worth noting that the aroma-active VOCs, 2,3-butanedione (1.9 %), 2-methoxy-4-vinylphenol (0.4 %), hexanoic acid (0.2 %), and methyl hexanoate (0.02 %) were also generated during fermentation in T28 P. Many lipid oxidation derived VOC, such as pentanal, hexanal, heptanal, octanal, nonanal decreased during fermentation in T 28 P, as did the branch chain aldehyde (3-methylbutanal), the aromatic aldehyde (benzaldehyde), and the alkanes (hexane and heptane). The lipid content of BSG is a source of VOC, such as propionic, acetic, and butyric acids, and fatty acids (Naibaho and Korzeniowska, 2021).

The profile of the T28 D sample differed, with 42 VOC identified, mainly consisting of ethyl acetate (33.8 %), acetoin (33.4 %), 2,3-butanedione (12.7 %), and 2-pentylfuran (3.0 %), with no other VOC above 2.5 %. The total abundance of VOC was ~58 % more than T0 and, therefore, slightly more than in the T28 P sample. The greatest difference between T28 D and T28 P was the lower amounts of ethyl acetate, acetic acid, 3-methyl butanal, hexanal, heptane, and greater amounts of acetoin and 2,3-butanedione. A higher amount of 2,3-butanedione and acetoin is expected in T28 D as *Pediococcus* was isolated from spoiled beer (Dobson et al., 2002). In comparison, a higher amount of acetate and acetic acid in T28 P was expected as POM1 is facultatively heterofermentative (Gobbetti, 1998). T28 P produced more acetic acid than T28 D, supported by Table 3 (~ 10X) and Fig.1c (~ 5X). LAB are suitable starters for plant-based yoghurt prototypes because they are robust under low pH conditions (Charalampopoulos et al., 2002) and can develop 'dairy-related' flavours (e.g., diacetyl, acetoin, acetaldehyde) (Prado et al., 2008; Salmerón et al., 2015). Recently, fermentation of BSG pressed liquid has been reported to reduce key off-flavour compounds of BSG while adding pleasant flavours such as fruity, chocolate and caramel notes (Shetty et al., 2023).

3.6. Liking and sensory profiles

The consumer sensory study was conducted with 119 untrained participants, 87 females and 32 males aged between 20 and 56 years, with a mean age of 27.4 years. Around 85 % of the participants reported having consumed plant-based yoghurt-like snacks. Upon visual inspection with the naked eye, all prototypes were of the same colour. They could be described as pale-brown colour, mostly due to the lightly roasted pilsner malt used to make the BSG. However, the colour became slightly lighter after blending, possibly due to the introduction of air bubbles.

The distribution of liking scores of the spoonable prototypes are shown in Fig. 4a–c. The participants preferred the control samples (both with purée, **C P,** and without purée, **C NP**) in all aspects: aroma, flavour, and overall. Among the bioprocessed samples, POM1 samples (with purée, **P,** or without purée, **P NP**) were more liked than DSM 14800 samples (with purée, **D P,** or without purée, **D NP**) in all aspects. Furthermore, in all aspects, the participants preferred prototypes with purées (**C P, P, D P**) over the versions without purées (**C NP, P NP, D NP**). Such influence due to the addition of flavouring is also common in dairy counterparts (Keating and White, 1990). Between aroma and flavour, flavour appeared to have a more substantial influence on overall liking. In this study, participants were able to differentiate between the unprocessed (control) and bioprocessed prototypes and even between the two bioprocessed prototypes. More importantly, only a few participants rated any prototypes as Dislike Extremely.

Following this, the potential aroma and flavour attributes influencing the liking scores of the prototypes were explored using CATA tasks (Fig. 5a–l). The response for CATA is binary: yes or no Results of the CATA indicate the proportion of participants who perceived a particular attribute, while it is not an accurate measure of the intensity of the attributes. The most common responses for aroma were sour, cereal, fruity, and berry. The flavour attributes with the most common responses were bitter, sweet, sour and cereal.

In the flavour CATA, up to 76 % of participants perceived the bioprocessed prototypes as bitter (Fig. 5a). This could be due to bioprocessing increasing hydrophobic amino acids, most of which are associated with a bitter taste (Table 2). The increase in polyphenols (Table 3a and 3d) is also associated with a bitter taste and astringency (Osakabe et al., 2024). Additionally, extensive protein hydrolysis has been reported to produce smaller (low molecular weight) peptides with a higher bitter intensity than those produced by limited hydrolysis. At the same time, amino acids such as proline in small peptides and dipeptides have also been linked to bitterness (Liu et al., 2022). This is because taste is a contact attribute, and small-sized peptides are more likely to interact with bitter receptors than larger peptides (Aluko, 2017). Hence, peptide size distribution could be an important parameter

Table 3
Volatile organic compounds profile (expressed as relative percentage).

Name	CAS	LRI	Ref LRI	Reference Odour Descriptor	Odour Ref.	T0	T4	T28 D	T28 P
Acid									
Acetic acid	64-19-7	692	690	Pungent, vinegar, sour	1	0,00	0,00	0,94	10,44
Butanoic acid, 3-methyl-	503-74-2	918	917	Cheesy, sweaty, rancid, goat, rotten fruit	1,2	0,00	0,00	0,36	0,00
Hexanoic acid	142-62-1	1053	1049	Acidic, sweaty, cheesy, sharp, goaty	1	0,00	0,00	0,07	0,23
Phenol									
2-Methyoxy-4-vinylphenol	7786-61-0	1413	1405	smoky, woody, phenolic, spicy, clove, carnation, peppery	1	0,00	0,00	0,80	0,42
Alcohol									
3-Buten-2-ol, 2-methyl-*	115-18-4	659		Herbal, earthy, oily	1	2,66	1,76	1,05	1,42
Aldehyde									
Butanal, 3-methyl-	590-86-3	693	692	Malty, cheese, green, dark chocolate, cocoa	1,2	7,39	4,43	1,69	2,56
Pentanal	110-62-3	736	733	Pungent, almond, malty	2	2,14	1,45	0,56	0,60
Hexanal	66-25-1	840	839	Green, grassy, herbal, lemon, tallow	1,2	15,29	10,79	1,90	2,95
Furfural	98-01-1	902	899	Sweet, woody, almond, caramellic, baked, bread	1,2	0,05	0,04	0,00	0,02
Heptanal	111-71-7	944	943	Fatty/oily, green, citrus, rancid	1,2	1,97	1,27	0,19	0,15
Benzaldehyde	100-52-7	1031	1031	Bitter almond, sweet cherry	1	0,77	0,56	0,07	0,33
Octanal	124-13-0	1048	1047	Waxy, citrus, soapy, green, fresh, fatty	1,2	0,32	0,19	0,09	0,05
2-Octenal, (E)-	2548-87-0	1120	1116	Fresh, cucumber, fatty, green, banana, waxy	1	0,19	0,14	0,00	0,17
Nonanal	124-19-6	1151	1150	Green, citrus, fatty, floral	1,2	0,58	0,39	0,18	0,24
Alkane									
Pentane	109-66-0	502	500			0,00	0,00	0,43	0,19
n-Hexane	110-54-3	601	600	Gasoline	3	0,24	0,10	0,06	0,09
Heptane	142-82-5	702	700	Sweet, ethereal	1	9,85	3,71	1,85	3,48
Octane	111-65-9	802	800	Gasoline	3	0,19	0,02	0,32	0,21
Benzene									
Benzene	71-43-2	687	682	Gasoline, sweet	1	0,08	0,07	0,05	0,07
Toluene	108-88-3	794	794	Nutty, bitter, almond, plastic	2	0,11	0,08	0,04	0,04
Ethylbenzene	100-41-4	892	890	Gasoline	3	0,10	0,07	0,03	0,06
Styrene	100-42-5	930	929	Balsamic, floral, plastic	1,2	0,32	0,33	0,16	0,17
Ester									
Ethyl acetate	141-78-6	643	642	Fruity, pineapple, apples, weedy, green	1,2	35,54	59,00	34,08	61,25
Methyl hexanoate	106-70-7	952	947	Green, solvent, estery, fruity, winey, Cognac	1,2	0,02	0,03	0,02	0,02
Ethyl benzoate	93-89-0	1233	1232	Fruity, dry, musty, sweet, wintergreen	1,2	0,12	0,02	0,03	0,03
Ether									
Ethyl ether	60-29-7	516	515	Pungent, ethereal	1	3,66	2,31	0,83	1,43
Furan									
Furan, 2-methyl-	534-22-5	635	615	Chocolate, ethereal, acetone,	1	0,71	0,62	0,13	0,20
Furan, 2-ethyl-	3208-16-0	720	720	Sweet, burnt, earthy, malty	1	0,24	0,25	0,28	0,51
2-n-Butyl furan	4466-24-4	913	909	Fruity, winey, sweet, spicy	1	0,25	0,23	0,17	0,21
Furan, 2-pentyl-	3777-69-3	1014	1012	Green bean, vegetable, earthy, metallic	1	5,44	4,25	3,07	3,73
Ketone									
Acetone	67-64-1	534	533	Solvent, ethereal, sour milk, apple	1	1,77	1,08	0,51	0,57
2,3-Butanedione	431-03-8	632	631	Buttery, creamy, sweet, pungent	1	0,00	0,00	12,85	1,87
Methyl Isobutyl Ketone	108-10-1	782	784	Sharp, solvent, green, herbal, fruity, dairy, spicy	1	0,48	0,38	0,00	0,27
Acetoin	513-86-0	786	778	Buttery, creamy, dairy, milky, fatty	1,2	0,70	0,58	33,71	1,97
2-Heptanone	110-43-0	936	936	Fruity, spicy, sweet, herbal, woody	1	1,93	1,24	1,12	1,27
Methyl heptanone	110-93-0	1034	1034	Citrus, green, musty, lemongrass, apple	1,2	0,81	0,67	0,53	0,58
Acetophenone	98-86-2	1146	1030	Must, flower, almond	1,2	0,05	0,03	0,02	0,02
2-Octanone	111-13-7	1038	1035	Earthy, woody, herbal, cheesy, parmesan	1	2,38	1,59	1,08	1,08
Sulfur									
Disulfide, dimethyl	624-92-0	779	771	Sulfurous, vegetable, cabbage, onion	1,2	0,22	0,12	0,10	0,10
Terpene									
α-Pinene	80-56-8	956	950	Pine, camphoreous, earthy, woody	1,2	0,05	0,03	0,02	0,03
β-Myrcene	123-35-3	1006	1004	Herbaceous, metallic	1	1,08	0,75	0,47	0,47
D-Limonene	5989-27-5	1055	1055	Fruity, citrus, orange, sweet, peely	1	0,13	0,09	0,05	0,06
o-Cymene	527-84-4	1058	1056			0,07	0,04	0,03	0,03
Humulene	6753-98-6	1534	1537	Woody	1,2	2,07	1,27	0,86	0,80
Other									
Methylene chloride*	75-09-2	563				0,03	0,04	0,01	0,02
Total (%)						100	100	100	100

Compound identification, chemical class, and average relative percentage measured ($n = 3$).
CAS: Chemical CAS (Chemical Abstract Service) (Blanks relate to isomers where we could not be 100 % sure of identification and therefore could not provide full identification. **LRI**: Linear Retention Indices as determined using the method by Van Den Dool & Kratz (van Den Dool and Kratz, 1963). **REF LRI**: These values were obtained from published papers or NIST 2014; **NA**: No published reference available to date (not many published as yet on a DB624 column); *tentative identification might be an isomer of this chemical compound. The database: http://www.thegoodscentcompany.com (1), http://www.flavornet.org/flavornet.html (2) and/or https://pubchem.ncbi.nlm.nih.gov/ were used as a source of reference odour descriptors

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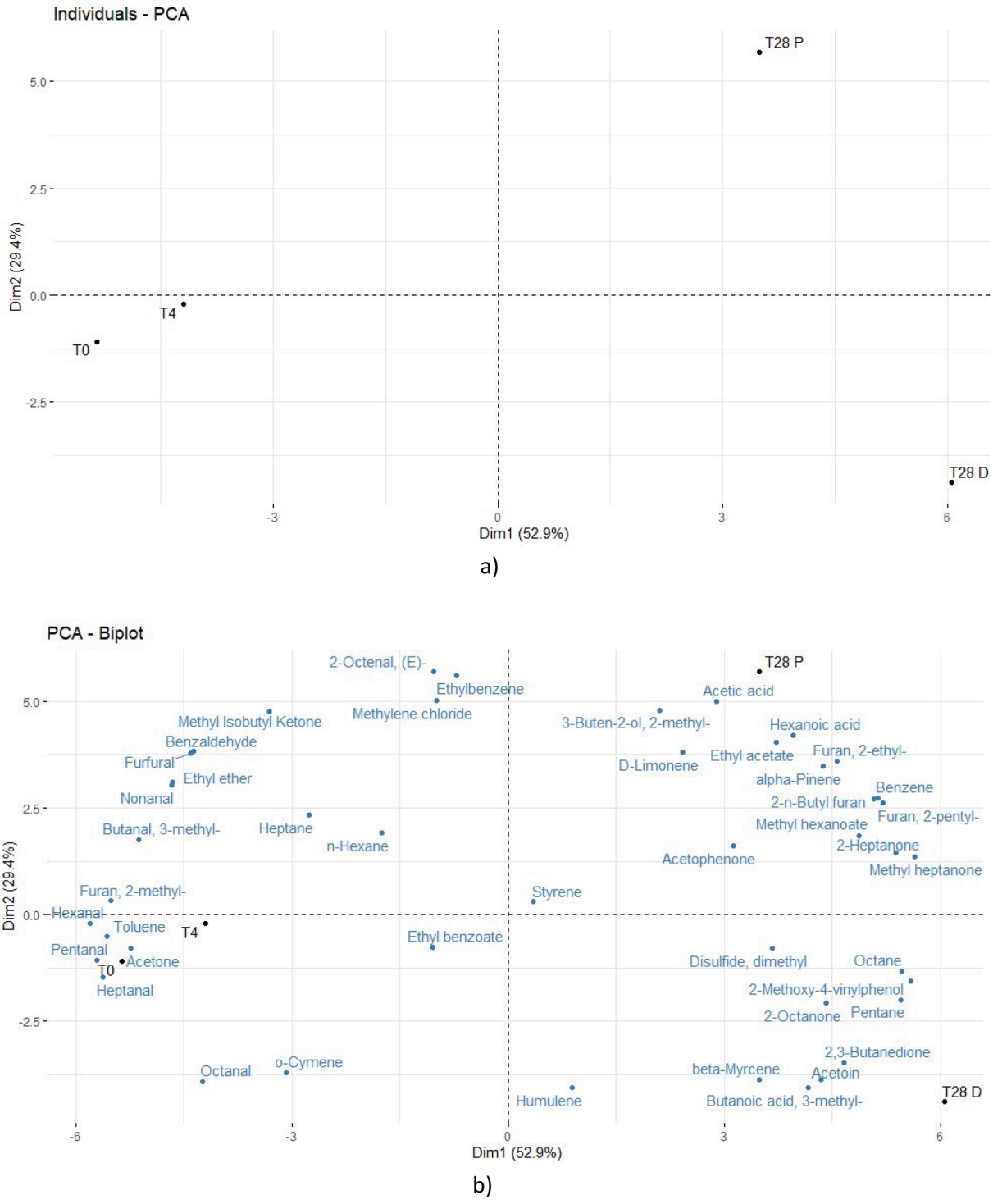

Fig. 3. Principal component analysis. a) Plot of separation based on volatile profile – samples, b) Bi-plot of separation based on volatile profile (samples + volatiles).

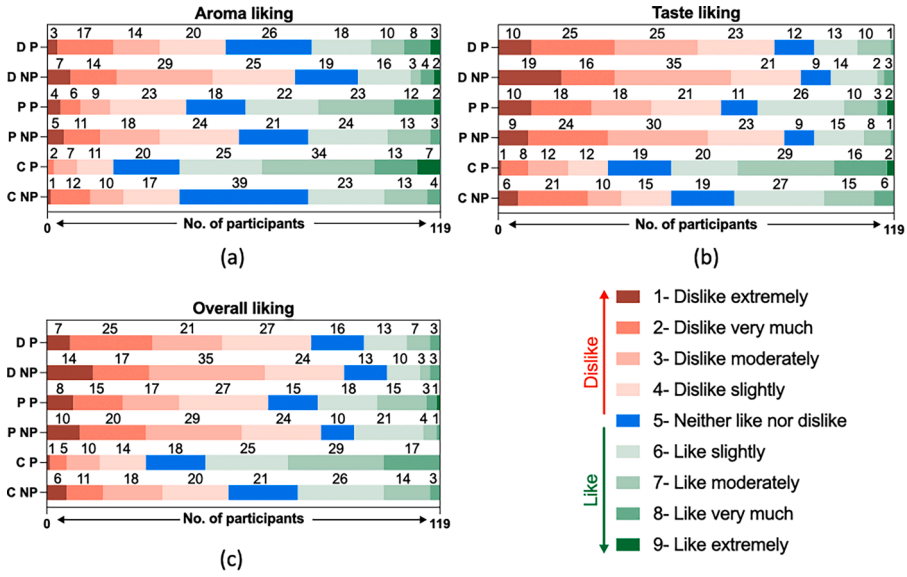

Fig. 4. The distribution of liking scores of the prototypes based on (a) Aroma, (b) Taste and (c) Overall experience. The values above the legends are the number of participants who assigned each hedonic score. **C NP** = control no purée, **C *P*** = control with purée, **P NP** = Fermented with POM1 no purée, ***P*** = fermented with POM1 with purée, **D NP** = fermented with DSM 14800 no purée, **D *P*** = fermented with DSM 14800.

to balance hydrolysis and off-flavours. Between the two bioprocessed samples, more participants reported the one fermented by *P. claussenii* DSM 14800 samples as bitter. These samples also contained twice as many free amino acids than those fermented with L. *plantarum* POM1 (T28 D vs T28 P, in Table 2). However, adding purée resulted in approximately a 10 % decrease in participants reporting bitterness for the bioprocessed samples and improved the liking scores. In the future, deactivating the enzymes (such as by heating) before fermentation could also be explored to control the hydrolysis and improve the flavour, as shown in the case of oat or soy-based yoghurt (Wang et al., 2023). In this study, enzymes were not deactivated after 4 h to limit the thermal treatment steps, achieving a simultaneous saccharification and fermentation condition.

More participants reported the control samples to be sweeter than the POM1 samples, followed by DSM 14800 samples (Fig. 5b). This could be because the control contained more sugars (glucose, mannose, and fructose) than the bioprocessed prototypes (Fig. 1d). The relative sweetness levels are fructose > sucrose (used in recipe) > glucose. The addition of purée increased the percentage of sweet responses by 27 % for control, ~20 % for POM1, and 12 % for DSM 14800. The greater increase in POM1 could be associated with a lower level of free amino acid and a reduced bitter response compared to DSM 14800. While >70 % of the participants reported the bioprocessed samples as sour, only 18 % reported the control as sour (Fig. 5j).

In the aroma CATA, up to 47 % of the panellists experienced a sour aroma in the bioprocessed sample, compared to only 13 % in the control samples (Fig. 5a). The response for yoghurt aroma was moderate at most, but it was more common in the bioprocessed sample than in the control (Fig. 5b). Interestingly, adding purée increased the yoghurt aroma response across all samples, with the control sample reaching up to 37 %, reflecting an increase of ~30 % from the original 6 %. A similar effect of purée was observed in the fruity and berry aroma responses, with the POM1 sample receiving the highest responses (Fig. 5a–b). As expected, adding the strawberry purée effectively enhanced the fruity and berry aroma responses, with control samples having the highest increase of up to 50 %. Regarding cereal aroma, fewer participants reported detecting it in the bioprocessed samples (up to 60 %) compared to the control samples (90 %) (Fig. 5c). The addition of purée decreased the cereal response by up to 30 % compared to the control sample. A small percentage of participants reported detecting a mushroom aroma, with the highest response in the DSM 14800 samples (34 %) (Fig 5f).

For cereal flavour, fewer participants reported the bioprocessed samples as having a cereal taste (up to 62 %) compared to the control (up to 90 %) (Fig. 5l). Adding purée to the control samples did not increase sour or decrease cereal responses to the same extent as in the bioprocessed samples. Sour taste perception results from the properties of weak acids, such as lactic acid, and is influenced by the total amount of acid present, as measured by titratable acidity (Zeece, 2020). Hence, lactic acid fermentation in this study conferred a sour sensory profile characteristic of conventional milk-based yoghurt (Montemurro et al., 2021). Between the two bioprocessed samples, T28 P was reported to have a more obvious sour taste, according to the CATA. These results align with the acidity of the samples: T28 P had higher levels of lactic acid, 4.5 times more acetic acid, a lower pH and higher TTA compared to T28 D.

Based on the CATA results, more participants reported presumably pleasant fruity, berry, and yoghurt aromas, while fewer reported cereal aromas in the bioprocessed samples. More participants also reported sour and bitter tastes, while fewer reported cereal flavour. For both the control and bioprocessed samples, the addition of purée increased the liking scores for aroma, taste, and overall sensory experience. In this study, the control samples received higher liking scores, which may be associated with the bitter taste identified in the bioprocessed samples. On the other hand, bioprocessing can enhance sensory quality by reducing both cereal aroma and flavour. Although an important sensory parameter, texture attributes were not included in this study. This was partially because it was challenging to decrease the BSG particle size during wet milling sufficiently, and the study was mostly focused on taste and aroma profile. However, this would be an important aspect to explore in future studies, with a much finer size reduction regime.

4. Conclusion

Developing plant-based dairy alternative snacks is challenging. Technological differences from those used in the traditional dairy industry are expected since plant materials possess different physicochemical qualities and more significant heterogeneity. Even more challenging is making them from side-streams, which could be categorised as upcycled dairy alternatives. L. *plantarum* POM1 and *P. claussenii* DSM 14800 were suitable starter cultures for enzymatically-

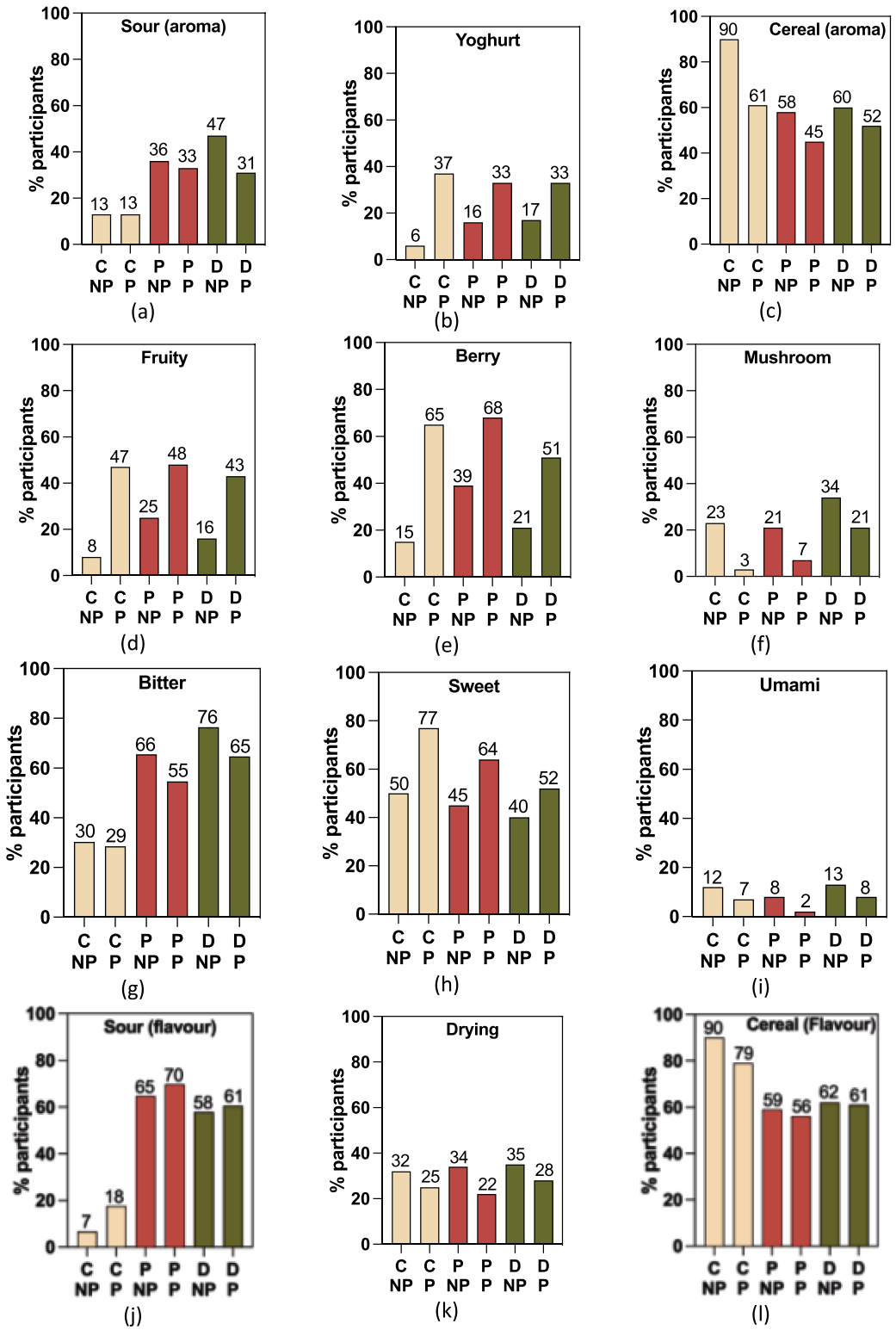

Fig. 5. CATA profile of the prototypes. a-*f* = Aroma CATA. ***a*** = sour, ***b*** = yoghurt, ***c*** = cereal, ***d*** = fruity, ***e*** = berry, ***f*** = mushroom. g-*l* = Flavour CATA. ***g*** = bitter, ***h*** = sweet, ***i*** = umami, ***j*** = sour, ***k*** = drying, ***l*** = cereal. **C NP** = control no purée, **C *P*** = control with purée, **P NP** = fermented with POM1 no purée, ***P*** = fermented with POM1 with purée, **D NP** = fermented with DSM 14800 no purée, **D *P*** = fermented with DSM 14800. The values above the bars represent the percentage of panellists who experienced the particular attribute.

hydrolysed BSG without any nutrient supplementation. Although both showed similar preferential consumption of carbohydrates, fermentation with L. *plantarum* POM1 resulted in higher levels of TTA and organic acids. Bioprocessing increased the phenolic content and antioxidant activity. A strain-dependent impact on BSG bioprocessing was clearly observed. BSG fermented by *P. claussenii* DSM 14800 showed a higher release of free amino acids (both essential and non-essential), while fermentation with L. *plantarum* POM1 showed higher GABA release. Enzymes had a more prominent impact on the dietary fibre profile but did not influence the VOCs and had a lesser impact on the FAA profile.

The two fermented samples were also distinctly separate on the VOCs PCA. The consumer sensory study indicated greater acceptability of unfermented spoonable snack prototypes than the two fermented ones. However, fermentation reduced the cereal flavour while introducing new, presumably desirable aromas. This showed that although bioprocessing improved the prototype nutritional content, bioactive compounds, and certain aroma and flavour attributes, the release of compounds perceived as bitter needs to be optimised or mitigated through a selection of specific starter cultures, possibly reducing bitter compounds formation and/or recipe (such as adding purée or similar ingredients) to obtain an acceptable sensory profile. These insights will help modulate upcycled food towards consumer preferences and increase food system sustainability.

More studies are needed to explore processing conditions further, such as investigating the effects of finer milling, implementing enzyme deactivation before fermentation, and thoroughly assessing enzyme activity and flavour compounds including flavonoids. Additionally, on the sensory side, future work could involve trained panellists for a more precise characterization of attributes, including texture and colour and an evaluation of different flavouring options.

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Ethical statement

The study protocol followed the ethical guidelines of the sensory laboratory of the Department of Food and Nutrition, University of Helsinki, approved by The University of Helsinki Ethical Review Board in the Humanities and Social and Behavioural Sciences (Statement 46/2016). A written informed consent was obtained from each participant. They were able to withdraw from the survey at any time without giving a reason.

CRediT authorship contribution statement

Kamaljit Moirangthem: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Antti Knaapila:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Youngsun Lee:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Mari Sandell:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Iwona Skibinska:** Writing – review & editing, Visualization, Investigation, Formal analysis. **Kieran N. Kilcawley:** Writing – review & editing, Resources, Formal analysis. **Paula M. O'Connor:** Writing – review & editing, Resources, Investigation. **Henry N. Maina:** Writing – review & editing, Resources, Methodology. **Katariina Niklander:** Writing – review & editing, Investigation. **Emily P. Verhulst:** Writing – review & editing, Investigation. **Dilip K. Rai:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Rossana Coda:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, 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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2025.100621.

Data availability

No data was used for the research described in the article.

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