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Effect of Co-Fermentation of *Saccharomyces boulardii* CNCM-I745 with Four Different Probiotic Lactobacilli in Coffee Brews on Cell Viabilities and Metabolic Activities

Mei Zhi Alcine Chan [1], Li Ting Tan [1], Shermaine Wan Qing Heng [1] and Shao Quan Liu [1,2,*]

[1] Department of Food Science & Technology, National University of Singapore, Science Drive 2, Singapore 117542, Singapore
[2] National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou 215123, China
* Correspondence: fstlsq@nus.edu.sg; Tel.: +65-6516-2687

Abstract: Amid trends in non-dairy probiotic foods and functional coffees, it is timely to develop a high-count probiotic, fermented coffee beverage. Here, we aimed to enhance the viabilities of different probiotic lactobacilli strains in coffee brews by co-culturing with the probiotic yeast, *Saccharomyces boulardii* CNCM-I745. The growth, survival, and metabolic activities of *Lactiplantibacillus plantarum* 299v, *Lactobacillus acidophilus* NCFM, *Limosilactobacillus fermentum* PCC, and *Lactobacillus gasseri* LAC-343 were monitored when cultured individually or co-cultured in coffee brews with *S. boulardii* CNCM-I745. In co-cultures, all four probiotic lactobacilli maintained viable populations above 5.5 Log CFU/mL for at least 6 months at 4 and 25 °C. In contrast, singly cultured lactobacilli populations generally could not be detected beyond 3 months of storage at either temperature. In co-cultures, vigorous nutrient uptake (glucose, glutamate, and alanine) by the yeast limited lactic acid accumulation by the lactobacilli. Co-culturing also led to accumulations in yeast-derived metabolites (ethanol, 2/3-methylbutanol, 2,3-dimethoxystyrene, and decanoic acid), and lactobacilli-derived metabolites (4-ethylphenol), but the coffee bioactive components (caffeine, trigonelline, and 5-O-caffeoylquinic acid) and antioxidant capacities were maintained. Overall, *S. boulardii* CNCM-I745 is effective in enhancing the viabilities of probiotic lactobacilli from different species, which may be useful in developing shelf-stable probiotic foods.

Keywords: probiotic yeast; lactic acid bacteria; survival; fermentation; commensal interaction; non-dairy

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1. Introduction

Probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [1]. Encompassing strains from a wide range of genera such as the recently reclassified genus *Lactobacillus*, as well as *Bifidobacterium*, *Bacillus* and *Saccharomyces*, probiotics are clinically evidenced to be safe and therapeutically effective when alive at an efficacious dose throughout a product's shelf life [2]. Commercially available probiotic strains, such as *Lactiplantibacillus plantarum* 299v, *Lactobacillus acidophilus* NCFM, *Limosilactobacillus fermentum* PCC, and *Lactobacillus gasseri* LAC-343, are demonstrated to be safe and clinically effective in areas relating to gut, immune, and respiratory health [3–6]. In addition, *Saccharomyces cerevisiae* var. *boulardii*, the only yeast with a probiotic status, has been clinically evidenced to ameliorate gastrointestinal diseases (e.g., irritable bowel syndrome), owing to its unique cell wall structures and bioactive metabolite secretions [7].

As the health benefits of probiotics are increasingly recognised by consumers, probiotic-fortified foods have seen rising popularity. Traditionally dominated by dairy-based formats, probiotic foods based on non-dairy food matrices (e.g., cereals, fruits, vegetables, soy, and

chocolate) have emerged in the last decade due to trends in veganism and concerns such as lactose intolerance and dairy allergies [8]. In parallel, health and wellness trends have also led to the emergence of retail coffees fortified with protein, medium chain triglycerides, collagen, L-theanine, etc. [9]. These coffees, which are termed as functional coffees, have been fortified with ingredients to produce additional functional benefit, beyond those that are inherent in coffee, e.g., the natural level of antioxidants [9].

Prompted by ongoing developments in non-dairy probiotic foods and functional coffees, we previously fermented coffee brews with the probiotics *Lacticaseibacillus rhamnosus* GG and *S. boulardii* CNCM-I745 [10,11]. In nutrient-scarce coffee brews, probiotics were incapable of growing. However, this was overcome with nutrient supplementation in the form of glucose and inactivated yeast extracts, which enabled probiotic growth beyond 7 Log colony-forming units (CFU)/mL. More remarkably, co-culturing the lactobacilli with the yeast was crucial in sustaining probiotic viabilities during storage, as *L. rhamnosus* GG maintained viable populations above 7 Log CFU/mL for an additional 11 weeks in co-culture with *S. boulardii* CNCM-I745, in contrast to that in the single culture.

The ability of yeasts to enhance lactobacilli viability has been observed by others, for example, between *Saccharomyces cerevisiae* and *Lacticaseibacillus paracasei*, or even *Bifidobacterium animalis* subsp. *lactis* [12–14]. Among the proposed mechanisms to explain the viability-enhancing properties of yeasts, co-aggregation is one of the most popular. By forming mixed-species biofilms between lactobacilli surface proteins and yeast surface mannan, co-aggregation enables the yeast to efficiently assimilate lactic acid produced by the lactobacilli, thereby protecting the lactobacilli from acid-induced stress [15,16]. Another well-evidenced mechanism is the provision of diffusible biofactors (e.g., amino acids and hydrophilic metabolites) by yeasts, independent of the lactobacilli. These diffusible biofactors may be derived from metabolically active yeasts, autolysed yeasts, cell-free yeast supernatants, or exogenous amino acid supplementation [17]. Separately, Hirai and Kawasumi [18] demonstrated that lactobacilli viability was improved by the ability of yeasts to scavenge reactive oxygen species (e.g., hydrogen peroxide), but not by physical contact/co-aggregation nor yeast metabolites.

Despite mechanistic evidence supporting the viability-enhancing effects of yeasts, it remains unclear if the same effects can be achieved upon co-culturing *S. boulardii* CNCM-I745 with other probiotic lactobacilli species/strains in coffee brews. In fact, dissimilar viability-enhancing effects were reported to be dependent on the strains involved in the yeast-lactobacillus pairings [19]. Clarifying such effects beyond *L. rhamnosus* GG would not only aid in the understanding of probiotic yeast-lactobacilli interactions but would also minimise an over reliance on a single probiotic strain in the event of a supply chain disruption. In addition, the viability-enhancing effects of yeasts have rarely been explored beyond a storage period of 2 months. A longer study duration (e.g., $\geq$6 months) would enable a more realistic assessment of whether yeasts are commercially viable in producing shelf-stable probiotic foods that can compensate for lag-times associated with processing, handling, operations, transportation, distribution, and storage.

Therefore, the aim of this study was to determine the ability of probiotic *S. boulardii* CNCM-I745 in conferring survival-enhancing effects to four different probiotic lactobacilli in coffee brews stored for 6 months. The four probiotic lactobacilli were as follows: *L. plantarum* 299v, *L. acidophilus* NCFM, *L. fermentum* PCC, and *L. gasseri* LAC-343. Growth, survival, and changes in non-volatile and volatile profiles were examined to determine patterns in substrate utilisation and metabolite production. Moreover, coffee alkaloids and phenolic compounds as well as in vitro antioxidant capacities were evaluated to assess the impact of probiotic fermentation on coffee brew bioactivities.

2. Materials and Methods

2.1. Microbial Strains, Cultivation, and Enumeration

Probiotics used in this study were as follows: *L. plantarum* 299v (trademarked as LP299v® by Probi AB, Lund, Sweden. Isolated from Jarrow Formulas Ideal Bowel Support

dietary supplement, Los Angeles, CA, USA), *L. acidophilus* NCFM (Danisco A/S, Copenhagen, Denmark), *L. fermentum* PCC (Chr. Hansen A/S, Horsholm, Denmark), *L. gasseri* LAC-343 (Morinaga Industries, Tokyo, Japan), and *S. boulardii* CNCM-I745 (isolated from "Florastor", Biocodex, Beauvais, France). For *L. plantarum* 299v and *S. boulardii* CNCM-I745, which were isolated from dietary supplements, the isolated colonies were Gram-stained and observed under a microscope to assess if microbial morphology was consistent with what was expected of the probiotic strain.

Microbial cultivation and enumeration procedures have been previously described [10,11]. Briefly, lactobacilli were cultivated in de Man, Rogosa, and Sharpe (MRS) broth (Oxoid Ltd., Hampshire, UK) while *S. boulardii* CNCM-I745 was cultivated in yeast malt broth (10 g/L glucose, 3 g/L yeast extract, 3 g/L malt extract, and 5 g/L bacteriological peptone; all from Oxoid Ltd.). Lactobacilli were enumerated on MRS agar (Oxoid Ltd.) that was spiked with 0.5 g/L natamycin (Danisco A/S), while *S. boulardii* CNCM-I745 was enumerated on potato dextrose agar (Oxoid Ltd.) that was spiked with 0.1 g/L chloramphenicol (Sigma-Aldrich, St Louis, MO, USA).

2.2. Fermentation Conditions and Design

Previously, we demonstrated that *S. boulardii* CNCM-I745 enhanced the survival of *L. rhamnosus* GG after 11 weeks in co-culture compared to single culture [11]. To evaluate if the same viability-enhancing effect can be extended to other probiotic lactobacilli, *L. plantarum* 299v, *L. acidophilus* NCFM, *L. fermentum* PCC, and *L. gasseri* LAC-343 were cultured with and without *S. boulardii* CNCM-I745.

First, pasteurised coffee brews were supplemented with 0.25 g/100 mL glucose (Thomas Coopers Breweries, South Australia, Australia) and 0.06 g/100 mL inactivated yeast extract (Optiwhite®, Lallemand Pty., Montreal, QC, Canada), according to Chan et al. (2020) [10].

Pasteurised coffee brews were then inoculated with either single or co-cultures of lactobacilli (~7 Log CFU/mL) and *S. boulardii* CNCM-I745 (~6 Log CFU/mL) comprising the following: *S. boulardii* CNCM-I745 (Sb), *L. plantarum* 299v (299v and 299v + Sb; single and co-cultures, respectively), *L. acidophilus* NCFM (NCFM and NCFM + Sb), *L. fermentum* PCC (PCC and PCC + Sb), and *L. gasseri* LAC-343 (LAC and LAC + Sb). Triplicate independent batches of each fermentation treatment were then incubated at 30 °C for 24 h, followed by 6 months of storage at 4 °C and 25 °C, as described previously [11]. In parallel, uninoculated coffee brews (blank) were subjected to identical fermentation and storage conditions to serve as a control against other probiotic fermented coffee brews, and to ensure the absence of exogenous microbial contamination with routine enumeration.

Microbial enumeration and pH measurements were conducted at 0 and 24 h (fermentation period), and once monthly thereafter (6-month storage period). For physicochemical analyses, samples were frozen at −20 °C and thawed prior to analyses. Time-points for non-volatile compound analyses and antioxidant capacity assays were 24 h and 1 month of stored samples (4 and 25 °C). While time-points for volatile compound analyses were 24 h and 1 month of stored samples (25 °C).

2.3. Non-Volatile Compound Analyses

As detailed previously [10], quantification of free amino acids was conducted on an ARACUS amino acid analyser (MembraPure, Berlin, Germany), while quantification of glucose, acetic acid, lactic acid, succinic acid, caffeine, trigonelline, and 5-caffeoylquinic acid were conducted on a high-performance liquid chromatography system (HPLC; Shimadzu, Kyoto, Japan) coupled to an ELSD-LT II evaporative light scattering detector (Shimadzu) and photo-diode array detector (Shimadzu).

Briefly, chromatographic separation of sugars was achieved with a 150 mm × 4.6 mm Zorbax Carbohydrate column (Agilent Technologies, Santa Clara, CA, USA) at 40 °C. The mobile phase was 80% (*v/v*) aqueous acetonitrile operating at an isocratic flow rate of 1 mL/min. Quantification of organic acids was conducted using a 300 mm × 7.2 mm

C-160H column (Supelco Co., Bellefonte, PA, USA) at 40 °C. The mobile phase was 0.1% (*v/v*) aqueous sulfuric acid at an isocratic flow rate of 0.4 mL/min. For phenolic compounds and alkaloids, a 150 mm × 4.6 mm Zorbax Eclipse C18 column maintained at 40 °C was used. The binary mobile phase consisted of 0.1% (*v/v*) aqueous acetic acid (solvent A) and methanol neat (solvent B), operating at 0.4 mL/min with the following gradient: 0–3 min, 5% B; 3–8 min, 5–20% B; 8–12 min, 20–30% B; 12–20 min, 30% B; and 20–30 min, 30–40% B. Alkaloids and phenolic compounds were detected at λ = 270 nm and 320 nm, respectively. Samples were diluted in their respective mobile phases, followed by centrifugation, and filtration through 0.20-µm filters prior to injection into the HPLC system.

2.4. Volatile Compound Analyses and Data Processing

Volatile compounds were analysed using headspace (HS)-solid phase micro extraction (SPME) combined with 7890A gas chromatography (GC) and a 5975C triple axis mass spectrometer (MS; Agilent Technologies, Santa Clara, CA, USA), as reported previously [10,11]. GC-MS instrument parameters and sample preparation procedures remained unchanged, except for a modification pertaining to the oven temperature ramp (50 °C for 5 min, increased to 230 °C at a rate of 3 °C/min thereafter). The concentration of butyl butyryl lactate (Mane SEA Pte Ltd., Singapore) as an internal standard was also reduced to 50 mg/L in coffee brews. The analysis blank comprised Ice Mountain water (Fraser and Neave Limited, Selangor, Malaysia) spiked with 50 mg/L of butyl butyryl lactate.

After GC-MS analysis, raw data were converted to mzXML format with ProteoWizard and imported into XCMS Online (https://xcmsonline.scripps.edu (accessed on 24 May 2021)) for feature extraction. The resulting feature list was exported to Excel, where features were filtered off if they were either not present in two-thirds of samples or if they possessed intensities less than 3-fold compared to the analysis blanks. The remaining unfiltered feature intensities were subtracted from the average analysis blank intensities, and missing values were replaced with half of the minimum value of each respective feature. Compounds with match probabilities > 70% were identified using NIST14 and Wiley275 libraries, and further confirmed with their linear retention index (LRI) calculated based on the retention time of a C10–C40 standard alkane mixture (Sigma-Aldrich). Features belonging to the same parent compound were removed, and compound intensities were represented by a single ion intensity (usually the base peak ion). Compound intensities were then normalised to butyl butyryl lactate and expressed as the following: (*m/z* fragment peak intensity of compound/base peak intensity of butyl butyryl lactate) × 1000.

2.5. Antioxidant Capacity Assays

Total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and oxygen radical absorbance capacity (ORAC) assays were performed according to the protocol by Singleton and Rossi [20], Brand-Williams et al. [21], and Žuvela et al. [22], respectively. For TPC and DPPH analyses, coffee brews were diluted 50-fold in deionised water, while a dilution factor of 10,000-fold in 75 mM phosphate buffer (pH 7.4) was followed for ORAC assays. Results were expressed as mg of gallic acid (for TPC assay) or Trolox (for DPPH and ORAC assays) equivalents per mL of coffee brew. Duplicate readings for each assay were conducted.

2.6. Statistical Analysis

Between-group comparisons were evaluated for statistical significance ($p < 0.05$) using ANOVA with Tukey's HSD post hoc test. Normality and equality of variances were analysed with Shapiro–Wilk test and Levene test, respectively. Statistical analyses were performed using IBM® SPSS® Statistics 20.0 software (Chicago, IL, USA), and experimental data from triplicate independent fermentations ($n = 3$) were presented as mean values ± standard deviation.

Normalised volatile compound peak intensities were log2 transformed and pareto scaled, prior to principal component analysis (PCA) using OriginPro 2019b, and heatmap

analysis with Euclidean distance measure and Ward-based hierarchal clustering using MetaboAnalyst (http://www.metaboanalyst.ca (accessed on 25 May 2021)).

3. Results and Discussion

3.1. Probiotic Growth and Survival during Fermentation and Storage in Coffee Brews

Figure 1 shows the growth, survival, and pH of *L. plantarum* 299v (299v), *L. acidophilus* NCFM (NCFM), *L. fermentum* PCC (PCC), and *L. gasseri* LAC-343 (LAC) when cultured with and without *S. boulardii* CNCM-I745 (Sb; 299v + Sb, NCFM + Sb, PCC + Sb, and LAC + Sb).

After 24 h, growth of single-cultured probiotic lactobacilli was consistently slightly higher compared to their co-cultures (1.3 vs. 0.6, and 0.9 vs. 0.5 and 0.8 vs. 0.6 and 0.7 vs. 0.4 Log increase for 299v, NCFM, PCC, and LAC single vs. co-cultures, respectively). This may be due to vigorous nutrient competition by *S. boulardii*, which limited the nutrient availability and subsequently, the growth of the lactobacilli in the co-culture (described in Section 3.2). Nevertheless, all probiotic lactobacilli achieved viable populations of at least 7.2 Log CFU/mL, and assuming a daily intake of 100 mL of the probiotic fermented coffee, it would mean a minimum intake of 9 Log CFU/serving. This minimum probiotic intake is within the range of 8–11 Log CFU/day reported to be effective in the available literature (including well-designed clinical trials, systematic reviews, and meta-analyses), as well as the general recommendations (9 Log CFU/serving) by regulatory bodies, such as in Canada and Italy [1,2].

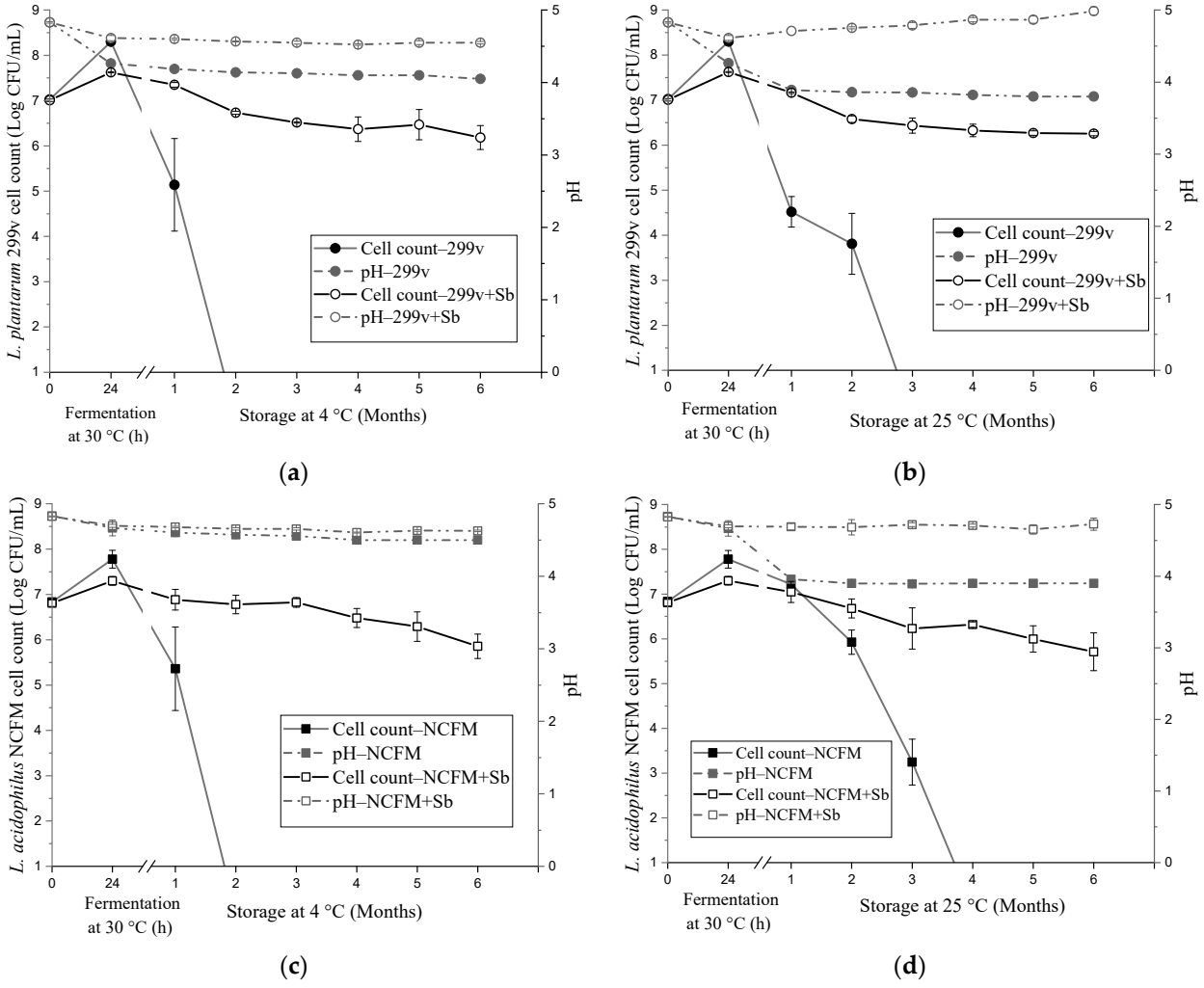

Figure 1. *Cont.*

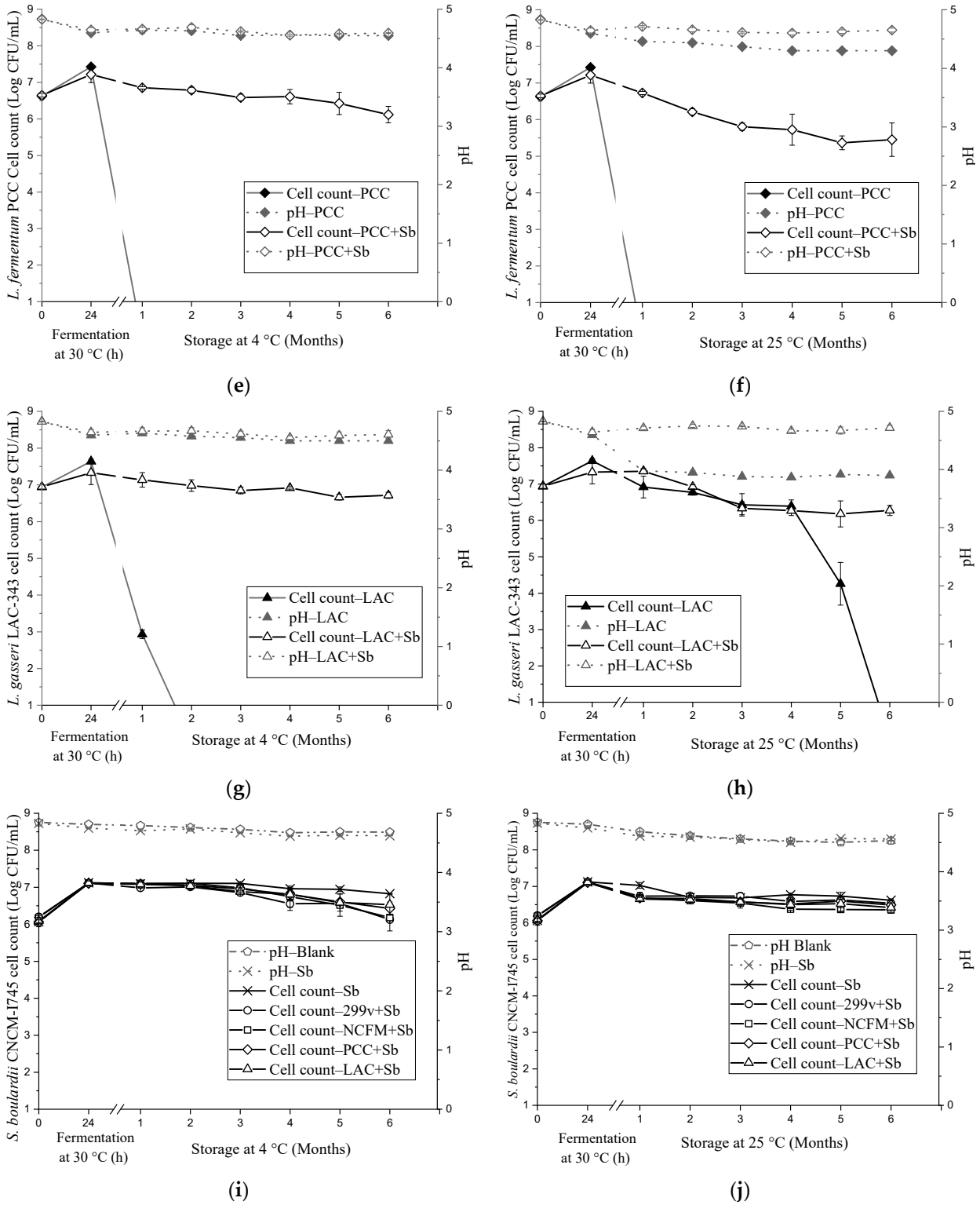

Figure 1. Growth, survival, and pH of different probiotic lactobacilli-*S. boulardii* CNCM-I745 pairings in coffee brews. *L. plantarum* 299v, *L. acidophilus* NCFM, *L. fermentum* PCC, *L. gasseri* LAC-343, and *S. boulardii* CNCM-I745 at 4 °C (**a**,**c**,**e**,**g**,**i**, respectively) and at 25 °C (**b**,**d**,**f**,**h**,**j**, respectively). Values are the mean of triplicate independent experiments (n = 3), with error bars representing the standard deviations of the mean values.

During the storage at 4 and 25 °C, the survival of co-cultured probiotic lactobacilli exceedingly surpassed that of the single cultures. After 6 months of storage, viable popu-

lations of probiotic lactobacilli were maintained in all pairings of 299v + Sb, NCFM + Sb, PCC + Sb, LAC + Sb (6.2, 5.9, 6.1, and 6.7 Log CFU/mL, respectively, at 4 °C, 6.3, 5.7, 5.5, and 6.3 Log CFU/mL, respectively, at 25 °C). This contrasts with their respective single lactobacilli single cultures, where populations were generally no longer detectable after 1 month of storage at 4 °C, and after 3 months of storage at 25 °C. An exception was the single-cultured *L. gasseri* LAC-343 (LAC), which displayed viable populations of 4.4 Log CFU/mL after storing for 5 months at 25 °C but could no longer be detected after 6 months. Therefore, the probiotic lactobacilli viability-enhancing effects conferred by *S. boulardii* CNCM-I745 are applicable to other probiotic lactobacilli species beyond *L. rhamnosus* GG. Such viability-enhancing effects are also effective at both 4 and 25 °C, justifying that co-cultured probiotic coffee brews are shelf-stable for at least 6 months without the need for costly cold-chain supply systems.

Interestingly, single lactobacilli cultures survived better under ambient storage than under refrigeration. At 2 months of storage, lactobacilli populations were no longer detectable at 4 °C, while viable populations of 3.8, 5.9, and 6.8 Log CFU/mL were observed for 299v, NCFM, and LAC, respectively, when stored at 25 °C. An exception was the single-cultured *L. fermentum* PCC (PCC), which was no longer detectable after a month of storage at 4 and 25 °C. Better lactobacilli survival at 25 °C compared to 4 °C was similar to what we had observed previously, where the survival of *L. plantarum* 299v and *L. acidophilus* NCFM increased by at least 5.4 Log-fold when stored at 25 °C, compared to 4 °C after 2 months in the same matrix [11]. Such observations are contrary to the expectation that viability losses of probiotic lactobacilli proceed at a slower rate at lower temperatures, due to reduced metabolic activities and accumulation of toxic metabolites (e.g., organic acids and hydrogen peroxide) [23]. Although the basis for this contradiction is unclear, a possible explanation could be the inability of these probiotic strains to tolerate cold stress, especially since *L. plantarum* 299v, *L. acidophilus* NCFM, and *L. gasseri* LAC-343 may be more adapted to mesophilic temperatures as isolates from the human intestinal mucosa [3,5,6]. While non-viable probiotics may still impart health benefits through their cell structures or metabolites [7], the consensus is that probiotics should be viable at an efficacious dose throughout a product's shelf life to confer a health benefit [2]. Therefore, a longer shelf life at 25 °C may be more desirable if single-cultured probiotic lactobacilli are used in coffee brew formulations.

The growth and survival of *S. boulardii* CNCM-I745 proved to be robust, regardless of co-culturing methods. Growth consistently reached 7.1 Log CFU/mL (~1.0 Log increase) by the yeast in single and co-cultures, and >6.1 Log CFU/mL was maintained throughout 6 months of storage at either 4 or 25 °C. Nevertheless, slight viability losses by the co-cultured yeast began to be apparent after storing for 6 months at 4 °C (maximum 0.7 Log CFU/mL difference between 299v + Sb and Sb), although such losses were not observed during storage at 25 °C.

The ability of *S. boulardii* CNCM-I745 to enhance the viabilities of probiotic lactobacilli, without suffering from significant viability losses itself for the most part of the storage duration, indicates commensalism, a relationship that we similarly observed with *L. rhamnosus* GG [11]. In commensalism, one microorganism is favoured by the interaction (lactobacilli in this study), while the other does not suffer from deleterious effects (*S. boulardii* CNCM-I745 in this study) [24]. Possible reasons behind the commensal interaction are discussed in Section 3.2.

It was also encouraging to note that the same viability-enhancing effects of *S. boulardii* CNCM-I745 were extendable to other probiotic lactobacilli subspecies, beyond *L. rhamnosus* GG [11], especially since dissimilar viability-enhancing effects have been observed with different yeast-lactobacillus pairings, being dependent on the probiotic species involved. For example, Liu and Tsao (2009) [19] observed that the yeast *Williopsis saturnus* failed to enhance the survival of *Lactobacillus johnsonii* and *Lactobacillus bulgaricus* in fermented milk stored for 9 weeks at 30 °C. In contrast, the same yeast enhanced the survival of *Lactobacillus acidophilus*, *L. rhamnosus*, and *Limosilactobacillus reuteri* by up to 10^6-fold in the same matrix.

By validating the viability-enhancing effects of *S. boulardii* CNCM-I745 on a wide range of probiotic lactobacilli species in this study, the over-reliance on a single probiotic strain is reduced in the event of supply chain disruption.

3.2. Changes in Glucose, Organic Acids, and Free Amino Acids

To monitor metabolite changes during fermentation and storage, glucose, organic acids, and free amino acids were quantified and are shown in Figure 2.

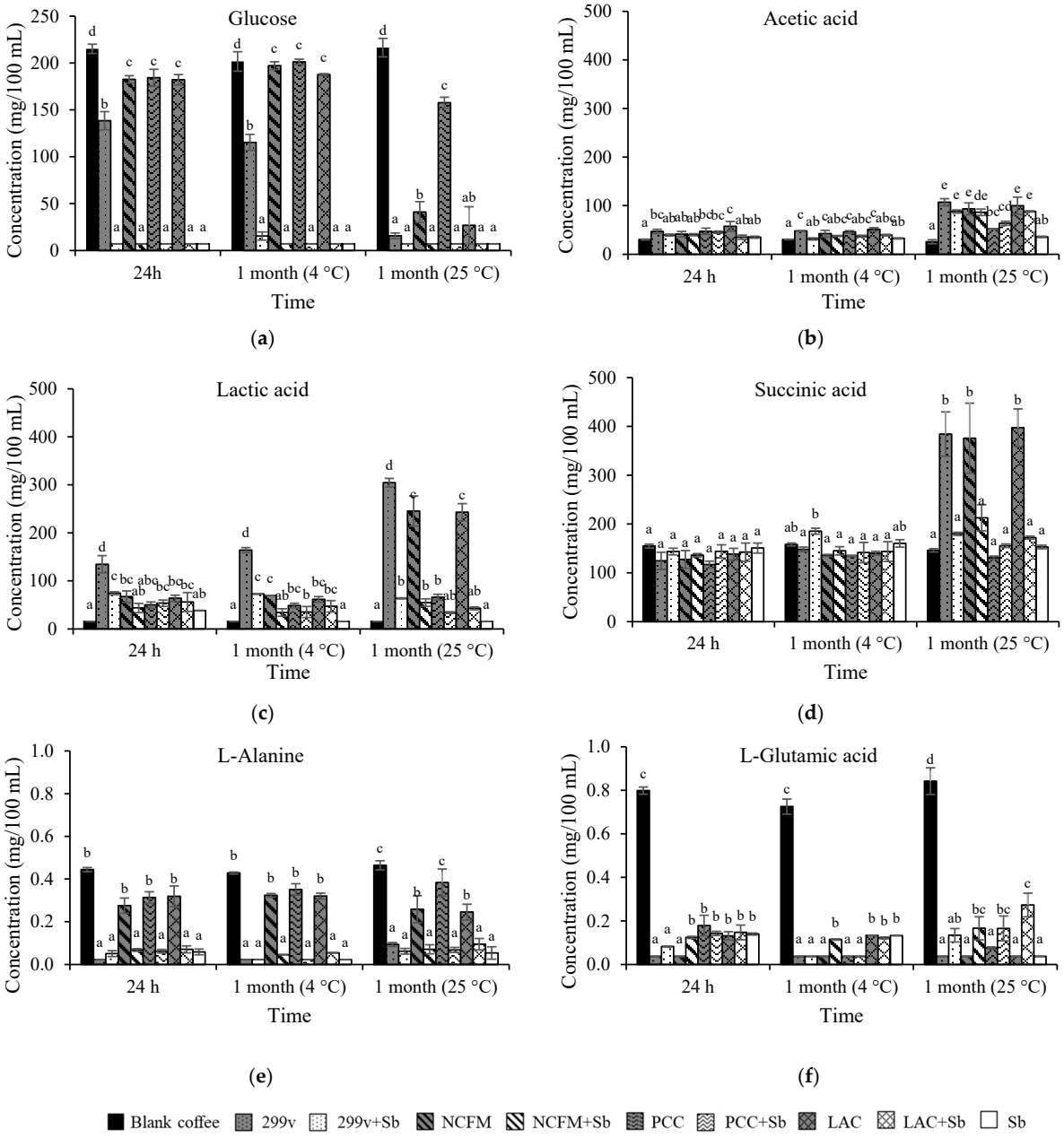

Figure 2. Changes in glucose, organic acids, and amino acids after fermentation and 1 month of storage of probiotic-fermented coffee brews. (**a**) Glucose, (**b**) acetic acid, (**c**) lactic acid, (**d**) succinic acid, (**e**) L-alanine, and (**f**) L-glutamic acid. Values are the mean of triplicate independent experiments ($n = 3$), with error bars representing the standard deviation of the mean values. Different lowercase letters indicate statistical differences ($p < 0.05$) between different fermentation setups within the same time point. Strain identities are as follows: *L. plantarum* 299v, *L. acidophilus* NCFM, *L. fermentum* PCC, *L. gasseri* LAC-343, and *S. boulardii* CNCM-I745.

From Figure 2, metabolite changes in terms of sugars, organic acids, and amino acids were not apparent after 1 month of storage at 4 °C, which is possibly due to the reduced biochemical and metabolic rates at cold temperatures [23]. Instead, more pronounced changes were reflected after 1 month of storage at 25 °C. An exception to the trend at 25 °C was single-cultured *L. fermentum* PCC (PCC), which did not have obvious metabolic changes due to early cell death (Figure 1f).

Looking at individual metabolites, glucose was partially consumed by lactobacilli single cultures (299v, NCFM, and LAC) after fermentation, depleting only after 1 month of ambient storage. Continual glucose utilisation by single cultures of 299v, NCFM, and LAC during 1 month of ambient storage resulted in accumulations of lactic, succinic, and acetic acids, with concomitant declines in the pH (Figure 1) during the same period. Accumulations of lactic and succinic acids by *L. acidophilus* NCFM (homofermentative), *L. plantarum* 299v, and *L. gasseri* LAC-343 (both facultative heterofermentative) can be explained by the conversion of glucose to pyruvate via the Embden–Meyerhof–Parnas pathway. Pyruvate is then converted to lactic acid via the homolactic pathway, or to succinic aid via the reductive arm of the partial Krebs cycle [25]. Additionally, a slight acetic acid formation was also evident by single cultures of 299v, NCFM, and LAC after storage for 1 month at 25 °C, presumably from catabolism of other substrates such as citrate, serine, acetyl-phosphate, and lactic acid [26,27].

For heterofermentative lactobacilli (*L. fermentum* PCC), glucose undergoes the phosphoketolase pathway to produce lactic acid, acetic acid, ethanol, and carbon dioxide [25]. However, acetic acid production at 25 °C was not evident in PCC, as indicated by the lack of glucose utilisation arising from early cell death (Figure 1f). Acetic acid production during ambient storage was also not evident in PCC + Sb, possibly due to competition for glucose and pyruvate by *S. boulardii* CNCM-I745, or the consumption of acetic acid by yeast. Acid accumulation by lactobacilli may induce undesired sourness and reduced product shelf life, especially since pH and sourness indices correlate highly with consumer rejection [10]. Therefore, vigorous glucose depletion by *S. boulardii* CNCM-I745 observed in co-cultures (299v + Sb, NCFM + Sb, PCC + Sb, and LAC + Sb, and Sb), may be an effective strategy to limit the production of lactic acid and succinic acid, thereby limiting the perceived sourness and consumer rejection.

Regarding free amino acids, L-alanine and L-glutamic acid were vigorously utilised by *S. boulardii* CNCM-I745 (299v + Sb, NCFM + Sb, PCC + Sb, LAC + Sb, and Sb) within 24 h. In yeasts, L-alanine and L-glutamate are precursors of key metabolic intermediates, with the former being converted to pyruvate via alanine aminotransferase, and the latter converted to α-ketoglutarate catalysed by NAD-dependent glutamate dehydrogenase [28,29]. L-Glutamic acid was also vigorously depleted within 24 h by singly cultured lactobacilli (299v, NCFM, PCC, and LAC), while L-alanine was rapidly utilised in 299v. However, utilisation of L-alanine was slower in NCFM and LAC, with appreciable amounts remaining after storing for 1 month at 25 °C. The assimilation of L-glutamic acid and L-alanine by lactobacilli can be explained by its racemisation to their D-enantiomers, for peptidoglycan cross-linking and cell wall formation [27,30]. In addition, while the l-glutamic acid (umami a d taste potentiating) and l-alanine (sweet) present in inactivated yeast extract may alter coffee flavour profiles [31], it is noted that the small amount of yeast extract used in this study (0.06 g/100 mL) did not result in perceived flavour changes during informal bench top tastings.

Overall, siphoning of nutrients (glucose, alanine, and glutamic acid) by *S. boulardii* CNCM-I745 may protect co-cultured probiotic lactobacilli from acid stress, particularly during storage at 25 °C, where the accumulation of lactic and succinic acids was prevented. Lactic acid accumulation is directly prevented by diverting glucose away from homolactic/heterolactic pathways. Lactic acid accumulation can also be indirectly prevented by siphoning alanine and glutamic acid away from the cell wall biosynthetic pathways in lactobacilli, thereby preventing cell replication and the formation of larger lactobacilli populations that have stronger lactic acid accumulation capabilities. Post-acidification is a

recurring issue in products containing live probiotic lactobacilli, as lower pH conditions promote the influx of undissociated organic acids and disrupt the transmembrane pH gradient, eventually resulting in probiotic cell death [32]. The siphoning of nutrients as a means to prevent post-acidification is consistent with our previous observations [11] and may explain how yeasts are effective in enhancing the survival of probiotic lactobacilli belonging to different species during ambient storage.

However, preventing post-acidification is inadequate in explaining enhanced probiotic lactobacilli viabilities when stored with *S. boulardii* CNCM-I745 at 4 °C. Similar pH values as well as lactic and succinic acids levels between single and co-cultures stored for 1 month at 4 °C suggest that other mechanisms are responsible. A possible mechanism that aligns with the commensal interaction observed in this study is that proposed by Ponomarova et al. [17]: the efflux of diffusible nutrients (e.g., amino acids) by yeast, as a means to regulate its own nitrogen metabolism independent of the presence of lactobacilli. We theorise that the continual secretion of diffusible nutrients by live yeast during storage would prevent nutrient depletion for the probiotic lactobacilli, something which cannot be achieved via the use of inactivated yeast extracts. Indeed, small amounts of amino acids such as aspartate and alanine were released by *S. cerevisiae* after prolonged cultivation beyond the stationary phase [33]. This may explain why the survival of co-cultured probiotic lactobacilli was enhanced compared to their single culture counterparts, although mechanistic studies are required to test this theory. Other mechanisms such as co-aggregation or yeast antioxidant capacity [16,18] also support the non-species-specific commensal interactions between *S. boulardii* CNCM-I745 and probiotic lactobacilli.

3.3. Changes in Volatile Components

To assess the effects of probiotic fermentation on coffee volatile profiles, principal component analysis (PCA) biplots were constructed and are presented in Figure 3. Additionally, to visualise the relative volatile metabolite intensities and identify volatile compounds specific to each fermentation treatment, a heatmap was constructed and is presented in Figure 4.

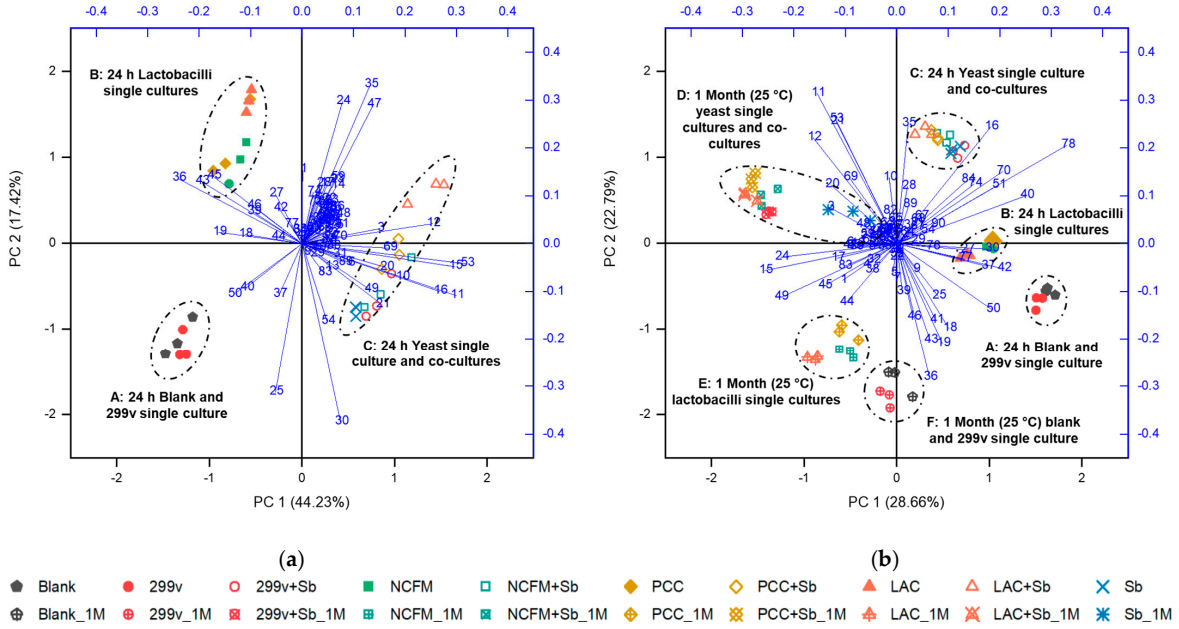

Figure 3. Biplot of principal component analysis of headspace volatile compounds in probiotic fermented coffee brews after (**a**) 24 h and (**b**) combination of 24 h and 1 month of ambient storage. Volatile compounds numbered 1 to 90 are listed in Table A1. Dashed circled regions indicate Groups A—F. Strain identities are as follows: *L. plantarum* 299v, *L. acidophilus* NCFM, *L. fermentum* PCC, *L. gasseri* LAC-343, and *S. boulardii* CNCM-I745. Blank denotes the blank coffee.

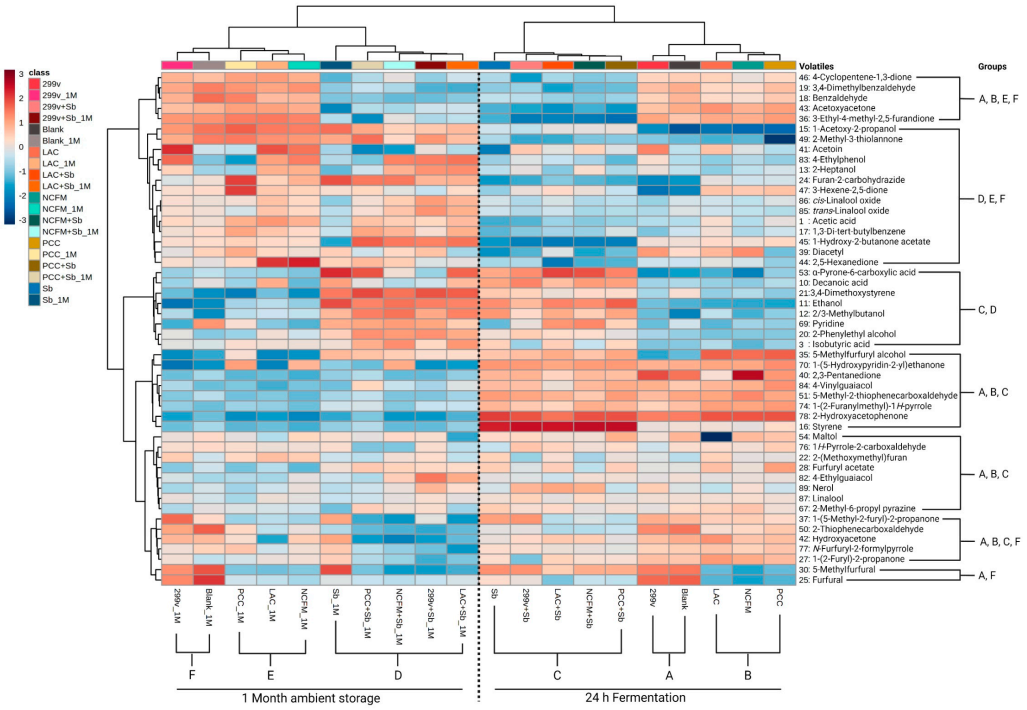

Figure 4. Heatmap of top 50 volatile compounds (based on ANOVA) associated with differentially fermented and stored probiotic coffee brews (Groups A−F). The colour scale represents normalised metabolite intensities autoscaled to samples, with dark red and blue representing high and low peak intensities, respectively. The dendrogram represents sample clusters based on Euclidean distance measure and Ward clustering. Strain identities are as follows: *L. plantarum* 299v, *L. acidophilus* NCFM, *L. fermentum* PCC, *L. gasseri* LAC-343, and *S. boulardii* CNCM-I745. Blank denotes the blank coffee.

First looking at volatile compound changes arising from the 24 h fermentation period, the samples clustered into three distinct groups according to their culturing methods, when represented by the first two principal components (44.2 and 17.4%, respectively; Figure 3a). The three groups were as follows: Group A, which consisted of the blank coffee and 299v, Group B, which consisted of singly cultured lactobacilli (NCFM, PCC, and LAC), and Group C, which consisted of the single and co-cultured *S. boulardii* CNCM-I745 (Sb, 299v + Sb, NCFM + Sb, and LAC + Sb). Clustering patterns by these three groups highlight the influence of culturing methods on volatile profiles. An exception to the trend was 299v, which unexpectedly clustered together with the coffee blank in Group A rather than in Group B, indicating the absence of major changes made by *L. plantarum* 299v to the original coffee aroma compounds. In addition, clustering patterns of the co-cultures together with Sb in Group C denote the dominance of *S. boulardii* CNCM-I745 over single lactobacilli cultures in modulating coffee volatile profiles.

The dominance of *S. boulardii* CNCM-I745 in modulating coffee volatile profiles is due to the yeast's ability to produce a wider variety of volatiles than the probiotic bacteria, as observed in Figure 4. In single and co-cultures of *S. boulardii* CNCM-I745 within Group C, yeast-derived metabolites included decanoic acid (10; 6.6–10.2-fold increase), ethanol (11; 92.6 to 305.2-fold increase), 2/3-methylbutanol (12; 82.0 to 248.2-fold increase), styrene (16; 28.5 to 55.6-fold increase), and α-pyrone-6-carboxylic acid (53; 107.3 to 801.0-fold increase). Exact structural annotations could not be made for 2/3-methylbutanol due to the retention time and GC-MS spectral similarities.

Decanoic acid may be released via fermentation as a result of either hydrolysis of coffee triglycerides, de novo formation from acetyl Co-A, or yeast autolysis [34,35]. Interestingly, decanoic acid secreted by *S. boulardii* elicited anti-fungal properties against *Candida albicans*, by inhibiting hyphae formation, candida adhesion, and biofilm formation [36]. Ethanol is derived from glucose via yeast alcoholic fermentation, while 2/3-methylbutanol is

produced from isoleucine/leucine via the Ehrlich pathway [37]. Notably, a supplementation level of 0.25% (*w/v*) glucose in this study was theoretically insufficient to exceed 0.5% (*w/v*) ethanol, which is a threshold that labels beverages as non-alcoholic in the United States [38] and the European Union [39]. Styrene is commonly produced by phenolic off-flavour positive (POF+) yeasts, from cinnamic acid via ferulic acid decarboxylase and phenylacrylic decarboxylase [40]. α-Pyrone-6-carboxylic acid may be produced by the catalytic action of catechol dioxygenase, resulting in the intradiol cleavage of pyrogallol, an intrinsic coffee phenolic compound [41,42].

Besides the production of yeast-derived volatiles, losses in endogenous coffee metabolites after 24 h of probiotic fermentation are expected. For example, significant losses in furfural (25) and 5-methylfurfural (30) were observed in all probiotic fermented samples, especially in lactobacilli single cultures (37.9 to 45.8-fold for furfural and 69.9 to 93.4-fold decreases for 5-methylfurfural after 24 h of fermentation). Significant losses of 5-methylfurfural in NCFM, PCC, and LAC were likely a result of the bacterial reduction to 5-methylfurfuryl alcohol (35), since 89.5 to 95.7-fold increases in the latter in NCFM, PCC, and LAC coincided with significant losses of 5-methylfurfural during the same time frame. An exception was 299v, which retained these coffee metabolites and was thus clustered together with the non-fermented coffee blanks in Groups A and F.

Next looking at volatile changes after 1 month of storage at 25 °C (Figures 3b and 4), three additional clusters (Groups D, E, and F) could be observed on the PCA biplot when represented by the first two principal components (28.7 and 22.8%, respectively). Group D comprised singly and co-cultured *S. boulardii* CNCM-I745 (Sb, 299v + Sb, NCFM + Sb, and LAC + Sb), Group E comprised singly cultured lactobacilli (NCFM, PCC, and LAC), while Group F comprised coffee blanks and 299v. The samples contained within Groups D, E, and F were thus analogous to Groups C, B, and A, respectively. The former three groups are representative of the effects of 1 month of ambient storage on headspace volatile profiles, while the latter three are representative of freshly fermented coffee brews. Based on these clustering patterns, culturing methods are the determining factor in influencing coffee headspace volatile profiles, although aroma changes are expected upon ambient storage.

After 1 month of ambient-storage, accumulations of lactobacilli-derived metabolites were evident. For instance, after 24 h fermentation, acetoin (41) and 4-ethylphenol (83) were produced ranging from 2.9- to 9.3-fold for acetoin and 44.8- to 192.5-fold for 4-ethylphenol. Upon 1 month of ambient storage, further accumulations of 4-ethylphenol (4.8 to 15.7-fold increase) and acetoin (7.0 to 12.4-fold increase) were apparent in singly cultured lactobacilli coffees, resulting in large concentrations detected in Group E. An exception to the trend was PCC, which did not reveal increases in 4-ethylphenol and acetoin possibly because of early cell death (Figure 1e). Perceptible aroma changes may thus arise from their continual accumulation by live lactobacilli during ambient storage, especially since 4-ethylphenol may be beneficial for retaining coffee brew aromas due to its smoky and spicy aroma [40].

In lactobacilli, 4-ethylphenol is formed from the strain-specific decarboxylation and subsequent reduction in *p*-coumaric acid [43]. Acetoin is formed either from pyruvate and subsequent enzymatic decarboxylation from α-acetolactate (pyruvate→α-acetolactate→acetoin), or via non-enzymatic decarboxylation with diacetyl as an intermediate (pyruvate→α-acetolactate→diacetyl→acetoin) [32]. Notably, the production of 4-ethylphenol and acetoin enables lactobacilli to adapt to acidic conditions, by regenerating NAD^+ and shunting pyruvate away from lactic acid production towards production of neutral acetoin [32,44].

Changes in the levels of yeast-derived volatile metabolites during 1 month of ambient storage were not evident, particularly for decanoic acid, ethanol, 2/3-methylbutanol, and α-pyrone-6-carboxylic acid. However, levels of styrene declined and could no longer be detected after 1 month of ambient storage. We postulate that its disappearance might be linked to its oxidation to 3,4-dimethoxysytrene (21), since elevated levels of 3,4-dimethoxysytrene (2.2 to 8.5-fold increases) coincided with a concomitant decrease in styrene within the same timeframe. The oxidation of styrene to 3,4-dimethoxystyrene associated with long storage periods may be toxicologically favourable, since styrene is classified as a class 2B carcinogen

by the International Agency of Research on Cancer [45], while 3,4-dimethoxystyrene is an approved flavouring agent for imparting sweet and floral notes [46].

During storage at 25 °C, losses in coffee aroma can be expected due to volatilisation of the following endogenous coffee volatiles across Groups D, E and F: maltol (54), 1*H*-pyrrole-2-carboxaldehyde (76), 2-(methoxymethyl)furan (22), furfuryl acetate (28), 4-ethylguaiacol (82), nerol (89), linalool (87), 2-methyl-6-propyl pyrazine (67), 1-(5-hydroxypyridin-2-yl)ethenone (70), 2,3-pentanedione (40), 4-vinylguaiacol (84), 5-methyl-2-thiophenecarboxaldehyde (51), 1-(2-furanylmethyl)-1*H*-pyrrole (74), and 2-hydroxyacetophenone (78). The loss of coffee aroma during ambient storage may also proceed more quickly for probiotic fermented coffees, since endogenous coffee volatiles including 1-(5-methyl-2-furyl)-2-propanone (37), 2-thiophenecarboxaldehyde (50), hydroxyacetone (42), *N*-furfuryl-2-formylpyrrole (77), and 2-furfuryl methyl ketone (27) were less prominent in probiotic-fermented samples (Groups D and E) than the control (Group F).

Nevertheless, there may be endogenous coffee volatile compounds that may be preferentially retained in 1-month-stored ambient samples (Groups D, E, and F). These include the following: 1-acetoxy-2-propanol (15), 2-methyl-3-thiolannone (49), 2-heptanol (13), furan-2-carbohydrazide (24), 3-hexene-2,5-dione (47), *cis*-linalool oxide (86), *trans*-linalool oxide (85), acetic acid (1), 1,3-di-tert-butylbenzene (17), 1-hydroxy-2-butanone acetate (45), diacetyl (39), and 2,5-hexanedione (44).

3.4. Changes in Coffee Bioactive Components and Antioxidant Capacities

Probiotic fermented coffees may potentially be a rich source of antioxidants, derived from endogenous coffee bioactive components (e.g., chlorogenic acids and hydroxycinnamic acids) and probiotic effector molecules (e.g., exopolysaccharides and phenolic compounds) [47,48]. Therefore, the quantification of phenolic compounds and alkaloids, as well as in vitro antioxidant capacities, were conducted and are presented in Figure 5. Coffee bioactive components such as caffeic, ferulic, and *p*-coumaric acids remained at trace levels and are not presented.

After 24 h of fermentation, the levels of caffeine, trigonelline, and 5-*O*-caffeoylquinic acid were preserved, although there were slight but statistically significant changes after cold and ambient storage. The coffee antioxidant capacities were similarly unaffected by probiotic fermentation, with unchanging levels of TPC, DPPH, and ORAC values after 24 h of fermentation and storage. However, slight but significant improvements in DPPH radical-scavenging activities were recorded for 299v + Sb and LAC + Sb after storing for 1 month at 25 °C.

The preservation of endogenous antioxidant capacities is unsurprising, given the unchanging levels of 5-O-caffeoylquinic acid and trigonelline, which are significantly correlated with DPPH radical-scavenging activities [47]. In addition, fluxes in volatile antioxidants (e.g., accumulation of 4-ethylphenol and losses of furfural and 5-methylfurfural; Figure 4) were not translated to antioxidant capacities, possibly because coffee volatiles have much weaker antioxidant capacities and are present in very low levels in relation to non-volatile coffee antioxidants (e.g., phenolics) [49].

While specific strains of lactobacilli are able to hydrolyse 5-*O*-caffeoylquinic acid via cinnamoyl esterase into caffeic and quinic acids [50], it appears that the strains tested here were incapable of doing so. Minimal changes in caffeine, trigonelline, and 5-O-caffeoylquinic acid as well as antioxidant capacities are consistent with our previous observations [10,11], suggesting that innate coffee bioactivities are preserved by common commercial probiotic strains.

3.5. Other Considerations

Overall, *S. boulardii* CNCM-I745 is an effective strategy to enhance the viability of probiotic lactobacilli belonging to different species, independent of storage temperature. To our knowledge, this is the first study that has demonstrated the long-term viability-

enhancing effects of yeast (6 months), which has broad applicability in developing shelf-stable, high-moisture probiotic foods, especially in communities lacking cold supply chains.

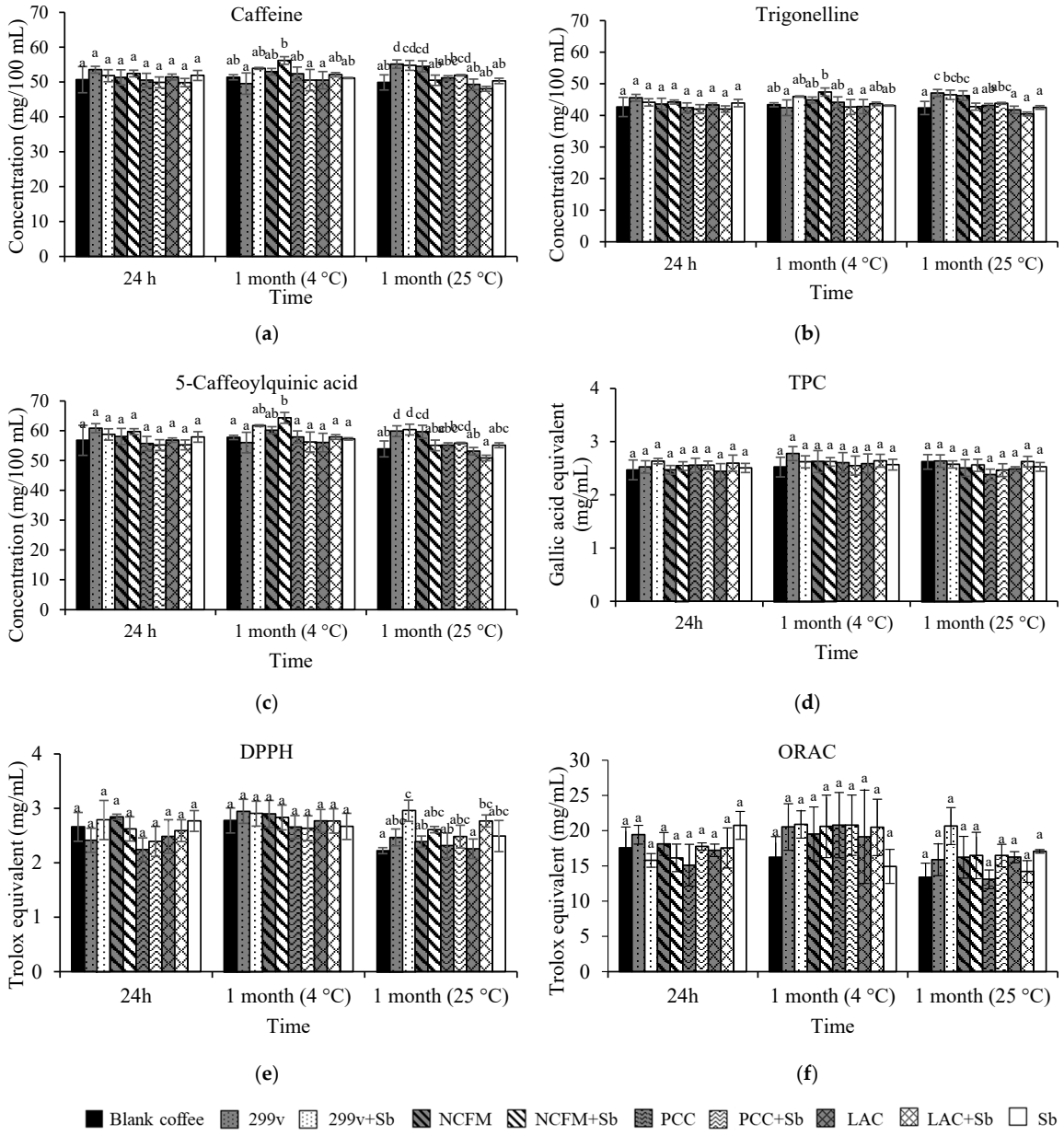

Figure 5. Changes in alkaloids, phenolic compounds, and antioxidant capacities after fermentation and storage of probiotic coffee brews. (**a**) Caffeine, (**b**) trigonelline, (**c**) 5-caffeoylquinic acid, (**d**) total phenolic content (TPC), (**e**) 2,2-diphenyl-1-picrylhydrazyl (DPPH), and (**f**) oxygen radical-scavenging assay (ORAC). Values are the mean of triplicate independent experiments (n = 3), with error bars representing the standard deviation of the mean values. Different lowercase letters indicate statistical differences ($p < 0.05$) between different fermentation setups within the same time point. Strain identities are as follows: *L. plantarum* 299v, *L. acidophilus* NCFM, *L. fermentum* PCC, *L. gasseri* LAC-343, and *S. boulardii* CNCM-I745.

Yet, the mechanisms behind such viability-enhancing effects remain elusive. Vigorous nutrient uptake by *S. boulardii* CNCM-I745 limited the lactic acid production and accumulation by the lactobacilli under ambient storage, which could have enhanced probiotic lactobacilli viability by preventing post-acidification. However, other mechanisms are expected, since preventing lactic acid accumulation by the yeast does not explain the enhanced

survivability of co-cultured lactobacilli during cold storage. Considering the commensal interactions and that the viability-enhancing effect is not dependent on lactobacilli species, we postulate that any of the mechanisms previously reported by others (co-aggregation, mixed-species biofilm formation, nutrient provision, and yeast antioxidant capacities) may be in play. While this paper was intended as a proof-of-concept, and was not mechanistic in nature, further work is required to fully elucidate the mechanisms behind the probiotic lactobacilli survival enhancement effect by *S. boulardii* CNCM-I745. In this perspective, cell morphological studies, co-aggregation assays, and omics approaches (e.g., transcriptomics, proteomics, and untargeted exometabolomic analysis) are warranted [15–17].

Furthermore, the co-cultured probiotic coffees presented here had a theoretical shelf life of at least 6 months solely based on probiotic viabilities. However, flavour changes as a result of probiotic fermentation and storage can also have a direct bearing on a product's shelf life since they would influence consumer acceptance. For example, for the purpose of enhancing probiotic lactobacilli viability by co-culturing with *S. boulardii* CNCM-I745, coffee aromas will inevitably be modified owing to accumulations in ethanol, 2/3-methylbutanol, decanoic acid, and 3,4-dimethoxystyrene. In addition, storage effects arising from the accumulation of flavour-active lactobacilli volatiles (4-ethylphenol and acetoin) and loss of endogenous coffee volatiles (e.g., 5-methylfurfural) may affect consumer perception during probiotic coffee brew storage. Therefore, sensorial studies are required to determine flavour changes and consumer acceptance in freshly fermented and stored probiotic coffee brews.

4. Conclusions

S. boulardii CNCM-I745 is effective in enhancing the viability of probiotic lactobacilli from different species in coffee brews, independent of storage temperature (4 and 25 °C). Probiotic lactobacilli in co-cultures maintained viable populations above 5.5 Log CFU/mL for at least 6 months, whereas single-cultured lactobacilli generally could no longer be detected beyond 3 months of storage at 4 and 25 °C. The viability-enhancing effects of *S. boulardii* CNCM-I745 may partially be attributed to its vigorous nutrient uptake (glucose, glutamic acid, and alanine), which limited lactic acid accumulation by the lactobacilli and prevented post-acidification during ambient storage. However, the results presented here suggest other implicit mechanisms, highlighting the need to further clarify the mechanisms behind lactobacilli–yeast interactions. In addition, further sensorial and consumer acceptance studies will be useful due to distinct changes in coffee aroma compound profiles arising from losses of coffee volatile components (furfural and 5-methylfurfural) and accumulations of flavour-active microbial metabolites (e.g., 2/3-methylbutanol, 2,3-dimethoxystyrene, decanoic acid, and 4-ethylphenol). Overall, we anticipate that the lactobacilli viability-enhancing effects of yeasts will be useful in the development of shelf-stable, high-moisture probiotic food products.

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Appendix A

Table A1. Headspace volatiles of coffee brews after 24 h and 1 month of storage at 25 °C.

No	Compound	LRI [1]	*m/z* [2]	Time [3]	Normalised Peak Intensities *									
					Blank Coffee	**299v**	**299v + Sb**	**NCFM**	**NCFM + Sb**	**PCC**	**PCC + Sb**	**LAC**	**LAC + Sb**	**Sb**
	Acids													
1	Acetic acid	1448	43	24 h	114.69 ± 2.47 a	138.95 ± 22.89 ab	122.52 ± 42.02 a	166.92 ± 43.34 b	145.11 ± 44.68 ab	203.37 ± 53.64 b	162.50 ± 8.63 b	265.31 ± 73.84 b	232.24 ± 56.50 b	79.69 ± 20.84 a
				1 M	167.79 ± 48.27 a	369.32 ± 57.31 abc	342.21 ± 98.32 abc	516.11 ± 141.00 bc	371.46 ± 79.57 abc	490.19 ± 198.12 bc	273.14 ± 55.79 ab	628.89 ± 103.02 c	408.02 ± 120.81 abc	108.01 ± 36.62 a
2	Propanoic acid	1536	74	24 h	5.09 ± 0.65 a	6.56 ± 0.63 a	4.78 ± 0.61 a	6.19 ± 1.50 a	5.38 ± 0.99 a	8.97 ± 3.15 ab	9.59 ± 1.45 b	10.79 ± 2.38 b	13.24 ± 1.41 b	7.35 ± 0.75 ab
				1 M	6.63 ± 3.44 ab	8.69 ± 1.58 ab	5.47 ± 0.98 ab	8.22 ± 2.00 ab	6.84 ± 1.70 ab	8.44 ± 1.48 ab	5.95 ± 0.75 ab	9.69 ± 2.41 b	7.71 ± 4.16 ab	2.99 ± 0.40 a
3	2-Methylpropanoic acid (Isobutyric acid)	1564	43	24 h	0.89 ± 0.09 a	1.05 ± 0.09 a	2.39 ± 1.32 a	1.18 ± 0.29 a	2.66 ± 0.66 b	1.39 ± 0.42 a	4.76 ± 1.19 b	1.92 ± 0.56 a	6.83 ± 0.37 b	4.06 ± 0.51 b
				1 M	1.67 ± 0.89 a	2.97 ± 0.20 ab	4.50 ± 1.36 bc	3.49 ± 0.83 abc	10.37 ± 0.64 c	3.60 ± 1.44 abc	4.81 ± 0.68 bc	3.23 ± 0.76 ab	9.67 ± 5.41 c	2.21 ± 0.58 a
4	Butanoic acid	1625	60	24 h	2.59 ± 0.19 ab	2.25 ± 0.30 a	2.27 ± 0.51 a	2.04 ± 0.11 a	2.12 ± 0.17 a	1.95 ± 0.56 a	4.3 ± 0.59 b	2.61 ± 0.35 ab	4.57 ± 1.64 b	2.52 ± 0.61 ab
				1 M	4.88 ± 2.07 b	5.22 ± 1.20 b	3.06 ± 0.93 a	3.61 ± 0.56 ab	4.37 ± 0.95 b	6.52 ± 3.09 b	4.37 ± 0.92 b	4.22 ± 0.62 b	2.52 ± 0.65 a	3.00 ± 0.14 a
5	3-Methyl-2-butenoic acid	1795	82	24 h	4.01 ± 1.45 a	5.50 ± 0.59 ab	5.66 ± 1.61 ab	6.25 ± 1.77 ab	4.46 ± 1.54 ab	5.98 ± 1.55 ab	5.78 ± 0.51 ab	6.35 ± 1.18 ab	8.20 ± 1.63 b	4.84 ± 0.22 ab
				1 M	6.44 ± 1.57 ab	8.93 ± 2.41 b	5.79 ± 2.25 ab	9.80 ± 2.21 b	3.91 ± 1.55 a	8.92 ± 3.51 b	5.14 ± 1.12 ab	10.24 ± 2.40 b	4.31 ± 0.94 a	4.64 ± 1.00 a
6	Hexanoic acid	1841	42	24 h	0.65 ± 0.21 abc	0.53 ± 0.06 ab	1.00 ± 0.07 ab	0.52 ± 0.06 a	0.85 ± 0.24 abc	0.45 ± 0.16 a	0.80 ± 0.30 abc	0.52 ± 0.08 ab	1.30 ± 0.52 c	0.86 ± 0.18 bc
				1 M	0.57 ± 0.05 a	1.66 ± 1.05 bc	1.32 ± 0.12 bc	1.35 ± 0.16 bc	1.32 ± 0.30 bc	1.12 ± 0.13 abc	1.61 ± 0.21 c	1.55 ± 0.45 c	0.80 ± 0.19 a	1.02 ± 0.03 ab
7	Heptanoic acid	1948	60	24 h	0.44 ± 0.15 a	0.32 ± 0.12 a	0.58 ± 0.15 a	0.35 ± 0.01 a	0.34 ± 0.08 a	0.34 ± 0.13 a	0.39 ± 0.06 a	0.39 ± 0.14 a	0.54 ± 0.04 a	0.49 ± 0.08 a
				1 M	0.53 ± 0.03 ab	0.78 ± 0.18 b	0.41 ± 0.11 ab	0.99 ± 0.04 b	0.27 ± 0.09 a	0.72 ± 0.34 b	0.30 ± 0.03 a	0.86 ± 0.39 b	0.29 ± 0.13 a	0.31 ± 0.08 a
8	Octanoic acid	2055	60	24 h	0.67 ± 0.10 a	1.17 ± 0.14 ab	1.43 ± 0.42 bc	1.88 ± 0.20 cd	1.17 ± 0.22 ab	1.05 ± 0.12 ab	1.27 ± 0.16 abc	1.19 ± 0.09 ab	2.06 ± 0.17d	1.23 ± 0.27 ab
				1 M	0.68 ± 0.11 a	1.69 ± 0.94 ab	1.66 ± 0.30 ab	2.35 ± 0.67 ab	1.05 ± 0.41 ab	2.46 ± 1.04 ab	1.76 ± 0.18 ab	2.88 ± 0.69 b	1.53 ± 0.83 ab	1.36 ± 0.62 ab
9	Nonanoic acid	2162	41	24 h	1.38 ± 0.14 a	1.23 ± 0.20 a	1.47 ± 0.37 a	1.46 ± 0.46 a	1.15 ± 0.37 a	1.08 ± 0.14 a	1.21 ± 0.36 a	1.08 ± 0.29 a	1.47 ± 0.54 a	1.21 ± 0.17 a
				1 M	1.71 ± 0.39 b	2.19 ± 1.22 b	0.91 ± 0.07 a	1.88 ± 0.78 b	0.63 ± 0.25 a	1.11 ± 0.39 ab	1.29 ± 0.13 ab	1.73 ± 0.55 b	0.60 ± 0.19 a	1.36 ± 0.34 ab
10	Decanoic acid	2268	73	24 h	0.23 ± 0.05 a	0.26 ± 0.02 a	2.39 ± 0.32 b	0.29 ± 0.03 a	1.87 ± 0.12 b	0.31 ± 0.03 a	1.77 ± 0.17 b	0.28 ± 0.01 a	1.85 ± 0.29 b	1.54 ± 0.58 b
				1 M	0.35 ± 0.10 ab	0.33 ± 0.23 a	0.47 ± 0.11 ab	0.21 ± 0.05 a	0.37 ± 0.18 ab	0.30 ± 0.10 a	0.33 ± 0.08 a	1.58 ± 0.30 b	1.47 ± 0.90 b	1.17 ± 0.69 b
	Alcohols													
11	Ethanol		45	24 h	0.68 ± 0.24 a	1.62 ± 0.30 a	62.63 ± 27.17 b	1.64 ± 0.07 a	111.13 ± 19.89 b	0.99 ± 0.33 a	201.28 ± 65.7 b	1.56 ± 0.60 a	206.33 ± 30.30 b	83.49 ± 27.42 b
				1 M	0.28 ± 0.21 a	0.20 ± 0.11 a	201.13 ± 52.14 b	4.38 ± 0.61 a	237.97 ± 75.44 b	4.68 ± 2.56 a	83.37 ± 21.58 b	5.44 ± 1.28 a	151.96 ± 26.15 b	124.48 ± 34.69 b
12	2/3-Methylbutanol	1214	55	24 h	0.46 ± 0.18 a	3.04 ± 0.77 a	37.67 ± 12.30 b	3.95 ± 0.88 a	45.94 ± 15.08 b	5.06 ± 1.71 a	84.99 ± 36.53 b	11.10 ± 4.88 a	113.96 ± 17.29 b	73.55 ± 9.17 b
				1 M	0.63 ± 0.52 a	8.55 ± 1.46 ab	90.15 ± 28.70 c	12.55 ± 2.78 ab	213.58 ± 85.00 c	9.65 ± 0.82 ab	98.63 ± 36.97 c	10.66 ± 1.56 ab	175.52 ± 102.92 c	27.03 ± 5.77 bc
13	2-Heptanol	1319	45	24 h	14.00 ± 8.45 a	23.79 ± 6.46 b	29.25 ± 6.00 b	17.23 ± 5.91 ab	34.68 ± 17.31 b	16.82 ± 6.71 ab	17.46 ± 1.30 ab	10.19 ± 0.72 a	19.77 ± 1.03 b	14.46 ± 1.67 a
				1 M	6.53 ± 1.60 a	44.34 ± 1.44 ab	87.75 ± 15.20 b	59.48 ± 8.41 b	61.58 ± 24.09 b	11.33 ± 0.65 a	9.56 ± 2.69 a	57.90 ± 18.10 b	81.92 ± 8.99 b	5.12 ± 0.30 a
14	1-Hexanol	1357	56	24 h	11.05 ± 3.30 a	15.03 ± 4.46 ab	17.52 ± 5.79 ab	19.35 ± 2.48 ab	17.23 ± 5.74 ab	27.26 ± 8.24 abc	28.82 ± 7.48 bc	27.55 ± 5.90 bc	40.95 ± 4.68 c	27.09 ± 5.58 abc
				1 M	12.63 ± 3.64 a	21.09 ± 2.72 abc	25.66 ± 6.83 bcd	30.12 ± 5.57 cd	27.62 ± 2.22 cd	27.20 ± 3.15 cd	24.78 ± 4.98 abcd	34.44 ± 4.21d	19.25 ± 4.84 abc	14.34 ± 0.76 ab
15	1-Acetoxy-2-propanol	1575	45	24 h	0.00 ± 0.00 a	0.04 ± 0.02 a	1.31 ± 0.40 b	0.05 ± 0.02 a	1.92 ± 0.70 b	0.03 ± 0.02 a	2.23 ± 0.31 b	0.05 ± 0.00 a	4.22 ± 2.31 b	0.89 ± 0.21 b
				1 M	11.86 ± 4.85 ab	21.41 ± 4.72 b	4.89 ± 1.53 a	19.87 ± 0.48 b	3.59 ± 0.56 a	25.82 ± 3.34 b	4.33 ± 1.40 a	20.80 ± 4.00 b	4.77 ± 1.06 a	10.62 ± 7.21 a
	Benzoyl derivatives													
16	Styrene	1252	104	24 h	0.34 ± 0.07 a	0.38 ± 0.03 a	15.22 ± 4.60 b	0.43 ± 0.04 a	16.36 ± 5.49 b	0.46 ± 0.04 a	14.97 ± 4.29 b	0.42 ± 0.02 a	19.15 ± 5.14 b	9.82 ± 0.69 ab
				1 M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
17	1,3-Di-tert-butylbenzene	1419	57	24 h	11.42 ± 5.00 ab	6.90 ± 1.52 aab	12.27 ± 4.41 ab	12.02 ± 2.75 ab	12.72 ± 5.11 ab	12.36 ± 2.55 ab	18.59 ± 2.94 ab	19.59 ± 6.51 b	19.05 ± 7.06 ab	6.89 ± 1.37 a
				1 M	16.94 ± 9.73 a	21.93 ± 3.70 ab	21.13 ± 4.65 a	24.96 ± 5.84 ab	37.67 ± 12.71 b	41.09 ± 17.70 b	30.34 ± 6.91 b	29.32 ± 5.45 b	36.47 ± 8.69 b	10.10 ± 4.65 a
18	Benzaldehyde	1526	105	24 h	7.62 ± 2.69 c	4.96 ± 0.68 bc	2.56 ± 0.48 ab	3.74 ± 0.35 bc	1.52 ± 0.28 a	4.50 ± 1.52 bc	1.95 ± 0.39 a	3.67 ± 1.04 bc	2.87 ± 0.52 ab	1.86 ± 0.44 a
				1 M	12.10 ± 5.36 b	10.95 ± 3.25 b	0.82 ± 0.24 a	7.86 ± 2.92 b	0.79 ± 0.24 a	12.61 ± 2.32 b	0.50 ± 0.12 a	6.73 ± 2.66 b	0.72 ± 0.09 a	0.75 ± 0.39 a
19	3,4-Dimethylbenzaldehyde	1817	105	24 h	6.20 ± 1.01 b	4.88 ± 1.73 b	1.39 ± 0.39 a	3.01 ± 0.28 b	0.85 ± 0.13 a	3.75 ± 1.13 b	0.96 ± 0.32 a	2.89 ± 0.67 ab	1.02 ± 0.05 a	0.79 ± 0.24 a
				1 M	7.26 ± 2.44 b	9.25 ± 1.77 b	0.43 ± 0.07 a	9.01 ± 1.15 b	1.02 ± 0.27 ab	7.98 ± 2.91 b	0.64 ± 0.20 a	7.17 ± 2.82 b	0.26 ± 0.08 a	0.50 ± 0.08 a
20	2-Phenylethyl alcohol	1925	91	24 h	4.20 ± 0.93 a	5.88 ± 0.68 a	25.71 ± 6.17 b	6.10 ± 1.14 a	19.34 ± 5.25 b	4.76 ± 1.36 a	15.43 ± 1.49 b	4.98 ± 0.87 a	27.59 ± 4.14 b	13.11 ± 0.92 ab
				1 M	2.97 ± 1.08 a	13.58 ± 2.84 a	50.60 ± 4.35 b	11.66 ± 4.51 a	47.03 ± 20.50 b	6.62 ± 2.10 a	32.24 ± 6.98 b	14.29 ± 5.45 a	32.61 ± 10.75 b	13.01 ± 3.07 a
21	3,4-Dimethoxystyrene (3,4-Dimethoxy-1-vinylbenzene)	2040	164	24 h	3.98 ± 1.30 ab	1.51 ± 1.00 a	9.63 ± 0.95 b	2.29 ± 1.05 a	7.49 ± 2.24 b	0.93 ± 0.32 a	7.10 ± 1.85 b	1.30 ± 0.46 a	9.66 ± 2.44 b	7.68 ± 0.93 b
				1 M	0.10 ± 0.02 a	0.70 ± 0.40 a	81.68 ± 10.86 b	0.48 ± 0.40 a	48.08 ± 14.76 b	0.22 ± 0.12 a	53.09 ± 10.79 b	0.71 ± 0.38 a	61.83 ± 13.40 b	16.55 ± 9.43 b
	Furans													
22	2-(Methoxymethyl)furan	1237	81	24 h	11.03 ± 2.02 a	17.13 ± 4.91 ab	15.09 ± 4.20 ab	18.89 ± 3.38 ab	14.61 ± 4.24 ab	17.01 ± 3.87 ab	22.33 ± 4.86 bc	24.38 ± 1.66 bc	30.19 ± 2.30 c	23.57 ± 1.97 bc
				1 M	10.99 ± 2.93 a	32.97 ± 9.74 b	22.91 ± 1.64 b	29.46 ± 8.38 b	21.51 ± 4.94 b	23.67 ± 5.53 b	8.58 ± 0.83 a	30.70 ± 3.58 b	11.79 ± 2.36 a	11.46 ± 1.84 a
23	2-Methyltetrahydrofuran-3-one (Coffee furanone)	1265	43	24 h	8.00 ± 1.71 a	11.18 ± 1.70 a	10.28 ± 4.30 a	10.34 ± 1.55 a	12.31 ± 4.85 a	17.52 ± 6.61 ab	25.79 ± 11.81 b	38.89 ± 17.20 b	35.43 ± 5.29 b	24.57 ± 2.84 b
				1 M	22.33 ± 5.82 a	16.12 ± 6.29 a	18.29 ± 7.77 a	27.07 ± 6.43 a	46.06 ± 21.71 a	22.37 ± 9.74 a	22.85 ± 10.35 a	18.69 ± 11.33 a	36.73 ± 29.86 a	6.80 ± 1.29 a

Table A1. *Cont.*

No	Compound	LRI [1]	*m/z* [2]	Time [3]	Normalised Peak Intensities *									
					Blank Coffee	**299v**	**299v + Sb**	**NCFM**	**NCFM + Sb**	**PCC**	**PCC + Sb**	**LAC**	**LAC + Sb**	**Sb**
24	Furan-2-carbohydrazide	1313	67	24 h	0.14 ± 0.03 a	0.16 ± 0.04 a	0.91 ± 0.12 ab	2.38 ± 0.47 b	0.98 ± 0.32 ab	1.86 ± 0.53 b	0.91 ± 0.35 ab	2.77 ± 0.27 b	2.44 ± 0.72 b	0.39 ± 0.08 a
				1 M	1.76 ± 0.31 a	2.85 ± 1.15 a	10.72 ± 2.76 ab	10.53 ± 2.41 ab	26.26 ± 2.48 b	42.32 ± 18.03 b	14.93 ± 1.57 b	5.27 ± 0.92 a	9.71 ± 4.99 ab	15.41 ± 0.44 b
25	Furfural	1468	96	24 h	692.23 ± 41.92 c	573.18 ± 104.82 c	97.87 ± 23.77 bc	15.12 ± 2.17 a	61.09 ± 14.53 abc	14.68 ± 4.67 a	59.87 ± 10.52 abc	18.26 ± 5.76 a	54.54 ± 15.7 ab	83.01 ± 10.71 bc
				1 M	623.60 ± 247.49 b	567.47 ± 92.09 b	45.64 ± 13.18 ab	38.47 ± 5.29 a	39.37 ± 3.87 a	53.56 ± 19.17 b	20.58 ± 4.82 a	40.05 ± 7.53 a	40.44 ± 9.60 ab	37.35 ± 14.91 a
26	2-Acetylfuran	1508	95	24 h	139.82 ± 5.81 a	119.71 ± 15.15 a	146.74 ± 31.79 a	146.33 ± 17.34 a	155.50 ± 52.37 a	143.11 ± 38.65 a	164.16 ± 12.77 a	163.96 ± 12.16 a	196.25 ± 33.32 a	132.18 ± 9.61 a
				1 M	134.90 ± 31.82 ab	166.24 ± 23.37 ab	188.96 ± 24.69 b	178.14 ± 19.96 b	194.03 ± 16.06 b	186.41 ± 28.50 b	165.34 ± 25.45 ab	183.83 ± 10.28 b	166.12 ± 12.69 ab	107.74 ± 24.50 a
27	1-(2-Furyl)-2-propanone (2-Furfuryl methyl ketone)	1521	81	24 h	5.84 ± 1.07 b	6.67 ± 1.22 b	2.14 ± 0.93 a	7.77 ± 0.71 b	4.74 ± 1.24 a	5.55 ± 0.57 ab	4.61 ± 0.48 a	8.65 ± 2.18 b	7.63 ± 1.51 b	3.86 ± 0.55 a
				1 M	1.42 ± 0.21 a	4.59 ± 1.52 b	0.92 ± 0.09 a	4.09 ± 0.76 b	1.74 ± 0.56 ab	2.86 ± 0.65 b	1.15 ± 0.43 a	3.43 ± 0.93 b	0.99 ± 0.18 a	3.80 ± 2.03 b
28	Furfuryl acetate	1537	52	24 h	1.98 ± 0.18 a	2.29 ± 0.74 a	2.69 ± 0.05 a	2.80 ± 0.93 a	2.14 ± 0.39 a	4.89 ± 1.12 b	3.90 ± 0.52 b	3.83 ± 0.29 b	4.96 ± 0.42 b	3.27 ± 0.33 ab
				1 M	1.49 ± 0.76 a	1.20 ± 0.44 a	3.39 ± 0.75 bc	1.11 ± 0.22 a	3.63 ± 0.82 c	1.19 ± 0.49 a	2.68 ± 0.44 abc	1.68 ± 0.75 a	2.63 ± 0.22 abc	1.92 ± 0.43 ab
29	1-(2-Furyl)-1-propanone (2-Propionylfuran)	1578	95	24 h	35.37 ± 6.83 a	35.74 ± 9.98 a	39.67 ± 8.77 a	32.95 ± 8.59 a	31.14 ± 2.49 a	27.19 ± 7.56 a	33.59 ± 2.01 a	29.19 ± 1.37 a	41.61 ± 4.41 a	30.74 ± 2.36 a
				1 M	29.11 ± 8.04 a	38.38 ± 8.23 a	31.08 ± 3.38 a	31.24 ± 2.39 a	29.62 ± 7.02 a	27.56 ± 9.01 a	25.41 ± 4.42 a	27.65 ± 4.29 a	22.01 ± 1.58 a	23.71 ± 7.30 a
30	5-Methylfurfural	1578	110	24 h	409.03 ± 31.49 b	345.28 ± 30.13 b	216.68 ± 43.58 b	4.38 ± 0.50 a	136.13 ± 25.90 ab	4.68 ± 1.49 a	135.76 ± 8.20 a	5.85 ± 0.13 a	143.53 ± 56.33 ab	154.01 ± 4.55 b
				1 M	340.22 ± 122.84 b	409.73 ± 63.47 b	2.45 ± 0.79 a	8.86 ± 2.52 b	2.41 ± 0.64 a	7.04 ± 1.11 ab	1.86 ± 0.13 a	4.95 ± 0.82 ab	1.80 ± 0.52 a	250.45 ± 131.30 b
31	Methyl 3-furancarboxylate	1580	126	24 h	0.81 ± 0.22 ab	0.62 ± 0.09 a	1.02 ± 0.12 ab	0.71 ± 0.25 a	0.90 ± 0.25 ab	0.56 ± 0.21 a	0.77 ± 0.04 ab	0.63 ± 0.10 a	1.26 ± 0.19 b	0.79 ± 0.03 ab
				1 M	0.57 ± 0.25 ab	1.08 ± 0.40 b	0.76 ± 0.06 b	0.66 ± 0.04 b	0.67 ± 0.26 ab	0.49 ± 0.12 a	0.65 ± 0.15 ab	0.45 ± 0.06 a	0.40 ± 0.07 a	0.46 ± 0.13 a
32	2-Acetyl-5-methylfuran	1617	109	24 h	5.53 ± 0.67 a	4.46 ± 0.15 a	6.90 ± 0.96 a	6.52 ± 1.53 a	5.75 ± 1.52 a	4.99 ± 1.50 a	4.92 ± 0.96 a	6.51 ± 1.34 a	7.16 ± 2.12 a	5.40 ± 0.63 a
				1 M	6.04 ± 1.22 a	8.21 ± 1.01 b	7.35 ± 1.74 ab	9.34 ± 1.59 b	8.56 ± 1.60 b	6.88 ± 1.74 ab	6.87 ± 0.64 ab	10.93 ± 4.73 b	5.45 ± 0.74 a	4.67 ± 0.98 a
33	2-Furanmethanol	1666	98	24 h	349.72 ± 8.64 ab	239.58 ± 25.76 a	365.44 ± 131.47 ab	293.10 ± 28.77 a	362.01 ± 114.35 ab	385.70 ± 136.71 ab	488.18 ± 42.42 b	494.59 ± 34.78 b	571.43 ± 164.84 b	331.08 ± 54.81 ab
				1 M	246.20 ± 73.76 a	303.91 ± 32.46 a	389.30 ± 86.60 a	476.61 ± 43.70 a	403.77 ± 108.71 a	446.97 ± 102.63 a	468.34 ± 192.24 a	455.12 ± 25.27 a	441.57 ± 94.38 a	238.93 ± 66.81 a
34	2-Methyl-5-propionylfuran	1684	109	24 h	5.54 ± 0.92 a	4.63 ± 0.45 a	6.05 ± 1.05 a	5.87 ± 1.32 a	5.17 ± 0.45 a	4.79 ± 1.43 a	5.18 ± 0.90 a	5.86 ± 1.72 a	7.38 ± 0.38 a	4.21 ± 0.73 a
				1 M	5.79 ± 0.64 a	6.43 ± 1.38 a	6.41 ± 0.69 a	6.39 ± 0.75 a	6.71 ± 3.12 a	6.00 ± 2.90 a	4.26 ± 0.64 a	6.08 ± 2.11 a	4.59 ± 1.27 a	5.17 ± 0.94 a
35	5-Methyl-2-furanmethanol (5-Methylfurfuryl alcohol)	1727	95	24 h	0.47 ± 0.10 a	0.45 ± 0.10 a	16.50 ± 3.86 ab	42.56 ± 6.05 b	13.53 ± 2.11 a	41.96 ± 16.21 b	21.88 ± 5.79 ab	44.95 ± 8.74 b	32.33 ± 8.89 b	9.91 ± 2.38 a
				1 M	0.24 ± 0.03 a	0.25 ± 0.11 a	7.43 ± 2.00 b	0.57 ± 0.30 a	11.16 ± 0.11 b	5.97 ± 0.76 ab	6.64 ± 0.23 b	0.25 ± 0.10 a	5.80 ± 1.47 ab	7.48 ± 2.62 b
36	3-Ethyl-4-methyl-2,5-furandione	1745	67	24 h	1.52 ± 0.40 ab	3.47 ± 0.12 b	0.10 ± 0.07 a	2.38 ± 0.50 b	0.06 ± 0.02 a	2.51 ± 0.93 b	0.06 ± 0.04 a	2.22 ± 0.23 b	0.18 ± 0.10 a	0.11 ± 0.05 a
				1 M	2.28 ± 0.61 b	6.61 ± 1.80 b	0.27 ± 0.12 a	5.37 ± 1.30 b	0.64 ± 0.20 ab	4.98 ± 1.23 b	0.01 ± 0.01 a	6.13 ± 1.51 b	0.26 ± 0.18 a	0.17 ± 0.04 a
37	1-(5-Methyl-2-furyl)-2-propanone	1781	95	24 h	11.31 ± 1.07 b	15.33 ± 0.29 b	19.82 ± 1.90 b	8.78 ± 3.52 ab	4.69 ± 0.93 a	5.29 ± 0.82 a	3.60 ± 0.65 a	7.22 ± 0.49 ab	5.65 ± 1.29 a	13.17 ± 1.23 b
				1 M	3.51 ± 0.95 b	59.17 ± 10.91 b	4.13 ± 0.49 b	2.23 ± 0.59 a	0.55 ± 0.32 a	1.84 ± 0.21 a	0.30 ± 0.14 a	2.28 ± 0.37 ab	0.32 ± 0.14 a	8.17 ± 2.55 b
38	4-(2-Furanyl)-3-buten-2-one (Furfural acetone)	1911	121	24 h	0.98 ± 0.31 a	0.74 ± 0.16 a	0.56 ± 0.06 a	0.73 ± 0.07 a	0.52 ± 0.08 a	0.68 ± 0.31 a	0.93 ± 0.06 a	0.81 ± 0.17 a	0.95 ± 0.30 a	0.70 ± 0.12 a
				1 M	0.95 ± 0.26 a	1.21 ± 0.29 a	1.17 ± 0.60 a	1.40 ± 0.16 a	1.17 ± 0.71 a	1.79 ± 0.29 a	1.14 ± 0.29 a	1.01 ± 0.30 a	1.11 ± 0.61 a	0.82 ± 0.41 a
	Ketones													
39	2,3-Butanedione (Diacetyl)		43	24 h	18.91 ± 4.57 b	54.14 ± 11.84 b	7.49 ± 0.78 a	53.25 ± 24.04 b	5.60 ± 0.63 a	5.15 ± 0.49 a	8.69 ± 3.33 a	30.47 ± 6.28 b	24.83 ± 3.61 b	3.27 ± 0.38 a
				1 M	15.70 ± 1.12 a	18.85 ± 9.43 ab	32.54 ± 5.61 b	19.46 ± 2.62 b	25.74 ± 12.91 b	38.28 ± 8.31 b	7.18 ± 3.63 a	22.23 ± 4.28 b	6.19 ± 1.48 a	15.72 ± 0.43 a
40	2,3-Pentanedione	1057	43	24 h	32.54 ± 7.48 b	54.86 ± 8.04 b	10.10 ± 1.01 b	81.73 ± 34.35 b	8.35 ± 0.39 a	10.29 ± 0.98 b	7.90 ± 0.59 a	3.11 ± 0.89 a	8.95 ± 0.47 ab	6.54 ± 0.76 a
				1 M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
41	3-Hydroxybutanone (Acetoin)	1289	45	24 h	0.47 ± 0.01 a	15.20 ± 5.80 b	4.34 ± 0.50 b	1.35 ± 0.20 a	3.43 ± 1.03 b	0.57 ± 0.15 a	1.69 ± 0.29 a	3.46 ± 0.50 b	3.25 ± 1.54 b	0.08 ± 0.02 a
				1 M	0.45 ± 0.19 a	107.29 ± 14.71 b	1.61 ± 0.25 b	16.75 ± 1.85 b	0.13 ± 0.11 a	0.97 ± 0.84 ab	0.02 ± 0.01 a	28.86 ± 1.65 b	0.41 ± 0.20 a	0.31 ± 0.16 a
42	1-Hydroxy-2-propanone (Hydroxyacetone)	1307	43	24 h	17.80 ± 3.01 b	13.16 ± 3.30 ab	9.78 ± 4.64 a	10.54 ± 1.38 a	10.62 ± 2.17 a	9.54 ± 2.40 a	7.47 ± 5.04 a	20.62 ± 1.23 b	17.98 ± 0.98 b	4.41 ± 1.06 a
				1 M	7.87 ± 3.49 b	20.86 ± 4.88 b	0.57 ± 0.42 a	10.93 ± 3.09 b	0.33 ± 0.25 a	6.23 ± 1.28 b	0.17 ± 0.07 a	0.67 ± 0.53 a	0.93 ± 0.87 a	10.45 ± 6.08 b
43	1-Hydroxy-2-propanone acetate (Acetoxyacetone)	1467	43	24 h	80.22 ± 2.63 b	57.25 ± 9.27 b	8.13 ± 1.66 a	66.77 ± 11.67 b	8.33 ± 2.84 a	71.56 ± 19.75 b	6.67 ± 1.14 a	88.12 ± 16.76 b	10.21 ± 0.99 ab	5.95 ± 0.15 a
				1 M	44.74 ± 23.15 b	78.73 ± 6.22 b	18.53 ± 2.60 ab	79.07 ± 9.79 b	12.15 ± 5.36 a	85.15 ± 13.92 b	6.01 ± 0.65 a	88.52 ± 5.12 b	12.97 ± 0.97 a	2.02 ± 0.51 a
44	2,5-Hexanedione	1505	43	24 h	1.69 ± 0.87 ab	1.89 ± 0.14 ab	2.25 ± 0.22 b	2.59 ± 0.73 b	1.35 ± 0.88 a	1.20 ± 0.62 a	1.35 ± 0.72 a	2.56 ± 0.10 b	1.13 ± 0.78 a	1.46 ± 0.17 a
				1 M	1.54 ± 0.21 a	4.64 ± 1.86 b	2.66 ± 0.88 a	35.88 ± 3.76 b	5.49 ± 0.98 b	4.01 ± 0.27 b	4.13 ± 0.10 b	30.78 ± 1.87 b	2.10 ± 0.11 a	2.90 ± 0.81 a
45	1-Hydroxy-2-butanone acetate	1534	43	24 h	11.43 ± 3.48 b	13.28 ± 2.30 b	2.31 ± 0.62 a	15.63 ± 1.81 b	1.87 ± 0.43 a	16.03 ± 5.62 b	2.11 ± 0.30 a	16.43 ± 2.36 b	3.29 ± 0.21 ab	1.82 ± 0.22 a
				1 M	9.11 ± 1.26 a	19.94 ± 3.65 ab	84.41 ± 7.95 b	18.47 ± 1.17 a	91.39 ± 15.02 b	21.06 ± 1.78 ab	81.12 ± 12.66 b	18.86 ± 8.69 a	90.83 ± 16.17 b	1.35 ± 0.04 a

Table A1. *Cont.*

No	Compound	LRI [1]	*m/z* [2]	Time [3]	Normalised Peak Intensities *									
					Blank Coffee	299v	299v + Sb	NCFM	NCFM + Sb	PCC	PCC + Sb	LAC	LAC + Sb	Sb
46	4-Cyclopentene-1,3-dione	1591	96	24 h	2.17 ± 0.24 c	2.10 ± 0.23 c	0.53 ± 0.14 a	1.57 ± 0.52 abc	0.80 ± 0.22 ab	1.76 ± 0.68 bc	1.00 ± 0.40 ab	2.06 ± 0.43 c	1.52 ± 0.11 abc	0.85 ± 0.26 ab
				1 M	1.84 ± 0.34 ab	4.34 ± 0.58 b	0.64 ± 0.12 a	3.63 ± 0.69 b	1.54 ± 0.81 ab	3.87 ± 1.11 b	0.46 ± 0.14 a	4.25 ± 0.98 b	0.99 ± 0.64 a	0.32 ± 0.06 a
47	3-Hexene-2,5-dione	1626	43	24 h	0.09 ± 0.02 a	0.09 ± 0.01 a	2.70 ± 0.78 ab	3.67 ± 0.74 b	2.49 ± 0.83 ab	3.34 ± 0.63 b	1.92 ± 1.14 a	3.53 ± 0.08 b	4.72 ± 0.74 b	0.77 ± 0.08 a
				1 M	1.58 ± 0.50 a	3.04 ± 0.41 b	1.32 ± 0.57 a	3.63 ± 0.39 b	1.87 ± 0.74 a	19.51 ± 3.43 b	1.68 ± 0.29 a	3.86 ± 1.39 b	2.18 ± 0.53 ab	1.42 ± 0.50 a
	Lactones													
48	Butyrolactone	1634	42	24 h	9.66 ± 1.84 a	6.47 ± 1.25 a	10.10 ± 4.29 a	8.11 ± 1.22 a	12.79 ± 3.27 ab	9.47 ± 2.26 a	13.48 ± 1.97 ab	11.02 ± 3.02 a	20.69 ± 4.69 b	8.59 ± 2.62 a
				1 M	9.52 ± 2.74 a	9.51 ± 0.35 a	18.00 ± 5.23 b	11.80 ± 2.88 ab	17.33 ± 2.40 b	16.33 ± 6.22 b	13.94 ± 2.59 b	11.12 ± 0.36 ab	17.91 ± 6.76 b	6.89 ± 2.23 a
	Organosulfur compounds													
49	2-Methyl-3-thiolannone	1527 [I]	60	24 h	0.33 ± 0.05 a	0.73 ± 0.14 ab	0.99 ± 0.46 b	0.79 ± 0.16 ab	1.33 ± 0.07 b	0.05 ± 0.00 a	1.64 ± 0.48 b	0.61 ± 0.27 a	1.73 ± 0.48 b	1.46 ± 0.34 b
				1 M	18.17 ± 3.07 a	24.26 ± 5.35 a	25.37 ± 2.39 a	22.52 ± 0.54 a	20.13 ± 17.22 a	29.25 ± 3.78 a	24.38 ± 3.81 a	23.57 ± 4.54 a	19.93 ± 16.85 a	11.30 ± 8.84 a
50	2-Thiophenecarboxaldehyde	1699	111	24 h	5.72 ± 0.33 c	4.24 ± 0.51 b	0.96 ± 0.14 a	1.15 ± 0.14 a	0.57 ± 0.14 a	1.21 ± 0.36 a	0.67 ± 0.21 a	0.85 ± 0.16 a	0.91 ± 0.29 a	0.66 ± 0.20 a
				1 M	4.20 ± 1.22 b	4.00 ± 0.70 b	0.12 ± 0.03 a	0.87 ± 0.22 b	0.15 ± 0.05 a	1.11 ± 0.09 b	0.07 ± 0.03 a	0.41 ± 0.00 ab	0.11 ± 0.00 a	0.28 ± 0.18 a
51	5-Methyl-2-thiophenecarboxaldehyde	1717	126	24 h	0.37 ± 0.04 a	0.26 ± 0.04 a	0.38 ± 0.13 a	0.44 ± 0.04 a	0.24 ± 0.08 a	0.26 ± 0.02 a	0.39 ± 0.11 a	0.31 ± 0.06 a	0.44 ± 0.13 a	0.31 ± 0.05 a
				1 M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
52	2-Acetylthiophene	1781	111	24 h	1.93 ± 0.26 a	1.41 ± 0.28 a	2.38 ± 0.55 a	1.80 ± 0.43 a	1.89 ± 0.63 a	1.60 ± 0.64 a	1.89 ± 0.19 a	1.99 ± 0.57 a	2.88 ± 0.52 a	2.00 ± 0.09 a
				1 M	2.00 ± 0.23 ab	2.68 ± 0.75 b	1.94 ± 0.47 ab	2.48 ± 0.47 b	2.17 ± 0.89 ab	2.46 ± 0.26 b	1.62 ± 0.40 ab	2.54 ± 0.60 b	1.27 ± 0.28 a	1.36 ± 0.13 a
	Pyranones													
53	α-pyrone-6-carboxylic acid (2-Pyrone-6-carboxylic acid)	1360	95	24 h	0.03 ± 0.01 a	0.03 ± 0.01 a	6.03 ± 1.17 b	0.05 ± 0.01 a	12.56 ± 3.67 b	0.18 ± 0.06 a	6.36 ± 1.61 b	0.12 ± 0.07 a	20.97 ± 2.43 b	2.81 ± 0.86 b
				1 M	0.15 ± 0.03 a	0.21 ± 0.05 ab	0.22 ± 0.02 ab	0.19 ± 0.00 ab	4.86 ± 7.98 bc	0.25 ± 0.03 bc	10.59 ± 2.64 c	0.20 ± 0.04 ab	12.80 ± 2.13 c	9.56 ± 0.23 c
54	Maltol (3-Hydroxy-2-methyl-4-pyrone)	1971	126	24 h	3.25 ± 0.55 cd	1.51 ± 0.53 ab	2.39 ± 0.19 bcd	3.09 ± 0.92 bcd	1.84 ± 0.33 bc	2.15 ± 0.42 bcd	3.69 ± 0.53d	0.06 ± 0.00 a	2.80 ± 0.97 bcd	1.78 ± 0.59 bc
				1 M	1.17 ± 0.40 ab	1.74 ± 0.69 ab	1.95 ± 0.93 b	2.30 ± 0.64 b	2.12 ± 0.83 b	1.32 ± 0.41 ab	0.74 ± 0.23 ab	0.73 ± 0.04 ab	0.19 ± 0.08 a	1.06 ± 0.40 ab
	Pyrazines													
55	2,5-Dimethylpyrazine	1322	42	24 h	80.26 ± 10.51 a	67.43 ± 3.51 a	94.55 ± 23.50 a	89.40 ± 14.16 a	82.78 ± 14.28 a	83.46 ± 25.46 a	97.16 ± 2.80 a	95.22 ± 3.61 a	113.60 ± 15.43 a	79.88 ± 1.90 a
				1 M	79.56 ± 17.60 ab	93.48 ± 16.37 ab	120.99 ± 13.18 b	103.62 ± 12.11 ab	109.90 ± 31.30 ab	99.40 ± 29.99 ab	99.14 ± 16.27 ab	100.22 ± 14.40 ab	89.57 ± 9.46 ab	63.86 ± 12.27 a
56	2,6-Dimethylpyrazine	1327	108	24 h	120.09 ± 11.66 a	99.62 ± 8.21 a	111.09 ± 24.45 a	132.20 ± 23.81 ab	153.84 ± 31.14 ab	150.72 ± 39.12 ab	149.77 ± 7.34 ab	140.10 ± 13.24 ab	189.89 ± 33.27 b	118.79 ± 7.86 a
				1 M	122.47 ± 29.12 ab	134.77 ± 20.98 ab	180.02 ± 23.50 b	158.80 ± 14.99 ab	120.00 ± 51.50 ab	155.33 ± 42.10 ab	149.02 ± 25.60 ab	150.88 ± 20.40 ab	136.47 ± 9.81 ab	96.89 ± 21.02 a
57	Ethyl pyrazine	1332	107	24 h	81.90 ± 4.93 a	64.34 ± 4.86 a	102.41 ± 19.68 a	85.14 ± 11.28 a	95.05 ± 38.61 a	80.28 ± 28.22 a	100.81 ± 7.26 a	97.34 ± 3.94 a	120.21 ± 23.34 a	81.05 ± 6.32 a
				1 M	76.14 ± 17.20 ab	94.93 ± 16.47 ab	112.01 ± 13.64 b	101.91 ± 14.46 ab	106.33 ± 18.49 ab	99.13 ± 26.13 ab	93.65 ± 15.72 ab	104.38 ± 11.98 ab	84.40 ± 2.68 ab	61.25 ± 14.78 a
58	2,3-Dimethylpyrazine	1346	108	24 h	16.51 ± 0.49 a	11.52 ± 1.54 a	21.21 ± 4.62 a	15.39 ± 2.76 a	16.04 ± 4.76 a	19.63 ± 7.19 a	19.17 ± 2.17 a	20.34 ± 1.44 a	25.55 ± 7.12 a	16.08 ± 3.11 a
				1 M	15.99 ± 5.14 a	14.38 ± 3.04 a	24.22 ± 4.29 a	17.89 ± 3.06 a	22.96 ± 5.76 a	20.28 ± 6.81 a	16.65 ± 2.69 a	19.41 ± 2.47 a	16.40 ± 0.63 a	11.38 ± 2.46 a
59	Pyrazine	1215	80	24 h	9.07 ± 3.04 a	10.49 ± 1.59 a	15.60 ± 5.30 a	19.00 ± 8.35 a	22.23 ± 8.73 a	22.79 ± 8.99 a	20.24 ± 7.40 a	26.93 ± 6.21 a	30.81 ± 10.72 a	19.29 ± 5.88 a
				1 M	15.22 ± 1.54 ab	18.16 ± 3.56 ab	26.64 ± 5.70 abc	23.88 ± 4.30 abc	34.62 ± 7.80 c	24.13 ± 5.38 abc	21.77 ± 6.10 abc	29.78 ± 10.62 bc	16.98 ± 2.92 ab	11.62 ± 2.74 a
60	Methyl pyrazine	1267	94	24 h	224.29 ± 29.07 a	181.42 ± 26.27 a	232.20 ± 67.29 a	246.11 ± 28.46 a	232.00 ± 47.88 a	235.21 ± 74.64 a	264.99 ± 84.17 a	298.04 ± 32.67 a	370.55 ± 82.46 a	234.12 ± 34.70 a
				1 M	209.44 ± 62.56 ab	258.45 ± 41.88 ab	345.60 ± 72.47 b	308.22 ± 41.39 ab	354.40 ± 8.63 b	342.54 ± 60.45 b	299.23 ± 63.17 ab	332.10 ± 29.30 b	319.95 ± 58.08 ab	172.34 ± 43.31 a
61	2-Ethyl-6-methylpyrazine	1382	121	24 h	109.72 ± 10.61 a	85.94 ± 2.86 a	128.83 ± 23.08 a	117.12 ± 20.55 a	118.68 ± 40.00 a	105.76 ± 35.39 a	118.97 ± 0.77 a	118.18 ± 14.48 a	153.08 ± 17.55 a	101.74 ± 3.55 a
				1 M	100.46 ± 20.15 a	110.95 ± 26.72 a	142.23 ± 14.52 a	127.16 ± 16.22 a	144.30 ± 32.09 a	115.76 ± 50.25 a	115.95 ± 19.69 a	113.95 ± 26.52 a	90.99 ± 23.42 a	79.84 ± 16.57 a
62	2-Ethyl-5-methylpyrazine	1387	56	24 h	8.52 ± 1.32 a	6.41 ± 0.47 a	8.42 ± 0.67 a	8.92 ± 1.33 a	9.43 ± 3.28 a	8.27 ± 3.30 a	9.79 ± 0.11 a	8.71 ± 1.15 a	12.37 ± 1.06 a	7.94 ± 0.58 a
				1 M	8.13 ± 1.97 a	8.66 ± 2.43 a	11.61 ± 1.28 a	10.20 ± 1.19 a	11.04 ± 3.18 a	11.66 ± 3.63 a	8.49 ± 1.83 a	8.23 ± 2.49 a	6.91 ± 2.39 a	6.53 ± 1.37 a
63	2-Ethyl-3-methylpyrazine	1401	122	24 h	60.52 ± 5.90 a	44.08 ± 2.35 a	72.37 ± 14.49 a	62.45 ± 10.77 a	65.11 ± 21.82 a	56.85 ± 18.53 a	66.71 ± 0.47 a	64.60 ± 6.68 a	88.57 ± 11.91 a	56.50 ± 2.62 a
				1 M	56.14 ± 10.79 a	51.56 ± 10.77 a	83.07 ± 9.20 a	63.48 ± 10.75 a	82.72 ± 17.87 a	65.01 ± 27.30 a	65.92 ± 12.23 a	57.07 ± 10.62 a	54.40 ± 10.99 a	45.33 ± 8.87 a
64	2,6-Diethylpyrazine	1428	135	24 h	14.61 ± 1.55 a	12.22 ± 0.90 a	16.58 ± 3.98 a	16.23 ± 2.48 a	15.56 ± 4.26 a	13.29 ± 3.54 a	15.33 ± 0.08 a	15.50 ± 2.74 a	19.21 ± 1.89 a	14.04 ± 1.07 a
				1 M	12.10 ± 2.18 a	13.47 ± 3.33 a	16.94 ± 2.70 a	14.50 ± 2.03 a	16.58 ± 4.55 a	17.38 ± 5.31 a	12.92 ± 2.52 a	12.76 ± 3.51 a	8.68 ± 2.63 a	10.54 ± 2.32 a
65	2,5-Dimethyl-3-ethylpyrazine	1441	135	24 h	71.56 ± 11.01 a	50.10 ± 1.59 a	82.88 ± 18.68 a	75.41 ± 14.56 a	77.21 ± 25.44 a	65.43 ± 21.47 a	75.68 ± 0.78 a	73.67 ± 12.70 a	99.08 ± 10.17 a	67.51 ± 4.79 a
				1 M	63.10 ± 11.99 ab	52.50 ± 12.37 a	93.42 ± 9.86 b	64.50 ± 11.17 ab	91.27 ± 26.37 b	87.94 ± 26.43 b	72.70 ± 15.60 ab	50.69 ± 18.35 a	55.82 ± 15.58 a	51.34 ± 9.58 a
66	2,3-Dimethyl-5-ethylpyrazine	1457	135	24 h	18.30 ± 2.65 a	12.49 ± 0.11 a	16.04 ± 0.78 a	19.03 ± 4.71 a	19.05 ± 6.24 a	16.25 ± 5.20 a	18.20 ± 1.40 a	18.53 ± 3.90 a	23.86 ± 1.33 a	16.92 ± 1.59 a
				1 M	15.94 ± 2.49 a	13.39 ± 3.16 a	23.08 ± 3.04 a	16.12 ± 2.66 a	23.08 ± 7.14 a	22.94 ± 7.91 a	18.44 ± 3.81 a	14.03 ± 3.38 a	13.22 ± 4.11 a	12.78 ± 2.24 a
67	2-Methyl-6-propyl pyrazine	1461	108	24 h	3.01 ± 0.16 a	2.65 ± 0.12 a	3.72 ± 1.29 ab	4.20 ± 0.69 b	3.05 ± 0.20 a	2.61 ± 0.19 a	3.53 ± 1.09 ab	3.6 ± 0.59 b	4.75 ± 0.71 b	3.61 ± 0.15 b
				1 M	1.87 ± 0.42 a	1.92 ± 0.65 ab	3.33 ± 0.77 b	2.57 ± 0.86 b	3.09 ± 0.68 b	2.60 ± 0.61 b	2.73 ± 0.16 b	2.36 ± 0.57 ab	1.38 ± 0.42 a	1.07 ± 0.22 a
68	2-Methyl-3,5-diethylpyrazine	1488	149	24 h	16.90 ± 1.96 a	12.85 ± 0.46 a	17.72 ± 3.92 a	16.44 ± 2.66 a	17.13 ± 4.81 a	14.20 ± 4.23 a	15.95 ± 0.50 a	16.29 ± 3.18 a	20.31 ± 2.11 a	15.20 ± 1.11 a
				1 M	12.48 ± 2.48 a	10.96 ± 2.34 a	15.92 ± 1.58 a	12.75 ± 2.13 a	15.37 ± 4.53 a	15.37 ± 4.30 a	13.00 ± 2.86 a	10.22 ± 2.33 a	9.48 ± 2.42 a	10.83 ± 2.60 a

Table A1. *Cont.*

No	Compound	LRI [1]	*m/z* [2]	Time [3]	Normalised Peak Intensities *									
					Blank Coffee	299v	299v + Sb	NCFM	NCFM + Sb	PCC	PCC + Sb	LAC	LAC + Sb	Sb
	Pyridines													
69	Pyridine	1194	52	24 h	1.19 ± 0.24 ab	0.52 ± 0.25 a	3.03 ± 1.16 b	1.21 ± 0.53 ab	9.64 ± 2.26 b	0.78 ± 0.33 a	4.64 ± 2.71 b	0.56 ± 0.08 a	12.73 ± 6.11 b	0.62 ± 0.21 a
				1 M	5.62 ± 1.90 b	0.37 ± 0.02 a	8.60 ± 2.71 b	0.62 ± 0.26 a	9.01 ± 3.27 b	2.60 ± 0.75 ab	5.24 ± 2.94 b	0.66 ± 0.07 a	3.51 ± 1.21 ab	3.37 ± 0.86 ab
70	1-(5-Hydroxypyridin-2-yl)ethanone	1639 [II]	122	24 h	0.92 ± 0.21 a	0.88 ± 0.04 a	1.32 ± 0.19 ab	0.89 ± 0.21 a	1.16 ± 0.21 ab	1.06 ± 0.20 a	1.22 ± 0.23 ab	0.86 ± 0.22 a	1.67 ± 0.15 b	0.94 ± 0.21 a
				1 M	0.00 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.00 ab	0.82 ± 0.12 b	1.12 ± 0.51 b	1.06 ± 0.24 b	0.01 ± 0.00 ab	0.00 ± 0.00 a	0.00 ± 0.00 a	0.32 ± 0.55 ab
	Pyrroles													
71	1-Methyl-1*H*-pyrrole-2-carboxaldehyde	1624	80	24 h	9.76 ± 2.52 a	9.23 ± 0.23 a	11.63 ± 2.82 a	11.35 ± 1.10 a	10.19 ± 0.83 a	13.27 ± 3.68 a	12.44 ± 0.94 a	11.73 ± 0.86 a	15.38 ± 1.95 a	10.71 ± 0.02 a
				1 M	9.50 ± 2.46 a	11.09 ± 1.40 a	13.20 ± 1.43 a	12.24 ± 1.08 a	12.15 ± 1.42 a	11.27 ± 3.42 a	11.50 ± 2.00 a	11.99 ± 0.60 a	10.17 ± 0.86 a	6.89 ± 0.62 a
72	1-Ethyl-2-formyl pyrrole (1-Ethyl-1*H*-pyrrole-2-carbaldehyde)	1610	123	24 h	3.52 ± 0.67 a	3.73 ± 0.27 a	4.24 ± 0.84 a	4.59 ± 0.28 a	4.02 ± 0.53 a	4.78 ± 0.40 a	4.40 ± 0.04 a	4.76 ± 0.53 a	5.11 ± 0.34 a	4.02 ± 1.15 a
				1 M	3.14 ± 0.26 a	4.07 ± 0.64 a	4.29 ± 0.63 a	4.40 ± 0.41 a	4.46 ± 0.56 a	4.19 ± 0.78 a	4.48 ± 1.36 a	4.05 ± 0.96 a	3.31 ± 1.36 a	2.80 ± 0.56 a
73	2-Acetyl-1-methylpyrrole	1657	123	24 h	6.47 ± 1.94 a	4.55 ± 1.33 a	7.43 ± 1.62 a	6.50 ± 1.79 a	6.53 ± 2.46 a	7.57 ± 1.12 a	7.39 ± 1.08 a	7.16 ± 2.11 a	10.05 ± 0.51 a	6.36 ± 0.86 a
				1 M	5.14 ± 2.01 a	6.10 ± 1.57 a	7.36 ± 1.72 a	6.38 ± 0.71 a	7.43 ± 2.30 a	8.45 ± 2.12 a	4.79 ± 1.33 a	4.84 ± 0.50 a	5.02 ± 0.60 a	5.81 ± 1.05 a
74	1-(2-Furanylmethyl)-1*H*-pyrrole	1831	81	24 h	8.79 ± 1.59 a	7.18 ± 0.59 a	9.11 ± 1.02 a	12.54 ± 1.73 b	11.90 ± 0.99 b	11.78 ± 2.40 b	10.63 ± 0.36 b	11.87 ± 0.94 b	11.03 ± 2.74 b	8.84 ± 0.44 a
				1 M	0.94 ± 0.69 a	1.65 ± 0.36 a	2.86 ± 0.44 a	1.53 ± 0.04 a	1.49 ± 0.22 a	1.99 ± 0.26 a	1.79 ± 0.35 a	1.60 ± 0.31 a	2.11 ± 0.26 a	4.27 ± 5.80 a
75	2-Acetylpyrrole (1-(1*H*-pyrrol-2-yl)-ethanone)	1976	94	24 h	8.62 ± 0.83 ab	6.50 ± 1.54 a	10.62 ± 1.00 b	8.43 ± 1.84 ab	9.27 ± 2.73 ab	6.71 ± 2.82 a	11.75 ± 1.02 b	9.18 ± 2.09 ab	14.26 ± 3.63 b	6.26 ± 0.35 a
				1 M	7.82 ± 2.46 ab	10.81 ± 3.85 ab	14.17 ± 2.04 b	9.72 ± 1.57 ab	12.32 ± 3.59 ab	10.73 ± 4.24 ab	10.41 ± 1.44 ab	9.91 ± 2.19 ab	9.16 ± 1.15 ab	6.29 ± 2.15 a
76	1*H*-Pyrrole-2-carboxaldehyde	2031	95	24 h	10.24 ± 3.88 a	7.44 ± 1.36 a	16.31 ± 2.98 a	10.79 ± 1.19 a	11.70 ± 3.73 a	12.19 ± 2.89 a	12.72 ± 1.59 a	11.22 ± 3.82 a	18.79 ± 7.01 a	6.97 ± 0.60 a
				1 M	8.12 ± 0.64 bc	12.48 ± 3.43 c	11.15 ± 1.29 c	10.93 ± 2.59 c	4.68 ± 2.01 ab	10.92 ± 1.47 c	2.50 ± 0.16 a	9.62 ± 0.80 bc	5.18 ± 0.4 ab	7.83 ± 1.47 bc
77	1-Furfuryl-2-formyl pyrrole (*N*-Furfuryl-2-formylpyrrole)	2255	81	24 h	4.82 ± 0.64 a	4.76 ± 0.67 a	4.71 ± 1.57 a	4.74 ± 1.07 a	3.70 ± 0.37 a	4.60 ± 1.05 a	3.63 ± 0.76 a	4.98 ± 0.85 a	5.30 ± 0.43 a	3.59 ± 0.65 a
				1 M	3.11 ± 0.26 b	4.00 ± 1.41 b	1.20 ± 0.13 a	3.78 ± 0.05 b	1.60 ± 0.37 a	5.17 ± 0.90 b	1.18 ± 0.32 a	2.48 ± 0.83 b	0.53 ± 0.20 a	3.32 ± 0.08 b
	Volatile phenols													
78	2-Hydroxyacetophenone	1806	121	24 h	0.72 ± 0.26 a	0.63 ± 0.12 a	0.88 ± 0.19 a	0.79 ± 0.11 a	0.88 ± 0.24 a	0.68 ± 0.23 a	0.82 ± 0.06 a	0.69 ± 0.14 a	0.88 ± 0.26 a	0.89 ± 0.06 a
				1 M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
79	Guaiacol (2-Methoxyphenol)	1863	124	24 h	8.25 ± 1.74 b	4.83 ± 1.29 a	9.63 ± 0.48 b	7.69 ± 1.38 ab	6.05 ± 0.95 a	7.06 ± 2.25 ab	7.98 ± 0.60 b	7.08 ± 0.90 ab	9.62 ± 2.24 b	6.74 ± 0.23 a
				1 M	5.73 ± 0.94 a	6.99 ± 1.02 a	11.11 ± 2.70 a	7.48 ± 1.38 a	8.97 ± 3.05 a	9.50 ± 1.86 a	8.26 ± 1.77 a	7.43 ± 1.39 a	6.06 ± 0.84 a	7.97 ± 0.89 a
80	2-Methylphenol (*o*-Cresol)	2006	107	24 h	1.46 ± 0.15 ab	1.17 ± 0.14 a	1.41 ± 0.29 ab	1.88 ± 0.20 bc	1.17 ± 0.22 a	1.05 ± 0.12 a	1.27 ± 0.16 a	1.19 ± 0.09 a	2.06 ± 0.17 c	1.23 ± 0.27 a
				1 M	0.82 ± 0.21 a	1.16 ± 0.17 a	1.65 ± 0.54 a	1.78 ± 0.27 a	1.15 ± 0.48 a	0.97 ± 0.27 a	1.36 ± 0.39 a	1.48 ± 0.33 a	1.50 ± 0.39 a	1.11 ± 0.05 a
81	Phenol	2009	66	24 h	3.84 ± 0.36 ab	2.78 ± 0.37 a	5.65 ± 0.68 b	3.83 ± 0.41 a	4.22 ± 1.15 ab	3.59 ± 1.35 a	4.66 ± 0.41 b	4.08 ± 0.65 ab	6.59 ± 1.37 b	3.64 ± 0.38 a
				1 M	4.21 ± 1.09 a	4.96 ± 1.26 a	5.45 ± 1.48 a	5.10 ± 0.70 a	5.92 ± 1.37 a	4.74 ± 1.32 a	4.66 ± 0.46 a	5.17 ± 0.98 a	4.02 ± 0.38 a	3.39 ± 1.13 a
82	4-Ethylguaiacol	2032	137	24 h	5.27 ± 1.27 a	5.06 ± 0.27 a	6.38 ± 1.31 a	6.01 ± 1.00 a	7.41 ± 3.10 a	5.20 ± 1.63 a	7.14 ± 1.91 a	5.27 ± 0.53 a	7.15 ± 0.21 a	5.01 ± 0.72 a
				1 M	2.07 ± 0.59 a	5.72 ± 3.27 ab	21.46 ± 2.23 b	5.75 ± 0.93 b	9.96 ± 5.77 b	3.61 ± 0.92 a	2.17 ± 1.06 a	3.10 ± 0.83 a	11.77 ± 2.72 b	3.09 ± 0.69 a
83	4-Ethylphenol	2178	107	24 h	0.83 ± 0.16 a	3.39 ± 0.38 b	3.46 ± 1.38 b	1.40 ± 0.15 ab	1.66 ± 0.79 ab	0.74 ± 0.27 a	1.54 ± 1.87 ab	0.85 ± 0.19 a	4.98 ± 3.38 b	0.96 ± 0.43 ab
				1 M	0.27 ± 0.05 a	52.51 ± 17.23 b	23.07 ± 2.87 b	22.00 ± 10.14 b	23.61 ± 11.89 b	0.35 ± 0.22 a	0.24 ± 0.12 a	12.22 ± 2.43 ab	23.81 ± 4.65 b	0.69 ± 0.03 a
84	4-Vinylguaiacol (4-Vinyl-2-methoxy-phenol)	2202	150	24 h	3.90 ± 0.85 a	6.17 ± 1.46 a	5.11 ± 1.08 a	5.45 ± 1.10 a	5.14 ± 0.72 a	6.35 ± 0.96 a	6.05 ± 0.96 a	4.50 ± 0.16 a	4.09 ± 0.54 a	4.20 ± 1.64 a
				1 M	0.34 ± 0.03 a	1.28 ± 0.68 b	1.04 ± 0.29 ab	0.82 ± 0.07 ab	1.55 ± 0.46 b	0.84 ± 0.35 ab	1.94 ± 0.59 b	0.46 ± 0.15 a	1.49 ± 0.11 b	0.51 ± 0.20 a
	Terpenes and terpenoids													
85	*trans*-Linalool oxide	1436 [III]	59	24 h	17.14 ± 0.03 a	13.58 ± 0.84 a	20.20 ± 6.97 a	18.47 ± 4.48 a	17.64 ± 3.95 a	14.94 ± 6.20 a	19.14 ± 2.25 a	18.28 ± 2.31 a	28.29 ± 3.39 a	17.83 ± 1.44 a
				1 M	16.81 ± 3.87 a	29.29 ± 5.21 ab	60.54 ± 8.59 b	31.38 ± 7.62 b	40.20 ± 19.39 b	19.76 ± 7.64 a	17.13 ± 1.56 a	35.22 ± 9.05 b	41.48 ± 1.52 b	12.30 ± 3.30 a
86	*cis*-Linalool oxide	1465 [III]	59	24 h	8.33 ± 0.33 a	6.25 ± 0.76 a	10.56 ± 4.20 a	9.49 ± 2.16 a	9.52 ± 2.15 a	10.09 ± 3.27 a	10.54 ± 1.09 a	9.70 ± 0.90 a	15.86 ± 1.91 a	9.63 ± 0.57 a
				1 M	8.61 ± 2.02 a	15.18 ± 2.45 ab	29.42 ± 4.18 b	16.68 ± 4.26 b	19.42 ± 9.12 b	13.37 ± 4.39 a	8.83 ± 0.96 a	19.25 ± 4.91 b	20.05 ± 1.64 b	4.84 ± 0.30 a
87	Linalool	1542	71	24 h	17.19 ± 0.30 a	18.32 ± 2.90 a	24.40 ± 4.33 a	19.72 ± 1.16 a	23.12 ± 7.13 a	16.85 ± 2.58 a	19.12 ± 1.62 a	18.28 ± 0.94 a	23.42 ± 3.11 a	17.61 ± 0.60 a
				1 M	11.47 ± 2.00 ab	18.79 ± 2.26 cd	19.75 ± 2.85d	18.23 ± 3.57 cd	17.66 ± 2.47 bcd	12.58 ± 2.56 abc	10.56 ± 1.16 a	17.60 ± 1.77 bcd	14.95 ± 1.06 abcd	11.44 ± 1.92 ab
88	α-Terpinenol	1692	59	24 h	4.33 ± 0.25 ab	3.07 ± 0.32 a	4.87 ± 0.62 b	3.97 ± 1.29 a	4.63 ± 1.46 ab	3.16 ± 0.83 a	3.82 ± 0.49 a	3.60 ± 0.78 a	5.87 ± 0.25 b	4.19 ± 0.53 ab
				1 M	3.81 ± 0.51 a	5.89 ± 1.28 a	5.69 ± 1.39 a	5.44 ± 1.66 a	5.96 ± 1.61 a	4.42 ± 1.79 a	3.46 ± 0.27 a	5.76 ± 1.31 a	3.52 ± 0.66 a	2.86 ± 0.65 a

Table A1. *Cont.*

No	Compound	LRI [1]	*m/z* [2]	Time [3]	Normalised Peak Intensities *									
					Blank Coffee	299v	299v + Sb	NCFM	NCFM + Sb	PCC	PCC + Sb	LAC	LAC + Sb	Sb
89	Nerol (2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-)	1806	69	24 h	0.83 ± 0.18 a	1.64 ± 0.02 b	2.26 ± 0.42 b	1.28 ± 0.23 ab	2.33 ± 0.79 b	0.78 ± 0.09 a	1.02 ± 0.11 a	0.80 ± 0.19 a	3.10 ± 0.32 b	0.90 ± 0.16 a
				1 M	0.61 ± 0.09 a	0.65 ± 0.08 a	2.31 ± 0.44 b	1.20 ± 0.25 b	1.21 ± 0.65 b	0.64 ± 0.13 a	0.53 ± 0.09 a	0.82 ± 0.04 ab	1.18 ± 0.39 b	0.57 ± 0.22 a
90	Geraniol	1855	69	24 h	3.34 ± 0.57 a	4.09 ± 0.40 a	4.92 ± 1.00 a	3.81 ± 0.23 a	4.57 ± 1.06 a	3.04 ± 0.79 a	2.87 ± 0.34 a	3.46 ± 1.34 a	4.96 ± 0.56 a	3.11 ± 0.59 a
				1 M	1.58 ± 0.73 ab	2.66 ± 0.77 ab	2.32 ± 0.24 ab	3.50 ± 0.68 b	2.69 ± 1.22 ab	2.04 ± 0.46 ab	1.48 ± 0.25 a	2.56 ± 0.25 ab	1.73 ± 0.58 ab	2.27 ± 0.28 ab

* Normalised peak intensities calculated according to the following equation: (*m/z* fragment peak intensity/internal standard base peak intensity) × 1000. Values are expressed as mean of triplicate independent experiments ± SD. Different lowercase letters in a row indicate statistical differences ($p < 0.05$) between differentially fermented coffee brews. ND: Not detected. Strain identities are as follows: *L. plantarum* 299v, *L. acidophilus* NCFM, *L. fermentum* PCC, *L. gasseri* LAC-343, and *S. boulardii* CNCM-I745. LRI, linear retention index, which was determined on a DB-FFAP column relative to C10-C40 hydrocarbons. [1] Reference retention index values from NIST Chemistry WebBook SRD 69 [51], unless otherwise indicated by [I] (Castro-Marín et al., 2018) [52], [II] (Baek and Cadwallader, 1998) [53], and [III] (Xiao et al., 2017) [54]. [2] Mass to charge (*m/z*) peak fragment used for semi-quantification. [3] Measurements taken after 24 h fermentation and 1 month (1 M) of storage at 25 °C.

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