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Tasting of traditional Polish fermented cucumbers: Microbiology, morpho-textural features, and volatilome

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ABSTRACT

In the present study, naturally fermented and unpasteurized cucumbers (*Cucumis sativus* L.) collected from 4 producers located in different regions of Poland were studied. The fermented cucumbers were characterized by significant nutritional features in terms of polyphenols content and antioxidant activity. Microbiological analyses revealed active bacterial populations of lactococci, thermophilic cocci, lactobacilli, and coagulase-negative cocci. The microbiological characterization of cucumber and brine samples through metataxonomic analysis allowed the dominant species to be detected, being *Lactococcus* and *Streptococcus* in cucumbers, and *Lactiplantibacillus*, *Leuconostoc*, *Pediococcus*, *Secundilactobacillus*, and *Lentilactobacillus* in brine. The isolation activity offered a clear picture of the main active lactic acid bacteria at the end of fermentation, being *Pediococcus parvulus* and *Lactiplantibacillus plantarum* group. All the studied isolates showed a good attitude in fermenting a cucumber-based broth, thus suggesting their potential application as starter or adjunct cultures for guided cucumber fermentation. Moreover, for the same isolates, strong aminopeptidase activity (due to leucine arylamidase and valine arylamidase) was observed, with potential effect on the definition of the final sensory traits of the product. Only a few isolates showed the ability to produce exopolysaccharides in synthetic medium. Of note, the presence of the *hdcA* gene in some *Pediococcus ethanolidurans* isolates also confirmed the need for a thorough characterization of starter candidates to avoid undesired adverse effects on consumer's health. No isolate showed the production of bacteriocins against *Listeria innocua* used as surrogate for *Listeria monocytogenes*. Based on the results of Headspace Solid-Phase Microextraction-Gas Chromatography/Mass Spectrometry analysis, a rich and complex volatilome, composed by more than 80 VOCs, was recognized and characterized. In more detail, the detected compounds belonged to 9 main classes, being oxygenated terpenes, alcohols, terpenes, ketones, acids, aldehydes, esters, sulfur, and sesquiterpenes.

1. Introduction

The fermentation of vegetables as effective preservation method has a long history dating back from 8,000 to 3,000 years b.c. (Ghazi Al-Shawi and Alneamah, 2021). Of note, Korean-style cabbage and kimchi fermentation date back to the primitive pottery age from withered vegetables stored in seawater, whereas the sauerkraut fermentation reached Europe during the Mongol invasion period in the 13th century (Ghazi Al-Shawi and Alneamah, 2021). As for fermented cucumbers, their production is reported in the Christian Bible, where pickles are cited many times (Ghazi Al-Shawi and Alneamah, 2021). Nowadays, cucumber-based products are very popular in Central European countries as the Czech Republic, Slovakia, Lithuania, Ukraine, and Poland (Wiśniewska et al., 2022). In these countries, the production of cucumber-based foods represents a historical legacy entwined with national identity. In more detail, in Poland, cucumbers and related food

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products are a pillar of the cuisine, with traditional dishes being the basis of the Christmas Eve dinner or other traditional ceremonies and festivals (Wiśniewska et al., 2022). Actually, many food preparations based on cucumbers are included in the list of traditional products supervised in Poland by the Ministry of Agriculture and are also recognized as important marketing tools (Wiśniewska et al., 2022).

To the authors' perspective, the distinction between pickled or fermented cucumbers is sometimes unclear since both these vegetable products are characterized by the acidic taste. As recently clarified by Moore et al. (2022), pickled cucumbers are acidified in a brine containing water, salt, and acetic acid. In such a process, the acidified cucumbers are then pasteurized, and preservatives are added to avoid fermentation (e.g., by lactic acid bacteria contaminating cucumbers from the environment after heat treatment) and, hence, spoilage. Conversely, the activity of lactic acid bacteria is highly desired in fermented cucumbers, hence, such acidified vegetables are obtained by soaking in a mildly acidic salt brine that allows lactic acid bacteria to multiply spontaneously (Moore et al., 2022). In more detail, cucumbers are thoroughly immersed into a 6 % salt (NaCl) brine; calcium chloride is usually added to the cover brine as to preserve the delicate texture and firmness of the cucumbers during fermentation and storage (Fleming et al., 1995). During fermentation, carbon dioxide could be produced by the natural microflora or via cucumber respiration and via malate decarboxylation (Fleming et al., 1995). At industrial level, air can be insufflated in the fermentation tank to remove excess carbon dioxide thus preventing the formation of bloaters (Ghazi Al-Shawi and Alneamah, 2021). Of note, since the salt concentration of the fermented cucumbers can be too high for direct human consumption, the salt concentration is usually reduced to ~ 2 % by water washing directly before packing and distribution. The brine resulting from the desalting process can be reused for further fermentation (back-slopping technique) (Ghazi Al-Shawi and Alneamah, 2021).

During cucumber fermentation, usually lasting from 10 to 21 days, lactic acid bacteria produce lactate, acetate, and a small amount carbon dioxide using fructose and glucose present in the vegetable matrix (Moore et al., 2022). Cucumbers are also a source of proteins for lactic acid bacteria; these macronutrients are hydrolyzed to peptides and amino acids through microbial enzymatic activities. Moreover, many other biochemical reactions are exerted by lactic acid bacteria in the vegetable matrix. Hence, the technological characterization of lactic acid bacteria through the study of their enzymatic activities is crucial for the selection of specific strains to be used as potential starter cultures (Abarquero et al., 2023).

Among the lactic acid bacteria involved in the natural fermentation of cucumbers, the genera *Lactiplantibacillus*, *Levilactobacillus*, *Weissella*, *Pediococcus*, *Leuconostoc*, and *Lactococcus* are those most detected (Moore et al., 2022). Of note, lactic acid bacteria (e.g., lactobacilli or pediococci) naturally occurring in fermented cucumbers can also have a protective effect towards some foodborne pathogens through the production of bacteriocins (Singh & Ramesh, 2008), thus contributing to obtain a safer product. Moreover, lactic acid bacteria can affect the morpho-textural properties of fermented cucumbers through the production of exopolysaccharides (EPS). Interestingly, pediococci isolated from fermented cucumbers showed the ability to produce dextran with anti-cancer properties (Shukla & Goyal, 2013). It is also known that, in fermented cucumbers, some lactic acid bacteria can be the causative agent of spoilage for the formation of gas inside the cucumber, leading to the bloater defect (Zhai & Pérez-Díaz, 2021).

Of note, fermented cucumbers are included in the group of fermented vegetables that can pose a risk of adverse health effects due to the presence of biogenic amines produced by microorganisms (Świder et al., 2023). Among the most detected biogenic amines, histamine, tyramine, putrescine, and cadaverine are included; these compounds can be the causative agents of hypo- or hypertension, headache, rashes, vomiting, or neurological disorders in the consumers (Świder et al., 2023), thus representing a serious risk to human health.

Hence, it is glaring that, among the critical factors affecting the success of cucumber fermentation, the natural microbiota represents a key element.

As recently reported by Ghazi Al-Shawi and Alneamah (2021), there is still a lack of knowledge of the microbiological, chemical, and physical factors implicated in cucumber fermentation. Hence, in the present study, naturally fermented and unpasteurized cucumbers (*Cucumis sativus* L.) from the Polish marked were studied. Samples were collected from 4 producers located in different regions of Poland, namely Lower Silesia, West Pomerania, Świętokrzyskie, and Małopolska. Cucumbers and their brine were subjected to microbiological characterization through viable counting and metataxonomic analysis. Moreover, cucumber and brine samples were also characterized for their physico-chemical and morpho-textural features, as well as volatile organic compounds (VOCs), these latter studied via Headspace Solid-Phase Microextraction-Gas Chromatography/Mass Spectrometry (HS-SPME-GC/MS).

Furthermore, to select lactic acid bacteria with potential pro-technological features (as starter or adjunct cultures), 60 lactic acid bacteria were isolated from cucumber and brine samples and characterized for: i) acidification performance; ii) key enzymatic activities; iii) presence of the *hdcA* gene of Gram-positive bacteria encoding for histidine decarboxylase; iv) production of sucrose-dependent and -independent EPS.

As reported by Kim et al. (2005), *Listeria monocytogenes* is an opportunistic human pathogen that can survive and grow in refrigerated foods (including pickled or fermented cucumbers) with pH values of approximately 4.0 to 5.0 and salt concentrations of 3 to 4 %. Hence, the lactic acid bacteria isolates were also tested for the production of bacteriocins against *Listeria innocua* utilized as surrogate for *L. monocytogenes* (ANSES, 2019).

2. Materials and methods

2.1. Sample collection

Samples of unpasteurized and ready-to-eat fermented cucumbers were collected from 4 Polish producers located in different regions of Poland, namely: Lower Silesia (producer 1), West Pomerania (producer 2), Świętokrzyskie (producer 3), and Małopolska (producer 4). Samples were codified as follows: CP1 (cucumbers from producer 1), BP1 (brine from producer 1), CP2 (cucumbers from producer 2), BP2 (brine from producer 2), CP3 (cucumbers from producer 3), BP3 (brine from producer 3), CP4 (cucumbers from producer 4), and BP4 (brine from producer 4).

For each producer, 2 production batches were collected; for each batch, 2 sample units of 500 g each were collected. Neither starter cultures nor preservatives were used in cucumber manufacturing. The sampled cucumbers were traditionally produced with 55–70 % organic cucumbers in brine, with variable amounts of garlic, horseradish, and dill, depending on the recipe. No further information on the manufacturing process of the samples was provided by the producers.

The size of the cucumbers was as follows: ~30 g weight, ~8 cm length, and ~ 2 cm diameter (CP1); ~45 g weight, ~8 cm length, and ~ 3 cm diameter (CP2); ~30 g weight, ~8 cm length, and ~ 2 cm diameter (CP3); ~30 g weight, ~7.5 cm length, and ~ 2 cm diameter (CP4).

Samples were kept refrigerated (+4°C) immediately after collection and analyzed within 2 days from sampling.

2.2. Physico-chemical analyses of cucumbers and brine

The pH of the samples was determined using a pH meter equipped with a HI2031 solid electrode (Hanna Instruments, Padova, Italy).

As for titratable acidity, 50 g of each cucumber sample (or brine) was added with 50 mL of deionized water and homogenized using a DI 18 Basic, IKA® Brasil at 1000 rpm for 5 min. The results were expressed as

amount (mL) of 0.1 M NaOH solution needed to titrate the pH of the cucumber homogenates to 8.3.

Acetic acid and lactic acid concentrations were measured using the Acetic Acid (Acetate Kinase Manual Format) and D-/L-Lactic Acid (D-/L-Lactate) test kits (Megazyme, Bray, Ireland), respectively, following the manufacturer's instructions. For each sample, the measurements were performed in duplicate, and the results were expressed as the mean ± standard deviation.

The concentration of salt (sodium chloride) was assessed by gravimetric analysis in accordance with the method suggested by the Italian *Istituto Superiore di Sanità* (Baldini et al., 1996).

Total phenolic content (TPC) was measured using the Folin–Ciocalteu colorimetric method previously described by Olędzki & Harasym (2023). Aliquots of 0.1 mL of Folin–Ciocalteu reagent and 1.58 mL of H_2O were added to the samples (0.02 mL). After 5 min of incubation, 0.3 mL of saturated sodium carbonate solution (Na_2CO_3) was added. After 20 min of incubation at 38 °C in the dark the total phenolic compounds were determined by measuring an absorbance of the resulting solution at 765 nm. The TPC results were presented in milligrams of gallic acid equivalent (GAE) per 1 g or mL of raw material used. All the samples were analyzed in duplicate.

The antioxidant capacity (against the 2,2-diphenyl-1-picrylhydrazyl radical) of the tested extracts was measured according to the method previously described by Olędzki & Harasym (2023). The measure of 0.035 mL of the test solution was measured and added to 1 mL of (0.1 mM) methanolic DPPH solution. The mixture was shaken and left at room temperature for 20 min, after which the absorbance was measured at 517 nm. The anti-radical activity was calculated from the calibration curve and expressed as the mg Trolox equivalent (TE) for 1 g or mL of raw material used. All the samples were analyzed in duplicate.

To test the reducing sugars content of cucumbers, homogenate was prepared as already described, except that, for cucumbers, the homogenate was centrifuged at 1500 rpm for 15 min at room temperature and the supernatant was taken. Reducing sugars content was measured using a modified method (Olędzki & Harasym, 2023), taking advantage of the reducing properties of sugars towards 3,5-dinitrosalicylic acid (DNS). A measure of 1 mL of DNS reagent was added to 1 mL of the test sample and mixed thoroughly. The resulting mixture was then heated in boiling water for 5 min. After the mixture cooled to room temperature, its absorbance at 535 nm was measured. The content of monosaccharides was expressed in g of glucose equivalent per 100 g or mL of sample tested. All the samples were analyzed in duplicate and expressed as mean ± standard deviation.

2.3. Morpho-textural analyses of cucumbers and brine

Color of cucumbers (the mesocarp) and brine were assessed using a Konica Minolta CR-300 chroma meter (Ramsey, NJ, USA). Color parameters (L*, a*, b*) were taken in triplicate, and expressed as mean ± standard deviation (Olędzki & Harasym, 2023).

Texture measurements were performed in cucumber using a mesocarp puncture test on a 5.0 mm height slice obtained from the center of cucumbers. The mesocarp puncture test was conducted on an AXIS texture analyzer FC20STAV500/500 (AXIS, Gdansk, Poland) using a 3-mm-diameter stainless steel probe to puncture. The mesocarp of one lobe of each slice was centered above a 3.1 mm hole in the base plate and the probe was lowered at a test speed of 2.5 mm s^{-1} through the sample. The test was conducted, and data analyzed using AXIS FM software (version 9.1.5. AXIS, Gdansk, Poland). The peak force of 8 samples per cucumber was averaged and recorded in Newtons (N) as the firmness value, and expressed as mean ± standard deviation.

The dynamic viscosity of brines was assessed with a rotational-oscillatory rheometer (MCR 102, Anton Paar, Stuttgart, Germany) with cylindrical cup-bob geometry with a 1-mm fluid gap. The temperature was 25 °C stabilized by a software-controlled unit connected to the rheometer ensuring correct and stable temperature control. The measuring units were controlled by RheoCompass v.1.24.584 (Anton Paar, Stuttgart, Germany) with data acquisition, analysis, storage, and retrieval. Samples were measured in triplicate.

2.4. Viable counts

For cucumbers, viable counts were performed by mixing 10 g of each sample with 90 mL of sterile peptone water (1 g L^{-1} of bacteriological peptone) homogenized using a Stomacher apparatus (400 Circulator, International PBI, Milan, Italy) for 3 min at 260 rpm. After cucumbers homogenization, and also for brine samples, ten-fold serial dilutions were prepared and viable counts of the following microbial groups were evaluated: i) presumptive lactobacilli on De Man Rogosa and Sharpe (MRS) agar (VWR Prolabo Chemicals, Leuven, Belgium) medium supplemented with cycloheximide (250 mg L^{-1}) incubated at 37 °C for 48–72 h; ii) presumptive lactococci on M17 agar (VWR) medium supplemented with cycloheximide (250 mg L^{-1}) incubated at 22 °C for 48–72 h; iii) presumptive thermophilic cocci on M17 (VWR) medium supplemented with cycloheximide (250 mg L^{-1}) incubated at 42 °C for 48–72 h; iv) coagulase-negative cocci on Mannitol Salt Agar (MSA) (Liofilchem, Roseto degli Abruzzi, Italy) incubated at 37 °C for 48–72 h; v) enterococci on Enterococcus Selective Agar (Merck KGAa, Darmstadt, Germany) incubated at 37 °C for 48 h; vi) Enterobacteriaceae on Violet Red Bile Glucose Agar (VRBGA) (VWR) incubated at 37 °C for 24 h; vii) eumycetes on Rose Bengal Chloramphenicol Agar (VWR) incubated at 25 °C for 72–96 h.

The results of two biological and three technical replicates were expressed as the log of colony forming units (cfu) per gram or mL of sample and reported as mean ± standard deviation.

2.5. Metataxonomic analysis of cucumbers and brine

2.5.1. DNA extraction and amplicon-based sequencing

Total DNA was extracted using the Master Pure complete DNA and RNA purification kit (Epicentre, Madison, WI, USA) according to the manufacturer's instructions. The quality of the extracted DNA was evaluated and quantified using a NanoDrop spectrophotometer (Thermo Scientific, Milan, Italy). Libraries of the V3-V4 region were constructed from the 16S rRNA gene region of bacterial DNA using primers and conditions previously described by Botta et al. (2020).

The PCR products were purified by means of an Agencourt AMPure kit (Beckman Coulter, Milan, Italy) and the resulting products were tagged with sequencing adapters using the Nextera XT library preparation kit (Illumina Inc, San Diego, CA), according to the manufacturer's instructions. Sequencing was performed using a MiSeq Illumina instrument (Illumina, San Diego, CA, USA) with V3 chemistry, which generated 2X250 bp paired-end reads. MiSeq Control Software, V2.3.0.3, RTA, v1.18.42.0, and CASAVA, v1.8.2, were used for the base-calling and Illumina barcode demultiplexing processes.

2.5.2. Bioinformatic analysis

The 983,860 raw-reads obtained from 16S rRNA amplicon-based sequencing were analysed in the R environment (R program version 4.1.1; https://www.r-project.org) using *DADA2* package (Callahan et al., 2016). A total of 544,369 reads passed the quality filtering parameters applied [*trimLeft = c(36,36); maxEE = c(2,2); minLen = c(50,50); truncQ = 10*]. After merging and *per-sample* chimera removal, all paired-end sequences shorter than 366 bp were discharged: 73.1 % of the filtered sequences were used to construct the frequency table of Amplicon Sequence Variants (ASVs), with an average value of 13,730 reads/sample and Good's coverage > 99 %. All parameters not reported for filtering/merging steps are intended as default *DADA2* setting.

Taxonomy was assigned with a confidence of 99 % sequence similarity through Bayesian classifier method (Wang et al., 2007) by matching ASVs with 2021 release (version 138.1) of Silva prokaryotic SSU reference database (https://zenodo.org/record/4587955#.YObFvh

MzZRE): the highest taxonomic rank level available was displayed when species was not reached. ASVs with unknown Phylum assignment or assigned to mitochondria-chloroplasts were removed from the frequency tables.

ASVs were aligned with *DECIPHER* package and an unrooted phylogenetic tree was constructed with *phangorn* package (Schliep, 2011; Wright, 2016). Alpha diversity metrics and weighted UniFrac beta-diversity distance were calculated with *phyloseq* and *picante* packages (Kembel et al., 2010; McMurdie and Holmes, 2013): the rarefaction limit was set to the lowest number of sequences/sample.

Metagenome inference was performed from ASVs frequency table with MetGEMs toolbox (Patumcharoenpol et al., 2021) using default parameters (https://github.com/yumyai/MetGEMs) and AGORA collection as reference database of genome-scale models (Magnúsdóttir et al., 2017). Gene family abundances were predicted and identified as KEGG orthologs (KO) and collapsed at level 3 of the KEGG annotations.

Sequencing data were deposited at the Sequence Read Archive of the National Center for Biotechnology Information under the bioproject accession number PRJNA997784.

2.5.3. Statistics

Statistical analyses and data plotting were performed using R program, unless otherwise stated. Normality and homogeneity of the data (Log-Transformed abundances, alpha-diversity metrics) were checked by means of Shapiro-Wilk's W and Levene's tests, respectively. Variations and differences between multiple groups were assessed with one-way ANOVA (coupled with Tukey's post-hoc test) and Kruskal–Wallis's test (coupled with pairwise Wilcoxon's test) for parametric and not parametric data, respectively. Pairwise comparisons were alternatively performed with Wilcoxon and T-tests according to data normality.

Weighted UniFrac beta-diversity distances was displayed in a PCoA graph, and the influence of sample type (brine, cucumber) and producer on the samples segregation has been significantly assessed with *Adonis* (PERMANOVA) function based on Brey-Curtis dissimilarity distance.

Enrichment analysis was performed with *GAGE* package on the predicted KO abundance table to identify biological pathways significantly overrepresented and underrepresented between brine and cucumber samples (Luo et al., 2009).

2.6. Isolation and characterization of lactic acid bacteria

2.6.1. Isolation and identification

Colonies of lactic acid bacteria grown on MRS agar (Merck) supplemented with cycloheximide (Merck) were randomly picked up and then sub-cultured to purity under the same conditions.

Isolates were obtained from all brine and cucumber samples and stored at −80 °C until further analysis.

Thawed lactic acid bacteria were then sub-cultured twice on MRS agar (Merck) at 30 °C for 48 h and subjected to DNA extraction according to Osimani et al. (2015); DNA purity and quantity were verified with a NanoDrop ND 1000 (Thermo Fisher Scientific, Wilmington, DE, USA). DNA extracts were standardized to a final concentration of 100 ng μL^{-1} and subjected to PCR in a My Cycler Thermal Cycler (BioRad), followed by electrophoresis as described by Osimani et al. (2015). The obtained amplicons were then shipped to Genewiz (Takaley, UK) for purification and sequencing. The basic local alignment search tool (BLAST) was then exploited to compare the obtained sequences with 16S rRNA sequences of type strains from GenBank DNA database (https://www.ncbi.nlm.nih.gov/). Finally, the sequences of the lactic acid bacteria cultures were submitted to the GenBank DNA database to obtain accession numbers.

2.6.2. Acidification in cucumber-based growth medium

To assess the acidification performance, lactic acid bacteria isolates were first sub-cultured twice in MRS broth (Merck) incubated at 30 °C for 18 h (Osimani et al., 2023). The cultures were then centrifuged at 1,610 × g for 5 min with a Rotofix 32A centrifuge (Hettich, Milan, Italy) and the pellets were washed with sterile physiological solution (0.9 % w v^{-1}) prior to resuspension in the same diluent. Bacterial cells concentration was established by measuring the optical density (OD) at 600 nm with a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). All the isolates were inoculated at 8 log cfu mL^{-1} in 10 mL of cucumber-based growth medium obtained as follows. The cucumber-based growth medium was prepared according to Johanningsmeier & McFeeters (2013) with some modifications. In more detail, cucumbers obtained from a local provider were processed in a mod. CE 11 centrifugal slow juicer (Girmi, Rimini, Italia) at 55 rpm speed. After juice extraction, NaCl and water were added to yield final concentrations of 67 % fresh cucumber and, according to Fleming et al. (1995), 6 % NaCl. The resulting cucumber-based growth medium was sterilized and stored at 4 °C until use.

The proximate composition of the cucumber-based growth medium was as follows: carbohydrates 1.24 %, protein 0.44 %, lipids <0.07 %, NaCl 6.07 %.

The pH values of the cucumber-based growth medium were measured prior to inoculation (t0) and daily (each 24 h) after incubation at 30 °C for 7 days.

2.6.3. Semi-quantitative assessment of enzymatic activities

In order to select starter or adjunct cultures of lactic acid bacteria isolates with the most suitable pro-technological enzymatic activities, the enzymatic activity profiles already proposed by Abarquero et al. (2023) were studied.

The enzymatic activities of the isolates were assessed with the semi-quantitative micromethod API® ZYM (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. In more detail, the API® ZYM galleries are designed to determine the activity of the following enzymes: 1 – control; 2 - alkaline phosphatase; 3 - esterase (C 4); 4 - esterase lipase (C 8); 5 - lipase (C 14); 6 - leucine arylamidase; 7 - valine arylamidase; 8 - cystine arylamidase; 9 - trypsin; 10 - alpha-chymotrypsin; 11 - acid phosphatase; 12 - naphthol-AS-BIphosphohydrolase; 13 - alpha-galactosidase; 14 - beta-galactosidase; 15 - beta-glucuronidase; 16 - alpha-glucosidase; 17 - beta-glucosidase; 18 - N-acetyl-ß-glucosaminidase; 19 - alpha-mannosidase; 20 - alphafucosidase.

The metabolic end-products produced during the incubation period were detected through coloured reactions revealed by the addition of reagents.

The API® ZYM galleries (bioMérieux) were inoculated as previously described by Osimani et al. (2023). After incubation for 4 h at 37 °C, 1 drop of ZYM A reagent (bioMérieux) and 1 drop of ZYM B reagent (bioMérieux) were added to each cupule until colour development (at least 5 min). For each cupule, a value ranging from 0 to 5 was given, corresponding to the colour developed: 0 corresponding to a negative reaction, 5 to a reaction of maximum intensity and 1, 2, 3, or 4 were intermediate reactions depending on the level of intensity (3, 4, or 5 being considered as positive reactions).

2.6.4. In-vitro EPS production

The isolates were screened for EPS production, based on the method already reported by Hilbig et al. (2019) with some modifications. In more detail, the isolates were first sub-cultured twice on MRS broth (Merck) at 37 °C for 48 h, then, EPS production was visually observed by adding aliquots (5 μL) of each bacterial culture on the following solid media: (i) MRS agar (Merck) added with 80 g L^{-1} sucrose (Serva, Heidelberg, Germany) to promote the synthesis of homopolysaccharides (HoPS); MRS agar (Merck) added with 10 g L^{-1} yeast extract (VWR Chemicals), 10 g L^{-1} meat extract (VWR Chemicals), 20 g/L galactose (VWR Chemicals), and 20 g/L lactose (Carlo Erba Reagents, Cornaredo, Italy) to promote the synthesis of heteropolysaccharides (HePS). After an incubation period of 48 h at 30 °C, the colonies were considered

positive if they had a mucoid appearance (evident shiny and slimy appearance) or a ropy consistency (able to produce visible filaments by using a sterile toothpick). The analyses were performed in duplicate for each isolate.

2.6.5. Detection of the hdcA gene of Gram-positive bacteria

The isolates were tested for the presence of the *hdcA* gene through qPCR performed using a CFX Connect Real-Time System machine (BioRad Laboratories, Hercules, CA, USA). The cycling conditions and primers used in the qPCR reactions were adjusted as previously described by Belleggia et al. (2021). *Lactobacillus parabuchneri* DSM 5987 was used as positive strain to create the standard curve. The qPCR analysis was performed in three technical replicates for each isolate, together with a blank and the results were expressed as presence (+) or absence (-) of the target gene.

2.6.6. Assessment of antimicrobial activity

The antimicrobial activity of the isolates was evaluated through the agar well diffusion assay already described by Osimani et al. (2023). Briefly, *Listeria innocua* was inoculated (2 %, v v^{-1}) into molten Brain Heart Infusion (BHI) soft agar (0.75 % agar) (VWR Chemicals). Then, 20 mL of the inoculated medium were transferred into 90 mm Petri dish (VWR Chemicals) until solidification. A cone of a 200 μL sterile tip (VWR Chemicals) was utilized to create wells of ~ 50 μL capacity on BHI soft agar (VWR Chemicals). The lactic acid bacteria to be tested were cultured twice in MRS broth (Merck) at 37 °C for 48 h, then, the broth cultures were added with 0.1 N NaOH (AppliChem, Darmstadt, Germany) solution to reach pH 7.0 to neutralise the organic acids produced during the growth of bacteria. A filtration step on sterile PES membrane filter of 0.22 μm pore size (Laboindustria S.p.A., Padova, Italy) was also performed. For each isolate, 4 wells were formed on BHI soft agar (VWR Chemicals), each containing: (i) 50 μL of the sub-cultured suspension; (ii) 50 μL of the neutralized suspension adjusted to pH 7.0; (iii) 50 μL of the filtered neutralized suspension; (iv) 50 μL of sterilized water as a negative control. Subsequently, the Petri dishes (VWR Chemicals) were incubated at 37 °C for 24 h and checked for the presence of zones of inhibition. In the case of positive results (presence of inhibition halo), 3 spots of 5 μL each of pepsin (Fluka™, Honeywell, Morristown, USA), trypsin (Fluka™), or Pronase (Merck) were laid along the circumference of the inhibition zone to evaluate the protein nature of the microbial-derived inhibitory compound. The Petri dishes were further incubated at the same conditions. The synthesis of bacteriocins by the tested lactic acid bacteria isolates was finally confirmed by the formation of crescents.

2.7. GC–MS analysis of volatile components

Headspace volatiles from each cucumber and brine samples were analyzed by HS-SPME-GC/MS, using a 7890 Agilent GC system coupled to an Agilent 5975 (Agilent Technologies, Santa Clara, California, USA) inert quadrupole mass spectrometer equipped with a Gerstel MPS2 autosampler (Gerstel, Mülheim, Germany), as described by Maoloni et al. (2021).

About 2 g collected from the sample, was shredded and placed in a 20 mL headspace vial. The sample was stirred for 2 min at 40 °C to accelerate equilibrium of headspace volatile compounds between the sample and the headspace. Then, volatile compounds extraction was carried out by injecting a 50/30 μm Divinylbenzene/Carboxen/Poly-DiMethylSiloxane (DVB/Carboxen/PDMS) SPME fiber (Supelco, Bellefonte, PA) into the vial and exposing it to the headspace for 15 min at 40 °C. Afterwards, the SPME fiber was desorbed directly into the injection port of the GC at 240 °C for 5 min in the splitless mode. Volatile compounds were separated using a capillary column HP Innowax (Agilent Technologies) (30 m × 0.25 mm id. × 0.25 μm film thickness); the carrier gas was helium with a flow of 1 mL min^{-1}. The temperature program of the GC oven was the following: 35 °C (hold 5 min), ramp to 150 °C at 5 °C/min, ramp to 240 °C a t 8 °C min^{-1} (hold 1 min). The injector, the quadrupole, the source and the transfer line temperature were maintained at 240 °C, 150 °C, 230 °C and 200 °C, respectively. Electron ionization mass spectra in full-scan mode were recorded at 70 eV electron energy in the range 31–350 amu. VOCs identification was achieved by comparing mass spectra with the Nist library (NIST 20) and by matching the retention indices (RI) calculated according to the equation of Van Den Dool & Kratz (1963) and based on a series of alkanes. The data were expressed as relative peak area respect to internal standard. Blank experiments were carried out in two different modalities: blank of the fiber and blank of the empty vial. Controls were processed every 4 analyses of the experimental samples. All the analyses were performed in duplicate and the results expressed as mean value of two technical replicates ± standard deviation.

2.8. Statistical analysis

To assess statistical differences within samples, the Tukey-Kramer's Honest Significant Difference (HSD) test (level of significance 0.05) was used by one-way analysis of variance (ANOVA). Tests were performed through JMP v11.0.0 software (SAS Institute Inc., Cary, NC).

3. Results

3.1. Physico-chemical analyses of cucumbers and brine

The results of the physico-chemical features of cucumbers and brine are reported in Table 1.

Regarding pH, the values of cucumbers ranged from 3.33 ± 0.05 (CP1, batch 2) to 3.71 ± 0.03 (CP4, batch 2), with samples from CP1 (batch 2) and CP4 (batch 2) showing the highest values, and samples from CP2 (batch 2) and CP4 (batch 1) showing the lowest values ($P < 0.05$). The pH of the brine samples ranged from 3.45 ± 0.02 (BP2, batch 2) to 3.86 ± 0.02 (BP4, batch 2), with samples from BP4 (batch 2) showing the highest value and those from BP2 (batch 2) and BP3 (batch 2) the lowest ($P < 0.05$). No statistically significant differences emerged between overall pH values of brine and cucumbers.

Titratable acidity ranged from 8.20 ± 0.4 (CP1, batch 1) to 10.03 ± 0.04 (CP4, batch 2) for cucumbers and 8.44 ± 0.01 (BP1, batch 1) 10.23 ± 0.18 (BP4, batch 2) for brine. Significant differences were observed between producers but not as much between batches ($P < 0.05$). Also the differences between the cucumbers and brines were negligible.

Lactic acid content for cucumbers differed from 0.952 ± 0.062 (CP1, batch 2) to 1.085 ± 0.001 (CP2, batch 1) g 100 g^{-1}, whereas for brine, the values were significant higher starting from 1.207 ± 0.004 (BP2, batch 1) to 1.357 ± 0.016 g 100 mL^{-1} (BP1, batch 1). For acetic acid content, the same trend was observed, however the differences between the cucumber and brine where far smaller than for lactic acid, and for cucumbers ranged from 0.192 ± 0.002 (CP1, batch 1) to 0.336 ± 0.000 g 100 g^{-1}(CP4, batch 1), whereas for brine the values were slightly higher ranging from 0.199 ± 0.001 (BP2, batch 2) to 0.353 ± 0.000 g 100 mL^{-1} (BP4, batch 2) ($P < 0.05$).

Concentrations of NaCl in cucumbers ranged from 0.64 ± 0.36 (CP3, batch 1) to 2.74 ± 0.73 (CP1, batch 1) g 100 g^{-1}, with samples from CP1 showing the highest value, and those from CP3 the lowest. Regarding brine, NaCl content ranged from 1.20 ± 0.74 (BP3, batch 1) to 3.59 ± 0.14 (BP1, batch 2) g 100 mL^{-1}, with no statistically significant differences among samples. In all the samples, the NaCl content was higher in brine than in cucumbers ($P < 0.05$).

The reducing sugars content in cucumbers ranged from 0.24 ± 0.01 (CP1, batch 1) to 0.77 ± 0.01 (CP4, batch 1) g GE 100 g^{-1}, with samples CP1 and CP3 showing the lowest value, and those from CP4 the highest ($P < 0.05$). In brine, the reducing sugars content ranged from 0.29 ± 0.01 (BP1, batch 1) to 1.01 ± 0.02 (BP4, batch 1) g GE 100 mL^{-1}, with no statistically significant differences among samples. In all the samples, the reducing sugars content was higher in brine than in cucumbers ($P <$

Table 1
Physico-chemical parameters of the analyzed fermented cucumber (C) and brine (B) samples.

Producer	Batch	Source	pH	Titratable acidity (mL of 0.1 N NaOH)	Lactic acid (g 100 g or mL^{-1})	Acetic acid (g 100 g or mL^{-1})	Antioxidant activity μM TE 100 g or mL^{-1}	Polyphenols mg GAE 100 g or mL^{-1}	Reducing sugars (g GE 100 g or mL^{-1})	NaCl (g 100 gor mL^{-1})
Producer 1	1	C	3.52 ± 0.05	8.20 ± 0.4	1.061 ± 0.010	0.318 ± 0.002	323.5 ± 3.5	26.93 ± 0.08	0.24 ± 0.01	2.74 ± 0.73
		B	3.67 ± 0.13	8.44 ± 0.01	1.357 ± 0.016	0.327 ± 0.003	319.6 ± 3.5	26.65 ± 0.07	0.29 ± 0.01	2.84 ± 0.99
	2	C	3.68 ± 0.06	8.31 ± 0.04	0.952 ± 0.062	0.304 ± 0.004	336.5 ± 0.7	27.02 ± 0.12	0.38 ± 0.07	2.12 ± 0.21
		B	3.64 ± 0.18	8.51 ± 0.10	1.355 ± 0.027	0.322 ± 0.013	332.5 ± 0.7	27.28 ± 0.13	0.50 ± 0.09	3.59 ± 0.14
Producer 2	1	C	3.56 ± 0.08	8.72 ± 0.00	1.085 ± 0.001	0.192 ± 0.002	372.5 ± 2.1	24.42 ± 0.26	0.52 ± 0.01	1.49 ± 0.25
		B	3.50 ± 0.14	9.03 ± 0.06	1.225 ± 0.004	0.200 ± 0.004	368.0 ± 2.1	24.66 ± 0.26	0.66 ± 0.01	2.40 ± 0.69
	2	C	3.33 ± 0.05	8.81 ± 0.04	1.035 ± 0.004	0.193 ± 0.001	373.0 ± 4.2	23.32 ± 0.25	0.48 ± 0.01	1.55 ± 0.52
		B	3.45 ± 0.02	9.07 ± 0.04	1.207 ± 0.004	0.199 ± 0.001	368.6 ± 4.2	23.32 ± 0.08	0.61 ± 0.01	1.83 ± 0.11
Producer 3	1	C	3.68 ± 0.06	9.70 ± 0.04	0.994 ± 0.007	0.268 ± 0.001	332.0 ± 8.5	27.80 ± 0.73	0.30 ± 0.02	0.64 ± 0.36
		B	3.74 ± 0.04	9.99 ± 0.04	1.226 ± 0.004	0.276 ± 0.003	328.0 ± 8.3	28.08 ± 0.75	0.37 ± 0.03	1.20 ± 0.74
	2	C	3.49 ± 0.01	9.47 ± 0.04	1.002 ± 0.000	0.260 ± 0.001	330.0 ± 1.4	29.85 ± 0.78	0.25 ± 0.01	0.79 ± 0.01
		B	3.47 ± 0.02	9.81 ± 0.11	1.224 ± 0.18	0.279 ± 0.002	326.1 ± 1.3	28.42 ± 0.74	0.31 ± 0.02	2.92 ± 0.04
Producer 4	1	C	3.37 ± 0.05	9.92 ± 0.03	1.056 ± 0.008	0.336 ± 0.000	235.5 ± 7.8	20.88 ± 0.68	0.77 ± 0.01	1.56 ± 0.01
		B	3.56 ± 0.06	10.17 ± 0.11	1.284 ± 0.007	0.345 ± 0.001	212.6 ± 7.8	20.28 ± 1.22	1.01 ± 0.02	2.01 ± 0.86
	2	C	3.71 ± 0.03	10.03 ± 0.04	1.063 ± 0.010	0.323 ± 0.001	224.0 ± 5.7	22.65 ± 2.62	0.73 ± 0.01	1.39 ± 0.15
		B	3.86 ± 0.02	10.23 ± 0.18	1.274 ± 0.005	0.353 ± 0.000	203.9 ± 4.9	22.42 ± 0.59	0.96 ± 0.02	2.78 ± 0.31

Values are expressed as means ± standard deviation.

0.05).

Total polyphenol content in cucumbers ranged from 20.88 ± 0.68 (CP4, batch 1) to 29.85 ± 0.78 (CP3, batch 2) mg GAE 100 g^{-1}, with samples from CP3 showing the highest average value, and those from CP4 the lowest ($P < 0.05$). As for brine samples, the detected values were in accordance with those of cucumbers ranging from 20.28 ± 1.22 (BP4, batch 1) to 28.42 ± 0.74 (BP3, batch 2) mg GAE 100 mL^{-1}.

The antioxidant activity vs DPPH was the lowest for all the samples from producer 4 regardless of type (cucumbers or brine) and batch ($P < 0.05$). The highest antioxidant activity of cucumbers was noted as 373.0 ± 4.2 (CP2, batch 2) and for the brine almost similar from the same sample − 368.6 ± 4.2. There were no differences between cucumbers and brines.

3.2. Morpho-textural analyses of cucumbers and brine

The results of color analysis of cucumbers are reported in Table 2.

The cucumbers color parameters differed mainly between producers with samples from CP4 having the lowest L* parameter ($P < 0.05$). Generally, the L* parameter ranged between 69.04 ± 0.01 (CP2, batch 2) and 60.92 ± 0.03 (CP4, batch 2). The a* parameter was highly pronounced towards green for the samples from CP1 and CP2, whereas for CP3 and CP4 the intensity of greenness was lower. In case of b* value, all the cucumbers were in the yellow zone with the highest values, attesting at 15.6 ± 0.3, noted for samples CP4, whereas CP2 samples showed the lowest b* value attesting at 7.5 ± 0.1 ($P < 0.05$).

The results of hardness analysis of cucumbers and viscosity analysis of brine samples are reported in Table 3.

The hardness measured by puncture test differed for all the producers, but not for batches, except for CP1. Despite the differences, the hardness was quite satisfying, with cucumbers of CP3 being the less

Table 2
Results of colour analysis of the analyzed fermented cucumber (C) samples.

Producer	Batch	Source	L*	a*	b*
Producer 1	1	C	66.05 ± 0.03	−12.3 ± 0.6	13.6 ± 0.3
	2	C	66.01 ± 0.01	−12.3 ± 0.1	13.6 ± 0.1
Producer 2	1	C	68.95 ± 0.08	−10.5 ± 0.4	7.7 ± 0.2
	2	C	69.04 ± 0.01	−10.9 ± 0.1	7.5 ± 0.1
Producer 3	1	C	61.11 ± 0.13	−11.7 ± 0.0	11.8 ± 0.0
	2	C	62.04 ± 0.06	−11.7 ± 0.0	11.8 ± 0.0
Producer 4	1	C	61.12 ± 0.12	−9.6 ± 0.2	15.6 ± 0.3
	2	C	60.92 ± 0.03	−9.8 ± 0.1	15.2 ± 0.0

Values are expressed as means ± standard deviation.
L* value describes the lightness; a* value describes the redness/greenness; b* describes the blueness/yellowness.

Table 3
Results of hardness analysis of the analyzed cucumber samples (C) and viscosity analysis of brine samples (B).

Producer	Batch	Source	Hardness [N]	Source	Viscosity mPa*s
Producer 1	1	C	8.24 ± 0.09	B	1.072 ± 0.005
	2	C	8.57 ± 0.06	B	1.067 ± 0.003
Producer 2	1	C	7.56 ± 0.06	B	1.052 ± 0.003
	2	C	7.48 ± 0.17	B	1.051 ± 0.008
Producer 3	1	C	7.06 ± 0.06	B	1.067 ± 0.005
	2	C	6.98 ± 0.04	B	1.066 ± 0.003
Producer 4	1	C	6.27 ± 0.10	B	1.133 ± 0.001
	2	C	6.15 ± 0.03	B	1.203 ± 0.109

Values are expressed as means ± standard deviation.

crunchy.

As for the brine samples, the detected values of viscosity were similar among all the batches and producers, with an average of 1.067 ± 0.003 mPa*s, except for BP4, where significant increase up to the average of 1.150 ± 0.056 mPa*s was observed ($P < 0.05$).

3.3. Viable counts of cucumbers and brine

The results of viable counts are reported in Table 4.

In more detail, counts of presumptive lactococci in cucumbers were comprised between 5.0 ± 2.1 (CP1, batch 2) and 6.5 ± 0.5 (CP3, batch 1) log cfu g^{-1}, with no statistically significant differences among cucumber samples. In brine, the counts of presumptive lactococci were comprised between 6.4 ± 1.8 (BP1, batch 2) and 7.3 ± 1.6 (BP4, batch 1) log cfu mL^{-1}, with no statistically significant differences among brine samples.

For presumptive thermophilic cocci, counts detected in cucumbers were comprised between 4.8 ± 1.8 (CP1, batch 2) and 6.5 ± 0.5 (CP3, batch 1) log cfu g^{-1}, with no statistically significant differences among cucumber samples. In the brine, the counts of presumptive thermophilic cocci were comprised between 5.8 ± 0.2 (BP2, batch 1) and 7.1 ± 1.7 (BP4, batch 2) log cfu mL^{-1}, with no statistically significant differences among brine samples.

Regarding presumptive lactobacilli, viable counts detected in cucumbers ranged from 5.7 ± 0.2 (CP4, batch 1) to 7.0 ± 0.3 (CP1, batch 2) log cfu g^{-1}, with CP1 samples (batch 2) showing the highest values and CP4 (batch 1) showing the lowest ($P < 0.05$). For brine, presumptive lactobacilli counts ranged from 7.0 ± 0.1 (BP4, batch 1) to 8.0 ± 0.4 (BP1, batch 2) log cfu mL^{-1}, with samples from BP1 (batch 2) showing the highest values, and those from BP4 (batch 1 and 2) showing the lowest ($P < 0.05$).

Coagulase-negative cocci counts in cucumbers were comprised between 0.6 ± 0.9 (CP1, batch 1) and 4.2 ± 1.0 (CP2, batch 2) log cfu g^{-1}, with samples of CP2 (batch 2) showing the highest values and those of CP1 (batch1) and CP2 (batch 1) showing the lowest ($P < 0.05$). Regarding brine, the counts were comprised between 2.0 ± 0.3 (BP3, batch 2) and 5.0 ± 0.2 (BP4, batch 1) log cfu mL^{-1}, with samples of BP4 (batch 1) showing the highest values and those of BP3 (batch 2) the lowest ($P < 0.05$).

Enterococci counts were < 1 log cfu g^{-1} in all the samples except for samples from producer 3 that had counts from 1.5 to 2.3 log cfu g^{-1}.

Enterobacteriaceae counts were < 1 log cfu g or mL^{-1} in all the analyzed cucumber and brine samples.

Finally, a high variability of eumycetes counts was observed with values ranging from < 1 to 2.7 ± 0.3 (CP4, batch 1) log cfu g^{-1} in cucumbers and from < 1 to 4.6 ± 0.4 (BP4, batch 1) log cfu mL^{-1} in brine. For brine, samples BP4 (batch 1) showed the highest values ($P < 0.05$).

In all the samples, the viable counts of presumptive lactococci, presumptive thermophilic cocci, presumptive lactobacilli, coagulase-negative cocci, and eumycetes were generally higher in brine than in cucumbers.

3.4. Microbiota composition of cucumbers and brine

Microbiota of the analyzed samples differed significantly (PERMANOVA, P[FDR] < 0.001) between brine and cucumbers (Fig. 1). Indeed, observing the PCoA ordination of beta-diversity distances, samples from these two substrates segregated in two phylogenetically distinct communities. On the other hand, the producer did not show a significant (PERMANOVA, P[FDR] < 0.001) segregation of the samples (data not shown).

Regarding the taxonomic composition, a core microbiota of twenty genera, which represented together more than 80 % of total abundance in all brine and cucumber samples, was observed (Fig. 2). Lactic acid bacteria were overall the dominant population, with *Lactococcus* and *Streptococcus* significantly (Wilcoxon's test; P-value [FDR adjusted] < 0.05) more abundant in the cucumbers, whereas the genera *Lactiplantibacillus*, *Leuconostoc*, *Pediococcus*, *Secundilactobacillus*, and *Lentilactobacillus* were more present in brine samples (Fig. 3). Moreover, cucumbers showed significantly different alpha-diversity metrics compared to brine, with greater richness, evenness, and phylogenetic diversity (Fig. 4).

The differential distribution of the microbiota of cucumber and brine samples determined a co-occurrence/-exclusion pattern, with genera from brine negatively correlated overall with those most abundant in cucumbers (Fig. 5).

Moreover, 28 inferred metabolic pathways were differentially represented between brine and cucumber samples in relation to the pairwise comparison of GAGE enrichment statistic ($P < 0.001$). Noteworthy, pathways related to monosaccharide/di-saccharide metabolism were presumptively overrepresented in brine samples, whereas pathways related to amino acids catabolism/biosynthesis were more abundant in cucumbers (Supplementary Table S1).

3.5. Characterization of lactic acid bacteria isolates

The closest relatives, percentage identities, and accession numbers of the sequences obtained from the 60 lactic acid bacteria isolated from cucumber and brine samples are reported in Table 5.

Table 4
Viable counts detected on fermented cucumber (C) and brine (B) samples.

Producer	Batch	Source	Presumptive lactococci	Presumptive thermophilic cocci	Presumptive lactobacilli	Coagulase-negative cocci	Enterococci	Enterobacteriaceae	Eumycetes
Producer 1	1	C	5.7 ± 0.3	5.4 ± 0.02	6.9 ± 0.2	0.6 ± 0.9	<1	<1	<1
		B	6.6 ± 0.0	6.5 ± 0.0	7.9 ± 0.1	3.0 ± 0.0	<1	<1	1.7 ± 0.0
	2	C	5.0 ± 2.1	4.8 ± 1.8	7.0 ± 0.3	1.3 ± 1.09	<1	<1	<1
		B	6.4 ± 1.8	6.2 ± 1.8	8.0 ± 0.4	2.5 ± 0.8	<1	<1	2.0 ± 0.3
Producer 2	1	C	5.5 ± 0.5	5.2 ± 0.7	6.5 ± 0.3	0.6 ± 0.9	<1	<1	<1
		B	6.6 ± 0.4	5.8 ± 0.2	7.7 ± 0.2	3.0 ± 0.2	<1	<1	<1
	2	C	6.0 ± 2.0	6.0 ± 1.6	5.8 ± 5.8	4.2 ± 1.0	<1	<1	<1
		B	6.8 ± 2.3	6.1 ± 2.4	7.3 ± 0.1	4.6 ± 0.7	<1	<1	2.0 ± 0.7
Producer 3	1	C	6.5 ± 0.5	6.5 ± 0.5	7.0 ± 0.1	1.3 ± 1.9	<1	<1	1.2 ± 1.6
		B	6.9 ± 0.3	6.9 ± 0.3	7.4 ± 0.0	2.7 ± 0.2	1.5 ± 0.3	<1	0.9 ± 1.3
	2	C	6.3 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	2.3 ± 0.0	2.0 ± 0.1	<1	<1
		B	7.0 ± 0.0	7.1 ± 0.1	7.1 ± 0.0	2.0 ± 0.3	2.3 ± 0.0	<1	<1
Producer 4	1	C	6.2 ± 1.7	5.1 ± 1.4	5.7 ± 0.2	3.8 ± 0.1	<1	<1	2.7 ± 0.3
		B	7.3 ± 1.6	7.1 ± 1.6	7.0 ± 0.1	5.0 ± 0.2	<1	<1	4.6 ± 0.4
	2	C	5.9 ± 2.1	5.7 ± 1.6	6.1 ± 0.2	3.0 ± 0.1	<1	<1	1.3 ± 1.8
		B	7.1 ± 2.1	7.1 ± 1.7	7.1 ± 0.0	4.0 ± 0.0	<1	<1	2.3 ± 3.3

Values are expressed as means of log cfu g^{-1} (cucumbers) or mL^{-1} (brine) ± standard deviation.

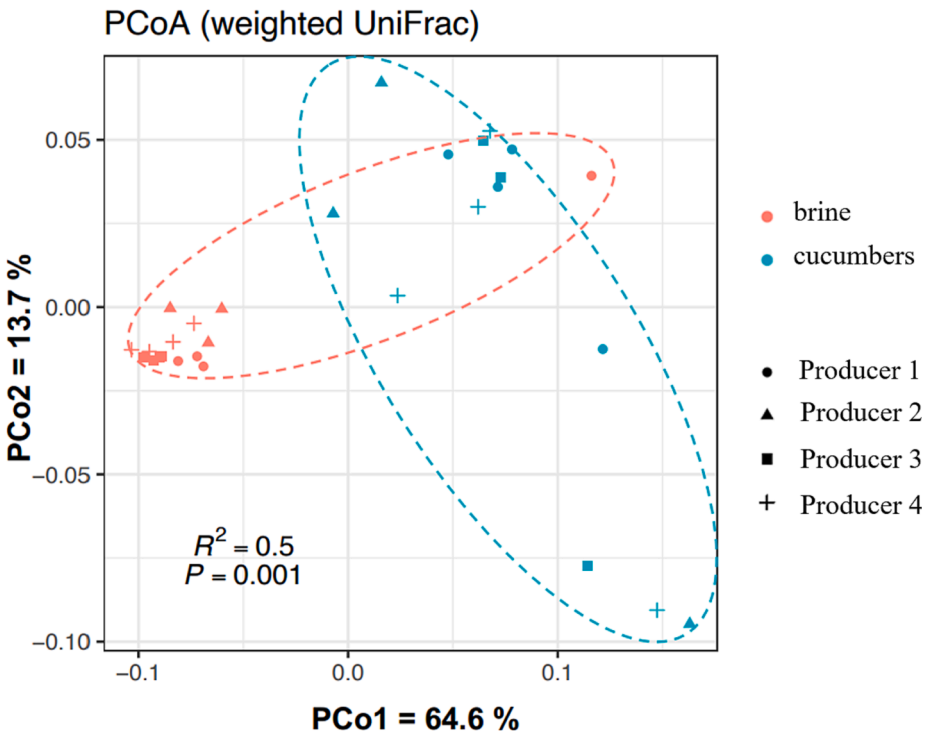

Fig. 1. PCoA charts displaying weighted UniFrac distance matrix (β-diversity). Sample types and producers are defined by different colours and shapes (legend); dashed ellipses are indicating significant different communities between brine and cucumbers ($P < 0.001$ [FDR adjusted], PERMANOVA; displayed in the graph).

In more detail, the closest relatives to *Pediococcus parvulus* represented the most frequently isolated lactic acid bacteria species (40 out of the 60 isolates), followed by the *Lactiplantibacillus plantarum* group (15 out of the 60 isolates) and *Pediococcus ethanolidurans* (5 out of the 60 isolates).

It is noteworthy that the *Lactiplantibacillus plantarum* group (basionym *Lactobacillus plantarum*) includes three closely related species, namely *Lactiplantibacillus plantarum*, *Lactiplantibacillus paraplantarum* (basionym *Lactobacillus paraplantarum*), and *Lactiplantibacillus pentosus* (basionym *Lactobacillus pentosus*) (Kim et al., 2020). Although the 16S rRNA target gene is still the most widely used method to assign bacterial phylogeny and taxonomy in PCR-based identification, it leads to misidentification of the *L. plantarum* group species due to the high genetic similarity of this gene (99.4 %–99.9 %) (Kim et al., 2020). Hence, in the present study, the *Lactiplantibacillus plantarum* group has been chosen for the taxonomic assignation of the isolated lactic acid bacteria.

Only a few isolates showed the ability to produce EPS in synthetic medium (Table 5). In more detail, the *P. parvulus* isolates PP4 and PP22 produced sucrose-independent EPS, whereas only PP22 produced sucrose-dependent EPS. As for the *L. plantarum* group, the isolates LP89 and LP90 showed the production of both sucrose-dependent and -independent EPS.

As for the presence of the *hdcA* gene (Table 5), only the isolates *P. ethanolidurans* PE77 and PE82 were positive for the target gene. Hence, these isolates were not further characterized since their use as potential starter or adjunct cultures was excluded.

As for enzymatic activity, according to the manufacturer's instructions, only the isolates showing color development scored 3, 4, or 5 were considered positive for the enzymatic activity assayed (Fig. 6).

Regarding esterase (C 4) and esterase lipase (C 14), only one isolate (PP5) showed a positive reaction.

As for proteases, 100 % of the isolates showed a strong activity for leucine arylamidase; moreover, 51 out of the 58 isolates were positive for valine arylamidase, and 12 out of the 58 isolates were positive for cystine arylamidase. The 12 positive isolates for valine arylamidase were only the closest relatives to *P. parvulus*.

Only a few isolates (7 out of 58) showed a weak positive reaction for acid phosphatase, whereas 8 out of the 58 isolates were positive for naphthol-AS-BIphosphohydrolase.

Most of the *L. plantarum* group isolates (13 out of 15) showed a strong beta-galactosidase activity, whereas only a few pediococci were positive for the same enzymatic activity.

Thirty-seven out of the 58 isolates were positive for alpha-glucosidase, whereas 51 of the 58 isolates were positive for beta-glucosidase.

A generally weak enzymatic activity was observed for the N-acetyl-ß-glucosaminidase in 22 out of the 58 isolates. No isolate was positive for alkaline phosphatase, lipase (C 14), trypsin, alpha-chymotrypsin, alpha-galactosidase, beta-glucuronidase, alpha-mannosidase, or alpha-fucosidase.

The results of acidification performance in cucumber-based medium of *P. parvulus* and *L. plantarum* group are reported in Fig. 7. In more detail, a high variability was observed among the tested *P. parvulus* cultures (data not shown), with progressive pH reduction from 24 h to 9 days (end of the experiment) of fermentation. After 9 days, all isolates showed pH values below 4.5, with isolates PP6, PP27, PP33, PP37, PP40, PP65, and PP74 reaching values equal or lower to 3.30.

Most of the *L. plantarum* group isolates were able of lowering the pH of the cucumber-based growth medium soon after 1 day of fermentation. Moreover, all the isolates showed pH values below 4.0 after 2 days of fermentation, reaching pH values below 3.5 at the end of the experiment (9 days).

pH values of sterile uninoculated control cucumber-based medium incubated at the same test conditions did not show variations during the 9-day assays.

Finally, regarding antimicrobial activity assessed through agar well diffusion assay, no isolate showed any inhibitory activity against *L. innocua* (data not shown).

3.6. Volatilome

The volatile compounds of the cucumber and brine samples

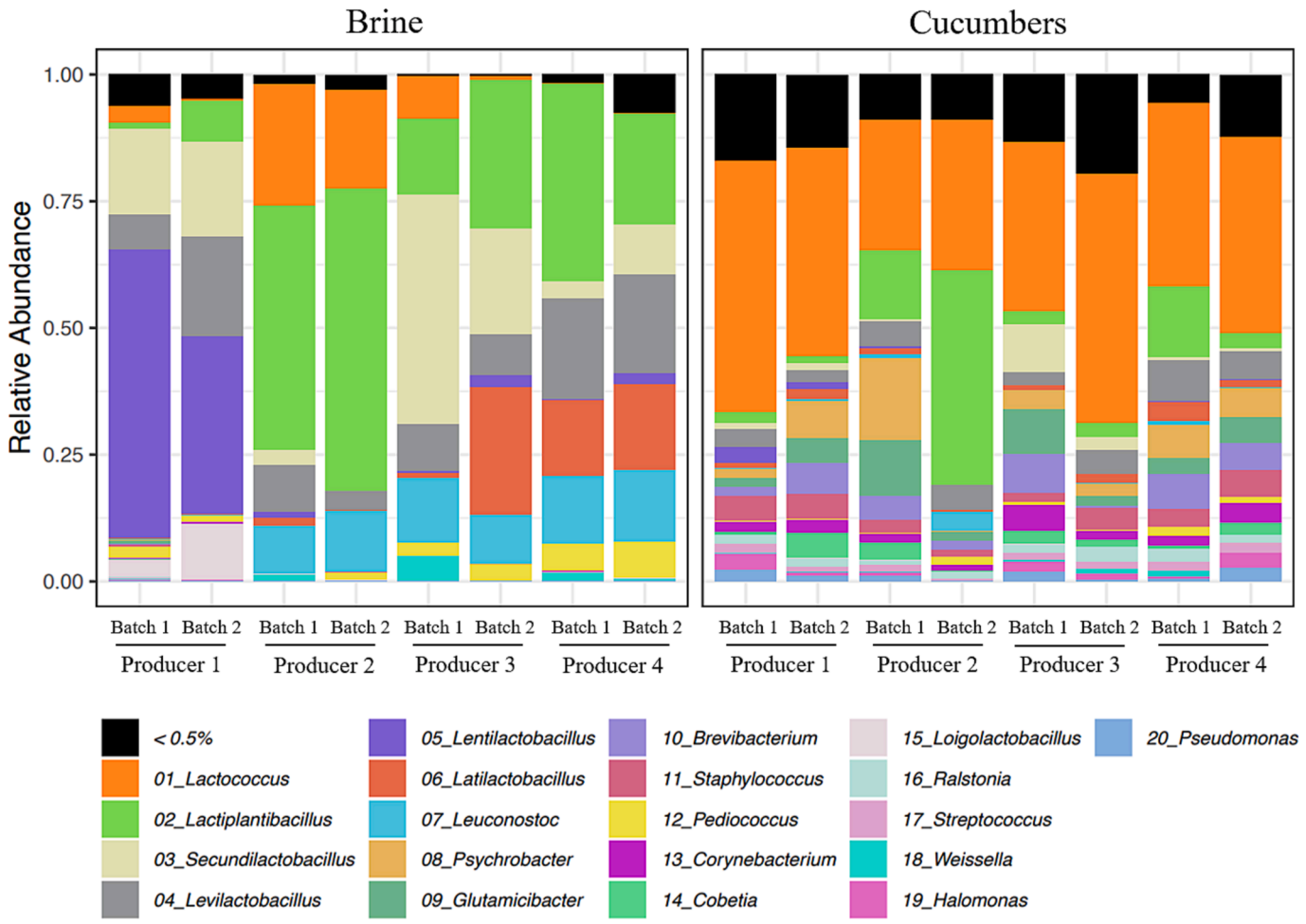

Fig. 2. Stacked bar plots showing core microbiota composition (relative abundance) at Genus rank level and relative colour coding key. Samples are grouped by sample types (brine, cucumbers) and displayed according to the producer ; taxa are sorted in the legend from the most to the least abundant (>0.5 % of average abundance).

manufactured by the 4 producers were identified through HS-SPME-GC/MS technique. The analysis allowed the detection and identification of more than 80 volatile compounds both in cucumbers and in brine (Table 6). The compounds belonged to 9 main classes, being oxygenated terpenes (17), alcohols (13), terpenes (12), ketones (8), acids (7), aldehydes (6), esters (6), sulfur (6), sesquiterpenes (2). Five compounds of other different classes were also found.

In general, all compounds were detected at higher amounts in cucumbers than in brine.

Aldehydes, ketones, and esters were found in moderate amounts. Among aldehydes the most represented were pentanal, propanal, and hexanal. Acetahaldehyde was found only in the samples of cucumbers and brines from producer 1.

Among the ketones, 2-propanone and 2-butanone were the most represented in all the samples. Among esters, ethyl acetate, methyl acetate, and propyl acetate were the most abundant.

Alcohols were found in high amount in all the samples. Ethanol was the most abundant alcohol, followed by 1-propanol and isoamyl.

Acids, represented mainly by acetic acid, were found in high amount in all the samples.

In all the samples, terpenoids were found in higher amount in cucumbers than in brine. The compounds a-pinene and limonene were found in all the samples. The samples of cucumber from producers 1 and 2 were characterized by the highest amount of terpene hydrocarbons, mainly limonene, o-cymene, a-phellandrene. Furthermore, the samples from producer 1 were characterized by the presence of other terpene hydrocarbons such as a-pinene, b-myrcene, a-terpinene, b-phellandrene, p-cymene.

Among oxygenated hydrocarbons, linalool, 4-terpineol, a-terpineol, carvone, isodihydrocarveol, dihydrocarvone cis 1, trans dihydrocarvone, and carvacrol were found in all the samples. Samples from producer 1 showed the highest amount of oxygenated terpens compared to the other samples.

Traces of sesquiterpenes were found only in the samples of cucumbers from producer 2 and 3.

Sulphur compounds were found mainly in the samples from producer 1 and 3 and the most represented were dimethylsulfide, allyl sulfide, allyl disulfide. Samples from producer 2 and 4 recorded only traces of allyl isothiocyanate, these samples were mainly characterized for sulphide, allyl methyl, and diallyl disulphide.

Among the other volatile compounds, dill ether was found in moderate amount in all the samples.

In order to better understand the differences between the cucumber and brine samples from the different producers (1, 2, 3, and 4), a PCA of the volatile compounds was performed (Fig. 8). The first two PCs explained about 55.23 % of the total variance of the data. As determined by the two PCs (factors), samples were located in different zones of the plane, highlighting that the samples had a different volatile composition. In particular, samples from producer 1 were located in the I square of the graph, samples from producer 2 in the IV square and samples from producer 2 and 3 in the II square.

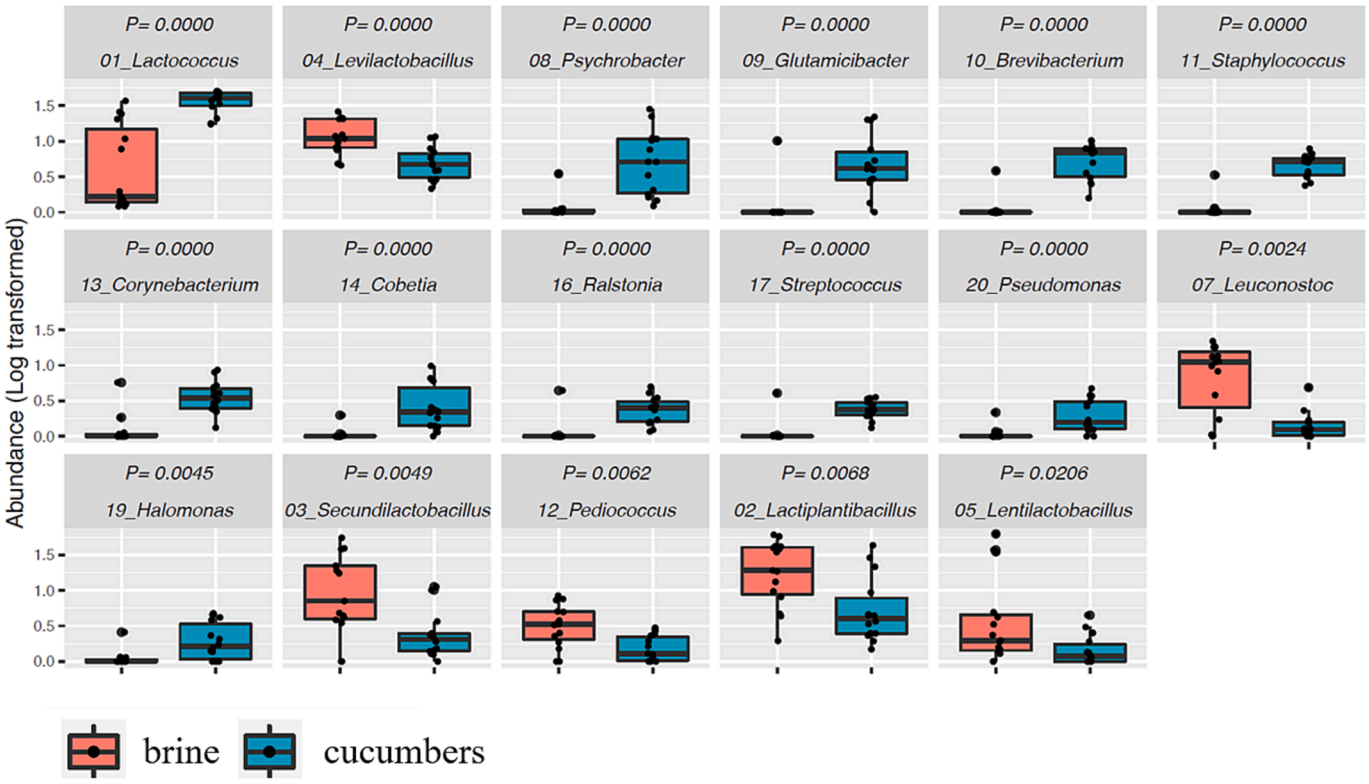

Fig. 3. Box plots of alpha-diversity metrics significantly different (Wilcoxon's test; P-value [FDR adjusted] < 0.05) between brine and cucumbers; *P*-value are reported in the graph.

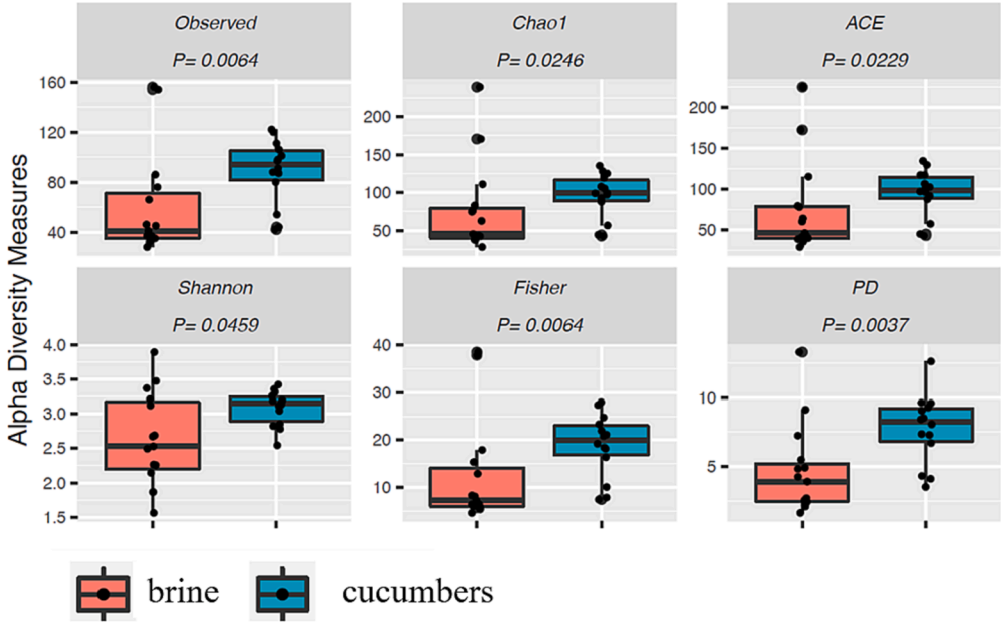

Fig. 4. Box plots of genera abundances significantly different (Wilcoxon's test; P-value [FDR adjusted] < 0.05) between brine and cucumbers; *P*-value are reported in the graph.

4. Discussion

Fermentation of vegetables represents one of the oldest methods of biopreservation and meets consumer demand for fresh, highly nutritious, healthy, and tasty ready-to-eat foods (Di Cagno et al., 2013).

The pH values detected in the analyzed samples were slightly higher than those reported by Di Cagno et al. (2013) for fermented cucumbers, with values between 3.1 and 3.5 at the end of fermentation. Of note, the low pH of fermented cucumbers and their brine is a result of the presence of organic acids and is pivotal for inhibiting pathogenic bacteria (Stoll et al., 2020).

As reported by Johanningsmeier & McFeeters (2013), the final pH and sodium chloride (NaCl) concentration exert a considerable impact on fermentation and microbial stability of cucumbers. Of note, salt-

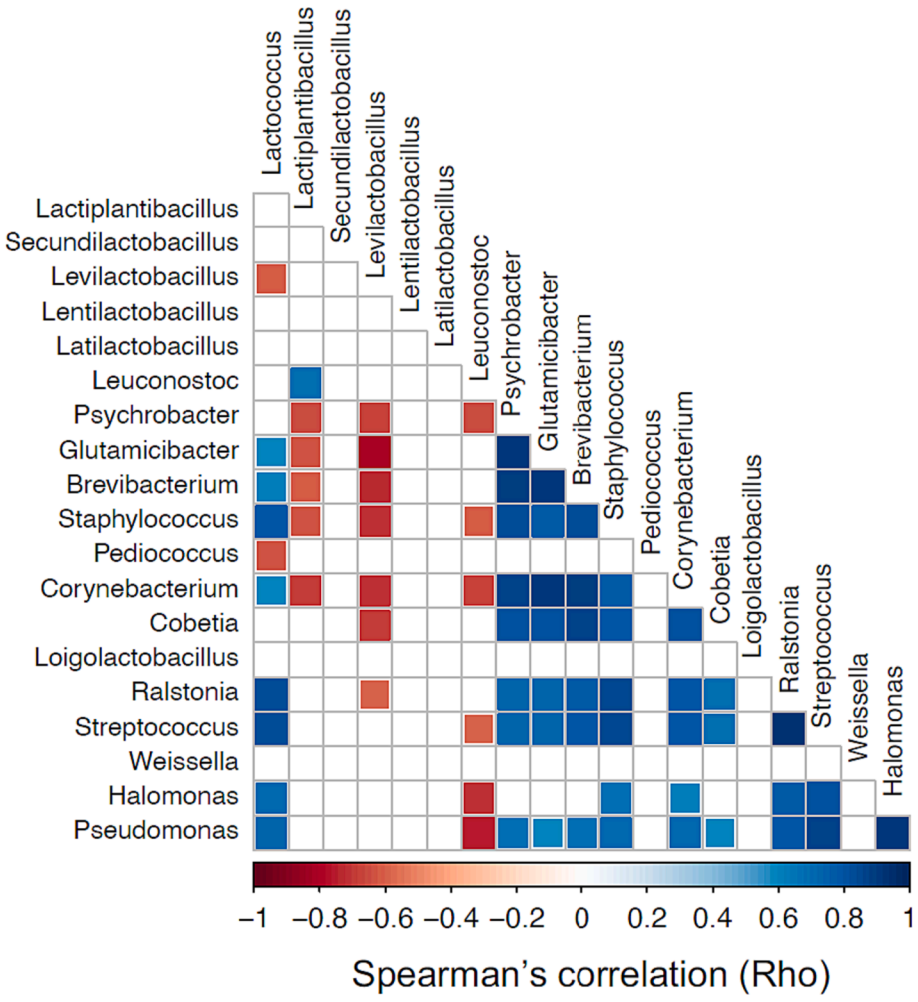

Fig. 5. Co-occurrence and co-exclusion pattern among core genera; only significant correlations (Spearman's moment rank correlation, P [FDR] < 0.001) are displayed in the heatmap. Colour coding key indicate correlation type (positive or negative).

reduced (from 0 to 4 % NaCl) fermented cucumbers with a final pH as low as 3.2 may be susceptible to spoilage under anaerobic conditions (Johanningsmeier et al., 2012). In the present study, the values of salt concentration of brine samples were comprised between 1.20 and 3.59 g 100 mL^{-1}, notwithstanding no spoilage was observed in the samples, although some alterative microbial species were detected as minority taxa by metataxonomic analysis.

The lactic and acetic acid contents are two indicators that attest a proper fermentation process based on lactic acid bacteria activity. The results of lactic acid content obtained for the analyzed samples were in accordance with those obtained by McMurtrie et al. (2019) and Nilchian et al. (2016), being, for lactic acid, in a range from 0.952 ± 0.062 to 1.085 ± 0.001 g 100 g^{-1} in cucumbers, and from 1.207 ± 0.004 to 1.357 ± 0.016 g 100 mL^{-1} in brine. Generally, the tested organic acids were more abundant in brine than the cucumbers. Of note, the acetic acid should be on low levels as it indicates the long fermentation or even initialized spoilage process as the effect of metabolization of the lactic acid (Franco et al., 2012). For acetic acid content the differences between cucumbers and brine where low and ranged from 0.192 ± 0.002 to 0.336 ± 0.000 g 100 g^{-1} for the cucumbers, whereas, for brine, the values ranged from 0.199 ± 0.001 to 0.353 ± 0.000 g 100 mL^{-1}.

Titratable acidity in cucumber and brine samples was in relation to the measured pH, as suggested by Yoo et al. (2006). Titratable acidity ranged from 8.20 ± 0.4 to 10.03 ± 0.04 mL of 0.1 N NaOH for cucumbers and from 8.44 ± 0.01 to 10.23 ± 0.18 mL of 0.1 N NaOH in brine samples. According to Nilchian et al. (2016), titratable acidity showed relation to NaCl content.

The reducing sugars indicate the residual fermentable carbohydrates and can be an indicator of progressing of fermentation. Reducing sugars content expressed as glucose equivalents were generally low, being higher in brine and lower in cucumbers. The values ranged from 0.24 ± 0.01 to 0.77 ± 0.01 g GE 100 g^{-1} in cucumbers and from 0.29 ± 0.01 to 1.01 ± 0.02 g GE 100 mL^{-1} in brine, with no statistically significant differences among samples. The results were in accordance with data obtained by Migut et al. (2018) and McMurtrie et al. (2019).

To the author's knowledge, there is a paucity of data for total polyphenol content (TPC) and antioxidant activity (AA) of spontaneously fermented vegetables. The data collected in the present study showed that for cucumbers TPC ranged from 20.88 ± 0.68 to 29.85 ± 0.78 mg GAE 100 g^{-1}, whereas AA from 203.9 ± 4.9 to 373.0 ± 4.2 μM TE 100 g^{-1}, respectively. There were no differences between cucumber and brine samples, whereas producers represented the major differentiating factor. The results herein collected were in accordance with those reported by Sayın & Alkan (2015) who tested 10 different spontaneously fermented vegetables for antioxidant characteristics.

As for color analysis, it is noteworthy that the pericarp and the mesocarp of fermented cucumbers are influenced by exterior conditions and, in result, the vivid green colour changes into greish olive green. During the fermentation, the chlorophylls undego a conversion to pheophorbids and partially to pheophytins. The differences in the color detected in the present study may occur due to location and growing conditions of cucumbers; moreover, the occuring changes can be

Table 5
Identification and characterization of lactic acid bacteria isolates from fermented cucumber (C) and brine (B) samples.

Producer	Batch	Isolation source	Isolate code	Species	% Identity[a]	Accession number[b]	*hdcA gene*	EPS production Sucrose-dependent	Sucrose-independent
Producer 1	1	B	PP1	*Pediococcus parvulus*	99.78 %	NR_113922	–	–	–
		B	PP2	*Pediococcus parvulus*	99.78 %	NR_113922	–	–	–
		B	PP3	*Pediococcus parvulus*	99.01 %	NR_113922	–	–	–
		B	PP4	*Pediococcus parvulus*	99.37 %	NR_113922	–	–	+
		C	PP5	*Pediococcus parvulus*	99.59 %	NR_113922	–	–	–
		C	PP6	*Pediococcus parvulus*	99.89 %	NR_113922	–	–	–
		B	LP7	*Lactiplantibacillus plantarum* group	99.15 %	NR_104573	–	–	–
		B	PP9	*Pediococcus parvulus*	98.32 %	NR_113922	–	–	–
		C	LP11	*Lactiplantibacillus plantarum* group	100.00 %	NR_025447	–	–	–
		C	PP12	*Pediococcus parvulus*	98.96 %	NR_029136	–	–	–
	2	B	PP14	*Pediococcus parvulus*	100.00 %	NR_113922	–	–	–
		B	PP16	*Pediococcus parvulus*	99.53 %	NR_029136	–	–	–
		C	PP17	*Pediococcus parvulus*	98.91 %	NR_029136	–	–	–
		C	PP18	*Pediococcus parvulus*	99.28 %	NR_113922	–	–	–
		B	PP19	*Pediococcus parvulus*	100.00 %	NR_113922	–	–	–
		B	PP20	*Pediococcus parvulus*	98.69 %	NR_029136	–	–	–
		B	PP21	*Pediococcus parvulus*	99.13 %	NR_029136	–	–	–
		B	PP22	*Pediococcus parvulus*	98.97 %	NR_113922	–	+	+
		C	PP24	*Pediococcus parvulus*	98.89 %	NR_029136	–	–	–
Producer 2	1	B	PP25	*Pediococcus parvulus*	98.30 %	NR_029136	–	–	–
		B	PP26	*Pediococcus parvulus*	99.72 %	NR_029136	–	–	–
		B	PP27	*Pediococcus parvulus*	99.23 %	NR_113922	–	–	–
		C	PP28	*Pediococcus parvulus*	100.00 %	NR_113922	–	–	–
		C	PP29	*Pediococcus parvulus*	100.00 %	NR_113922	–	–	–
		B	PP30	*Pediococcus parvulus*	99.88 %	NR_113922	–	–	–
		B	PP31	*Pediococcus parvulus*	100.00 %	NR_029136	–	–	–
		B	PP33	*Pediococcus parvulus*	99.91 %	NR_029136	–	–	–
	2	B	PP36	*Pediococcus parvulus*	100.00 %	NR_113922	–	–	–
		B	PP37	*Pediococcus parvulus*	99.78 %	NR_113922	–	–	–
		B	PP38	*Pediococcus parvulus*	99.78 %	NR_113922	–	–	–
		B	PP39	*Pediococcus parvulus*	100.00 %	NR_113922	–	–	–
		C	PP40	*Pediococcus parvulus*	99.35 %	NR_113922	–	–	–
		C	PP41	*Pediococcus parvulus*	99.75 %	NR_029136	–	–	–
		B	PP42	*Pediococcus parvulus*	99.49 %	NR_029136	–	–	–
		B	PP45	*Pediococcus parvulus*	97.45 %	NR_029136	–	–	–
		C	PP47	*Pediococcus parvulus*	99.63 %	NR_029136	–	–	–
Producer 3	1	B	LP48	*Lactiplantibacillus plantarum* group	99.18 %	NR_104573	–	–	–
		B	LP49	*Lactiplantibacillus plantarum* group	98.62 %	NR_104573	–	–	–
		B	LP50	*Lactiplantibacillus plantarum* group	99.39 %	NR_104573	–	–	–
		B	LP51	*Lactiplantibacillus plantarum* group	98.55 %	NR_104573	–	–	–
		C	LP52	*Lactiplantibacillus plantarum* group	99.25 %	NR_104573	–	–	–
		C	LP53	*Lactiplantibacillus plantarum* group	98.23 %	NR_104573	–	–	–
		B	LP54	*Lactiplantibacillus plantarum* group	97.57 %	NR_104573	–	–	–
		B	LP55	*Lactiplantibacillus plantarum* group	98.64 %	NR_104573	–	–	–
		B	LP57	*Lactiplantibacillus plantarum* group	99.58 %	NR_104573	–	–	–
	2	B	LP85	*Lactiplantibacillus plantarum* group	99.82 %	NR_104573	–	–	–
		B	LP89	*Lactiplantibacillus plantarum* group	99.26 %	NR_104573	–	+	–
		C	LP90	*Lactiplantibacillus plantarum* group	99.50 %	NR_104573	–	+	+
Producer 4	1	B	PP60	*Pediococcus parvulus*	99.34 %	NR_029136	–	–	–
		B	PE63	*Pediococcus ethanolidurans*	99.02 %	NR_043291	–	–	–
		B	PP64	*Pediococcus parvulus*	99.66 %	NR_029136	–	–	–
		C	PP65	*Pediococcus parvulus*	99.35 %	NR_029136	–	–	–
		C	PP71	*Pediococcus parvulus*	100.00 %	NR_029136	–	–	–
	2	B	PP74	*Pediococcus parvulus*	99.74 %	NR_113922	–	–	–
		B	PE76	*Pediococcus ethanolidurans*	99.81 %	NR_043291	–	–	–

(*continued on next page*)

Table 5 (*continued*)

Producer	Batch	Isolation source	Isolate code	Species	% Identity[a]	Accession number[b]	*hdcA gene*	EPS production Sucrose-dependent	Sucrose-independent
		C	PE77	*Pediococcus ethanolidurans*	99.35 %	NR_043291	+	–	–
		B	PP80	*Pediococcus parvulus*	94.36 %	NR_029136	–	–	–
		B	PE82	*Pediococcus ethanolidurans*	99.72 %	NR_043291	+	+	+
		C	LP83	*Lactiplantibacillus plantarum* group	99.26 %	NR_104573	–	–	–
		C	PE84	*Pediococcus ethanolidurans*	99.44 %	NR_043291	–	–	–

-, negative; +, positive colonies.
n.d., not determined.
[a] Percentage of identical nucleotides in the sequence obtained from the lactic acid bacteria strains and the sequence of the closest relative found in the GenBank database.
[b] Accession number of the sequence of the closest relative found by BLAST search.

associated with photooxidation during brining and storage, resulting in yellowing of the colour. The cucumber color parameters differed mainly between producers, thus suggesting slight differences in the raw materials or fermentation conditions, according to producer.

The hardness measured by puncture test differed for all the producers, but not between batches of the same producer, thus attesting the consistency of the production process within each producer. The detected differences may arise from the maturity stage of the cucumbers used, the fertilization method as well as particular composition of brine or processing temperatures applied in cucumbers preparation.

Interestingly, the results of brine viscosity were in accordance with those reported by Fasina et al. (2002), and were generally close to the values reported for water, except for samples of producer 4 that showed values up to 1.150 ± 0.056 mPa*s. This latter result can origin from the presence of dissolved polysaccharides or proteins, being the indication of some shifting in fermentation or even spoilage, as reducing sugars also indicated the highest values for samples of producer 4.

The results of microbiological analyses highlighted metabolically active microbial groups in both the brine and cucumber samples herein studied. It is noteworthy that, as reported by Mattos et al. (2005), microorganisms occurring in cucumbers are mainly located on the exocarp of the fruit; this evidence likely explains the difference between microbial counts of cucumbers and brine, in which microorganisms survive as planktonic cells. Of note, the differences between viable counts of presumptive lactococci, presumptive thermophilic cocci, presumptive lactobacilli, coagulase-negative cocci, and eumycetes in brine and in cucumbers were in accordance with the data reported by Correa-Galeote et al. (2022) for olives fermented in salt brine. Interestingly, Correa-Galeote et al. (2022) observed that the NaCl concentration in brine was able to modulate the size and the structure of the dominant bacterial genera (Correa-Galeote et al., 2022). Of note, the high counts of protechnological microorganisms in the brine samples herein analyzed confirm the exploitability of this matrix as source of natural starter cultures in fermentation of cucumbers carried out via back-slopping technique.

As for lactic acid bacteria (lactococci, thermophilic cocci, and lactobacilli), the counts detected in the analyzed samples were in accordance with those reported by Stoll et al. (2020) and Pérez-Díaz et al. (2017) in fermented cucumbers at the end of fermentation, with values up to 6–7 log cfu g^{-1}. In raw cucumbers, lactic acid bacteria represent a minor part of the autochthonous microbiota, with counts of ~ 2 log cfu g^{-1} (Franco et al., 2017); however, as soon as brine is added, their number steadily increases and fermentation progresses, since lactic acid bacteria are able to survive in this extreme environment (Franco et al., 2017). It is noteworthy that, as reported by Stoll et al. (2020), many lactic acid bacteria species are described as halophilic or halotolerant, being able to grow in foods containing 2–4 % (e.g., lactococci) to 10 % NaCl (e.g., *Weissella* spp.); hence, the high counts detected in the samples herein studied suggest the presence of well-adapted halotolerant lactic acid bacteria species. In fermented cucumbers, the metabolic activity of lactic acid bacteria produces organic acids, thus leading to the inhibition of spoilage or pathogenic bacteria naturally occurring in the raw materials (Franco et al., 2017). In more detail, in cucumber fermentation, homofermentative species produce lactic acid from fructose or glucose, whereas heterofermentative lactic acid bacteria use the phosphoketolase pathway to produce lactic acid, acetic acid, ethanol, and carbon dioxide (CO_2) (Franco et al., 2017). During cucumber fermentation, lactic acid bacteria can also produce EPS with inhibitory activity against some major foodborne pathogens as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp., and fungal species (e.g., aspergilli) (Kavitake et al., 2023).

Regarding coagulase-negative cocci, to the author's knowledge, there is a lack of knowledge of the occurrence of this heterogeneous group of bacteria in fermented cucumbers. However, coagulase-negative cocci (namely, *Staphylococcus saprophyticus*, *Staphylococcus succinus*, and *Staphylococcus xylosus*) have already been isolated in fermented foods of vegetable origin (e.g., fermented soybean) (Jeong et al., 2016). Further research is needed to better understand the role of these microorganisms in fermented cucumbers.

As for enterococci, *Enterococcus* species have already been isolated from fresh cucumbers by Pérez-Díaz et al. (2019). Interestingly, in the present study, samples with enterococci counts > 1 log cfu g^{-1} were those with the lowest salt content (producer 3), thus suggesting the presence of *Enterococcus* species whose growth benefited from the lower quantity of salt in the same samples (Heo et al., 2019).

As for the counts of Enterobacteriaceae, all the analyzed samples revealed counts below 1 log cfu g or mL^{-1}. It is noteworthy that raw cucumbers can be naturally contaminated by Enterobacteriaceae, with counts up to 4 log cfu g^{-1} (Franco et al., 2017). Although members of this bacterial family can be the causative agents of cucumber spoilage at the beginning of natural fermentation (e.g., the CO_2-mediated bloater defect) (Zhai & Pérez-Díaz, 2021), Enterobacteriaceae are not acid-resistant; hence, a rapid drop in pH caused by the lactic acid bacteria metabolic activity usually avoids Enterobacteriaceae growth in the fermenting matrix. Moreover, the presence of Enterobacteriaceae in ready-to-eat food is an indicator of poor hygiene during production. Hence, the low counts revealed in the samples herein analyzed suggest that good manufacturing practices were applied during the manufacture of fermented cucumbers.

In the samples herein analyzed, from very low (<1 log cfu g^{-1}) to high counts of eumycetes (yeasts and molds) were detected. However, the highest loads of eumycetes detected in the present study were in accordance with the counts reported by Pérez-Díaz et al. (2019), that attested at 3.6 log cfu g^{-1} in cucumbers. As reported by Franco & Pérez-Díaz (2012), in fermented cucumbers, yeasts (e.g., *Pichia manshurica* and *Issatchenkia occidentalis*) are naturally present in the cucumber fruit and can be the causative agents of spoilage during bulk storage due to the production of secondary metabolites as propionic and/or butyric acids. In fermented cucumbers, yeasts can utilize organic acids produced by lactic acid bacteria, with a subsequent increase in the pH of the brine to

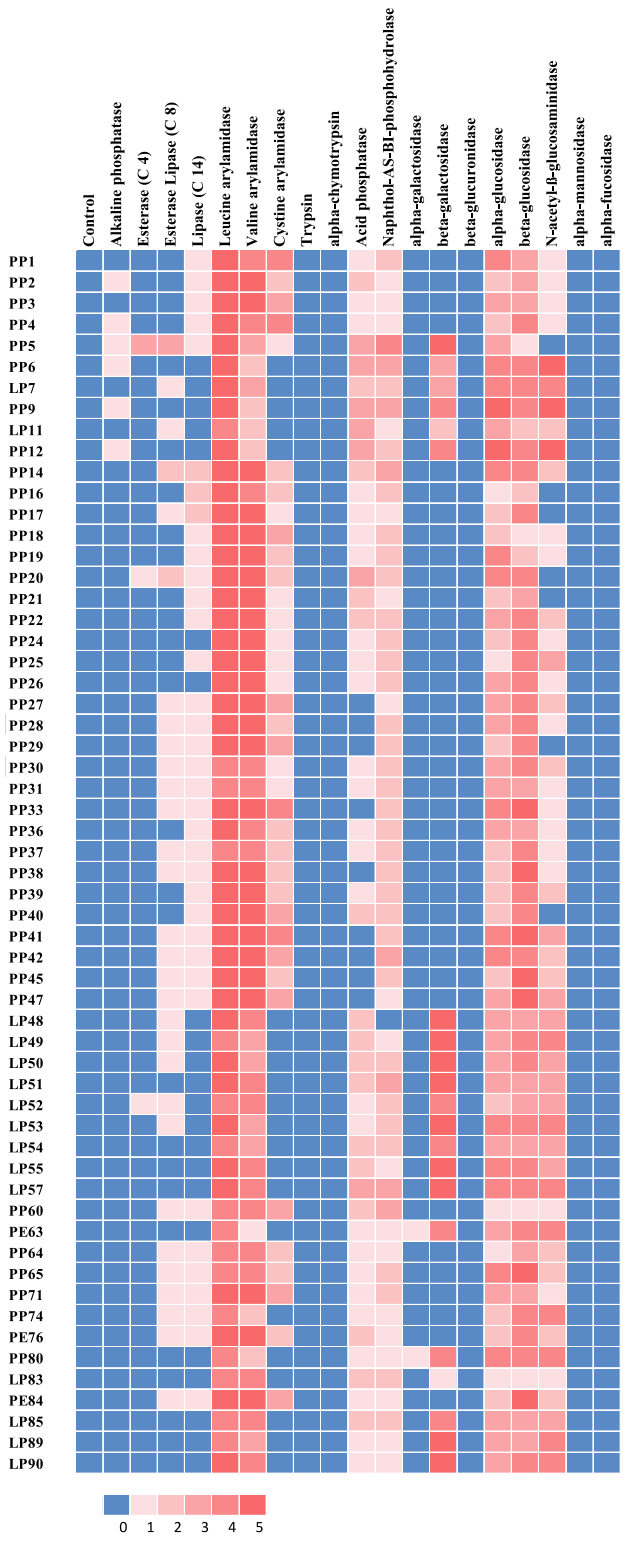

Fig. 6. Heat map representing the results of semi-quantitative assessment of enzymatic activities of lactic acid bacteria isolated from cucumber and brine samples.For each enzymatic reaction, a value ranging from 0 to 5 was assigned, corresponding to the colors developed: 0 corresponds to a negative reaction (dark blue dots), 5 to a reaction of maximum intensity (dark red dots), and values 1, 2, 3 or 4 are intermediate reactions depending on the level of intensity (3, 4, or 5 being considered as positive reactions). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

values above 4.5 (Franco & Pérez-Díaz, 2012). Hence, the presence of yeasts is detrimental to the quality and safety of the product.

To the authors' knowledge, only a few papers dealing with metataxonomic analysis of fermented cucumbers are available in the scientific literature. In the present study, the metataxonomic analysis confirmed the neat separation of the microbiological features of brine and cucumbers, irrespective of the producer.

Among the dominant microbial taxa in cucumbers, *Lactococcus* and *Streptococcus* were detected.

In more detail, the occurrence of *Lactococcus* confirms the high counts of lactococci detected through viable counting. *Lactococcus lactis* has already been detected by Pérez-Díaz et al. (2017) in the cover brine of fermented cucumbers produced at commercial scale, although isolates of *L. lactis* were detected only during the early stage of fermentation (from 1 to 7 days), thus suggesting that this lactic acid bacteria species might not be able to survive as cucumber fermentation progresses. Interestingly, the bacteriocin-producing *Lactococcus garvieae* strain, active against *E. coli* and *S. aureus*, has already been isolated by Gao et al. (2015) in traditional Chinese fermented cucumbers, thus suggesting the potential application of this lactic acid bacteria species for the biopreservation of fermented foods.

To the authors' knowledge, there is a lack of data in the scientific literature on the occurrence of *Streptococcus* species in fermented cucumbers for further comparison of results. However, the high occurrence of *Streptococcus* in the cucumber samples herein analyzed is consistent with the high loads of presumptive thermophilic cocci detected through viable counting. *Streptococcus* has recently been identified as one of the dominant species in pickled swamp fruit and *jeruk*, a type of fermented pickle locally made in Malaysia (Ajibola et al., 2023). Further research is needed to better elucidate the function of streptococci in cucumber fermentation.

In the brine samples analyzed in the present study *Leuconostoc*, *Secundilactobacillus*, *Lentilactobacillus Lactiplantibacillus*, and *Pediococcus* were the dominant taxa.

Leuconostocs are hetero-fermentative lactic acid bacteria commonly found in fermented vegetables as kimchi and sauerkraut (Eom et al., 2007). In fermented vegetables, *Leuconostoc* species contribute to the development of flavor compounds through the synthesis of organic acids, alcohols, CO_2, and mannitol (Eom et al., 2007). The presence of *Leuconostoc* has already been reported by Kao et al. (2023) among the dominant lactic acid bacteria in naturally fermented cucumbers, with the highest relative abundance during the last fermentation period (aging of fermented cucumbers). However, Singh & Ramesh (2008) reported the presence of *Leuconostoc* isolates only during the early stage of cucumber fermentation. The apparently controversial results reported by Kao et al. (2023) and Singh & Ramesh (2008) clearly highlight the importance of combining the results of culture-based methods (microbial isolation and identification) with those of culture-independent methods. Indeed, if metataxonomic analysis can provide a detailed overview of the microbial taxa occurred along the entire fermentation process, the isolation of cultivable microorganisms can provide information on the metabolically active microbiota at a specific time of production.

As for *Secundilactobacillus*, the occurrence of this genus of hetero-fermentative lactic acid bacteria in fermented cucumbers has already been reported by Świder et al. (2023), although as a minority taxon. To the authors' knowledge, there is a lack of studies regarding the presence of *Secundilactobacillus* in fermented vegetables; however, *Secundilactobacillus collinoides,* recently isolated from spoiled ropy basque cider, proved to be able to produce heteropolysaccharides with a potentially beneficial role (Puertas et al., 2023). Interestingly, a novel pentose-fermenting and GABA-producing *Secundilactobacillus* species (namely, *Secundilactobacillus angelensis* sp. nov) was recently isolated from a solid-state fermented *zha-chili*, a traditional Chinese fermented food produced with crushed rice/corn flour, fresh chili, and salt (Zhang et al., 2022), suggesting the adaptation of *Secundilactobacillus* to salty foods.

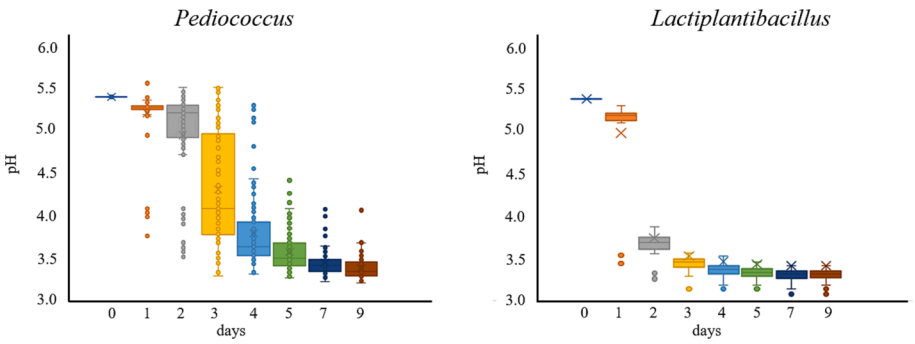

Fig. 7. Box plots summarizing the results of acidification activity of isolated *Pediococcus* and *Lactiplantibacillus* species in cucumber-based growth medium from 0 to 9 days of fermentation.For each box, the bottom whisker marks the minimum value, the bottom of the box marks the location of first quartile, the line within the box refers to the median value, the top of the box marks the location of the third quartile, the top whisker marks the maximum value in the data set, the "X" symbol marks the average value, and circles indicate the outliers.

Concerning *Lentilactobacillus*, this genus of lactic acid bacteria has already been detected by Świder et al. (2023) in fermented cucumbers. Interestingly, *Lentilactobacillus* was detected among the dominant lactic acid bacteria occurring in brine samples of fermented black olives (Penland et al., 2021) and Spanish-style green table olives (Correa-Galeote et al., 2022; Tzamourani et al., 2022), confirming the brine of fermented foods as a natural source for the isolation of this microorganism.

In the samples herein analyzed, *Lactiplantibacillus* and *Pediococcus* represented the most isolated lactic acid bacteria. It is noteworthy that the spontaneous fermentation of cucumbers represents the most used method to stabilize and preserve raw vegetables as cucumbers; notwithstanding, in vegetables of industrial significance, the interest in using selected microorganisms as starter or adjunct cultures has recently increased (Di Cagno et al., 2013). Indeed, several factors can affect the success of fermentation, thus leading to inadequate inhibition of spoilage and pathogenic microorganisms and unwanted or unexpected variations in the sensory, nutritional, and morpho-textural characteristics of the product (Di Cagno et al., 2013). Moreover, the microorganisms used as starter or adjunct cultures should also be able to avoid the occurrence of the secondary fermentation during bulk storage of cucumbers, which leads to lactic acid loss, pH increase, and discarding of fermented fruits (Franco et al., 2012). Among the potential causative agents of secondary fermentation, *Pichia manshurica* and *Issatchenkia occidentalis* were identified among the yeasts, whereas *Lactobacillus buchneri*, *Clostridium* sp., and *Pediococcus ethanolidurans* were identified as potentially relevant in different stages of the secondary fermentation (Franco et al., 2012; Medina et al., 2016).

In fermented cucumbers, species of *Lactobacillus* and *Pediococcus* have already been detected as dominant genera emerging during late stages of fermentation (Singh & Ramesh, 2008) and represent the most suitable lactic acid bacteria to be used as starter cultures, depending on their metabolism (Behera et al., 2020). Of note, *L. plantarum* is pivotal in completing the final stage of fermentations due to its acid tolerance, that is higher than that of other lactic acid bacteria (Lu et al., 2003).

In the present study, 40 *P. parvulus*, 5 *P. ethanolidurans*, and 15 *L. plantarum* group were isolated.

Regarding the presence of the *hdcA* gene in two *P. ethanolidurans* isolates (PE77 and PE82), it is noteworthy that Świder et al. (2023) have recently used a strain of *P. ethanolidurans* (namely KKP 2998) as a positive control for *hdcA* gene detection in lactic acid bacteria to be used in cucumber fermentation, thus confirming the presence of this target gene in the species *P. ethanolidurans*. To the authors' knowledge, a paucity of studies on histamine production by *P. ethanolidurans* is available in the scientific literature for further comparison of data, notwithstanding, pediococci are included among the histamine-producing lactic acid bacteria together with *Oenococcus* and *Lactobacillus* (Lucas et al., 2008); hence, their use as starter or adjunct cultures should be carefully evaluated.

Regarding EPS production, only a few isolates of *P. parvulus* and *L. plantarum* group showed the ability to produce sucrose-dependent or -independent EPS. EPS produced by lactic acid bacteria have been classified into two main types such as homopolysaccharides (fructan, galactan, glucan, and mannan) and heteropolysaccharides (constituted by different sugar units in the structure) (Kavitake et al., 2023). EPS are microbial secondary metabolites with potential antioxidant, antimicrobial, anti-biofilm, antiviral, and cryoprotective activities (Kavitake et al., 2023).

The ability of producing EPS by *P. parvulus* strains has already been reported by Velasco et al. (2006). In more detail, these authors observed that EPS production by *P. parvulus* was not directly linked to its growth, since production was observed also during the stationary phase, provided that glucose was present in the growth medium (Velasco et al., 2006). Moreover, Coulon et al. (2012) reported that some *P. parvulus* strains were able to synthesize a β-glucan, with negative effects on wine quality as a ropy texture.

As for EPS-producing *L. plantarum* group isolates, the metabolic feature of EPS production by *L. plantarum* strains is widely acknowledged (Jiang & Yang, 2018). Interestingly, EPS-producing *L. plantarum* strains have already been isolated from fermented cucumbers, pickled vegetables, and kimchi (Ahmed et al., 2021; Seo et al., 2015; Zhou et al., 2016), as well as in rotten jackfruit (Dilna et al., 2015).

Regarding enzymatic activities of the isolates, to the authors' knowledge, there is little information in the scientific literature on the activity of proteases from *P. parvulus*. However, a strong activity of leucine arylamidase and valine arylamidase has already been observed in *Pediococcus* species (e.g., *Pediococcus acidilactici*) (Jang et al., 2021). Moreover, leucine arylamidase and valine arylamidase have been observed in *L. plantarum* strains by Abarquero et al. (2023). Of note, the activity of the bacterial aminopeptidases detected in the isolates herein studied could result in efficient release of amino acids from peptides, thus contributing to the development of the flavor of fermented cucumbers.

Most of the isolates showed beta-glucosidase activity, which is pivotal for cellulose hydrolysis (Fernandes et al., 2022). Of note, highly efficient beta-glucosidase-producing lactic acid bacteria have already been isolated from Korean kimchi by Jang et al. (2010).

It is noteworthy that all the isolates showed the absence of beta-glucuronidase whose activity could lead to the development of carcinogenic compounds in the colon of the consumer, thus also improving the possibility of cancer induction (Tasdemir & Sanlier, 2020).

Although most isolates exhibited a weak N-acetyl-β-glucosaminidase activity, 11 isolates (PP6, LP7, LP9, PP12, LP49, LP53, LP57, PP74, PP80, LP89, and LP90) showed a strong activity for this enzyme. N-acetyl-β-glucosaminidase is a hydrolase whose activity breaks down oligosaccharides and is correlated with carbohydrate catabolism (Sirini

Table 6
Volatile organic compounds (VOCs) identified by solid phase microextraction/gas chromatography-mass spectrometry in cucumber and brine samples from 4 different producers.

RI	Compounds	Producer 1				Producer 2				Producer 3				Producer 4			
		Cucumber batch1	batch2	Brine batch1	batch2	Cucumber batch1	batch2	Brine batch1	batch2	Cucumber batch1	batch2	Brine batch1	batch2	Cucumber batch1	batch2	Brine batch1	batch2
Aldehydes																	
670	acethaldehyde	237.74 ± 1.76	177.87 ± 22.36	147.32 ± 4.74	160.14 ± 14.19	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
801	propanal	144.07 ± 4.54	127.39 ± 7.35	113.16 ± 6.13	106.61 ± 3.15	112.00 ± 6.86	98.75 ± 4.76	nd	nd	nd	nd	0.97 ± 0.03	1.29 ± 0.04	12.802 ± 1.17	17.26 ± 0.67	3.07 ± 0.26	3.25 ± 0.22
1003	pentanal	583.34 ± 37.23	558.63 ± 64.95	236.42 ± 8.85	176.80 ± 7.72	276.93 ± 18.86	232.60 ± 19.42	133.39 ± 0.35	119.70 ± 6.78	354.33 ± 22.87	10.92 ± 0.16	2.12 ± 0.06	nd	92.15 ± 6.26	132.39 ± 14.96	198.91 ± 3.76	195.74 ± 2.86
1121	hexanal	47.06 ± 4.38	28.37 ± 2.18	26.67 ± 0.38	24.24 ± 0.79	55.83 ± 4.91	54.74 ± 0.78	15.49 ± 1.52	20.70 ± 1.79	26.50 ± 0.01	21.10 ± 5.88	6.66 ± 0.46	7.50 ± 0.65	40.63 ± 1.89	44.06 ± 1.47	10.49 ± 0.25	14.21 ± 0.71
1642	4-methyl-benzaldehyde	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.77 ± 0.03	1.12 ± 0.01
1513	benzaldehyde	48.87 ± 0.78	28.86 ± 0.50	48.31 ± 4.11	33.52 ± 3.69	17.13 ± 1.27	20.39 ± 0.18	7.39 ± 0.17	7.44 ± 0.36	10.13 ± 0.25	8.47 ± 1.08	4.35 ± 0.09	3.56 ± 0.09	7.67 ± 0.11	12.61 ± 0.36	9.29 ± 0.08	13.77 ± 0.67
	Total	**1061.08**	**921.13**	**571.86**	**501.31±**	**461.89**	**406.48**	**156.28**	**147.84**	**390.97**	**40.49**	**14.09**	**12.36**	**153.27**	**206.33**	**222.53**	**228.09**
Ketones																	
814	2-propanone	96.52 ± 6.51	75.31 ± 8.71	120.48 ± 8.95	72.82 ± 0.98	61.36 ± 3.45	85.41 ± 0.24	42.14 ± 6.32	57.20 ± 0.30	58.85 ± 4.71	37.51 ± 3.47	69.52 ± 7.86	58.06 ± 0.46	91.60 ± 6.17	135.55 ± 0.50	89.67 ± 9.10	71.25 ± 6.55
884	2-butanone	nd	99.12 ± 2.43	18.68 ± 0.30	21.86 ± 1.49	98.37 ± 5.62	128.53 ± 5.25	157.67 ± 14.48	104.14 ± 2.04	75.14 ± 4.14	31.61 ± 2.94	91.09 ± 4.59	55.37 ± 0.17	20.37 ± 0.59	15.92 ± 1.78	31.52 ± 0.73	30.80 ± 4.70
1048	1-penten-3-one	43.80 ± 1.47	37.70 ± 1.62	10.09 ± 0.69	11.72 ± 1.34	27.93 ± 2.88	27.09 ± 1.20	nd	nd	33.60 ± 2.21	13.68 ± 1.44	12.55 ± 0.22	16.40 ± 0.05	13.95 ± 1.36	14.12 ± 0.24	3.03 ± 0.57	3.14 ± 0.31
1073	2.3-pentanedione	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	6.82 ± 0.12	5.92 ± 0.36	5.67 ± 0.32	8.87 ± 0.39
1206	4-methyl-2-heptanone	nd	nd	nd	nd	nd	nd	0.53 ± 0.01	0.51 ± 0.02	nd	nd	1.61 ± 0.02	1.41 ± 0.01	nd	nd	nd	nd
1298	1-octen-3-one	29.27 ± 2.72	15.22 ± 1.82	nd	nd	26.24 ± 0.43	32.96 ± 2.32	4.96 ± 0.35	5.92 ± 0.22	nd	3.50 ± 0.29	nd	0.59 ± 0.02	4.37 ± 0.40	4.10 ± 0.12	1.58 ± 0.03	1.13 ± 0.05
1322	2.3-octanedione	10.42 ± 0.44	15.59 ± 1.75	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1327	6-methyl-5-hepten-2-one	9.68 ± 0.12	7.40 ± 1.01	nd	nd	5.75 ± 0.28	5.41 ± 0.41	nd	nd	3.64 ± 0.39	2.60 ± 0.09	nd	1.95±	nd	nd	nd	nd
	Total	**189.69**	**250.34**	**149.25**	**106.40±**	**219.64**	**279.40**	**205.30**	**167.78**	**171.23**	**88.90**	**174.78**	**133.79**	**137.12**	**175.61**	**131.46**	**115.19**
	Esters																
821	methyl acetate	112.10 ± 4.5	109.52 ± 0.82	134.45 ± 8.51	115.77 ± 2.67	36.48 ± 4.51	35.07 ± 4.04	17.28 ± 1.74	14.83 ± 0.45	36.09 ± 0.94	13.61 ± 0.42	112.59 ± 12.09	116.04 ± 13.61	108.02 ± 0.73	116.80 ± 2.46	104.26 ± 8.73	100.53 ± 7.26
870	ethyl acetate	239.69 ± 7.25	193.56 ± 16.30	142.80 ± 2.72	184.36 ± 4.05	174.02 ± 19.16	142.40 ± 4.00	205.06 ± 15.53	162.65 ± 23.89	220.36 ± 14.99	95.45 ± 5.22	140.15 ± 7.75	167.11 ± 15.68	116.58 ± 1.36	99.12 ± 2.95	130.73 ± 9.18	105.52 ± 3.24
953	propyl acetate	nd	nd	nd	nd	211.13 ± 12.47	187.38 ± 4.72	102.48 ± 1.78	88.51 ± 8.72	nd	292.70 ± 20.24	158.02 ± 3.90	235.60 ± 0.95	367.04 ± 4.16	531.32 ± 38.33	186.86 ± 24.89	283.59 ± 3.90
1137	isoamyl acetate	34.47 ± 2.98	31.27 ± 3.75	13.51 ± 0.50	20.92 ± 1.06	nd	nd	nd	nd	4.91 ± 0.66	2.36 ± 0.04	1.79 ± 0.03	1.67 ± 0.02	9.36 ± 0.13	11.91 ± 1.08	3.24 ± 0.19	3.60 ± 0.35
1311	methyl lactate	nd	nd	4.11 ± 0.23	4.26 ± 0.37	3.18 ± 0.06	4.07 ± 0.13	5.88 ± 0.78	8.55 ± 0.22	6.24 ± 0.64	5.40 ± 0.44	2.85 ± 0.06	4.37 ± 0.08	6.77 ± 0.05	4.62 ± 0.06	4.28 ± 0.39	2.78 ± 0.10
1339	ethyl lactate	36.81 ± 5.3	22.68 ± 2.40	50.54 ± 6.57	32.75 ± 3.87	60.45 ± 8.02	89.87 ± 7.19	83.60 ± 10.44	104.40 ± 3.27	38.03 ± 0.67	25.79 ± 0.18	15.74 ± 0.20	21.48 ± 0.22	30.04 ± 0.59	31.09 ± 0.72	36.50 ± 1.76	45.31 ± 3.50
	Total	**423.07**	**357.02**	**345.41**	**358.07**	**485.26**	**458.80**	**414.30**	**378.95**	**305.62**	**435.30**	**431.15**	**546.27**	**637.80**	**794.86**	**465.86**	**541.32**
Alcohols																	
940	ethanol	2394.32 ± 1.10	1838.59 ± 65.65	2821.46 ± 329.36	1820.58 ± 81.54	1557.08 ± 141.95	1660.56 ± 181.06	1393.43 ± 118.00	1487.41 ± 40.11	3084.99 ± 18.63	2020.81 ± 130.14	2074.73 ± 85.04	2080.33 ± 22.12	1265.24 ± 2.50	963.86 ± 72.71	1102.62 ± 34.13	1005.80 ± 12.88
1053	1-propanol	382.76 ± 32.26	529.25 ± 57.23	433.79 ± 30.77	683.46 ± 41.45	469.56 ± 36.39	486.42 ± 57.30	463.96 ± 48.67	441.04 ± 19.74	88.77 ± 9.92	nd	nd	nd	1934.72 ± 26.34	3217.36 ± 207.90	2010.09 ± 126.47	3208.96 ± 191.61

(*continued on next page*)

Table 6 (*continued*)

RI	Compounds	Producer 1				Producer 2				Producer 3				Producer 4			
1173	1-butanol	nd	nd	nd	nd	nd	nd	nd	nd	31.16 ± 0.93	24.26 ± 3.42	15.20 ± 1.61	17.48 ± 0.98	58.38 ± 2.77	71.60 ± 3.29	52.49 ± 4.26	69.86 ± 6.57
1187	1-penten-3-ol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	7.65 ± 0.57	8.19 ± 0.20	2.67 ± 0.16	3.08 ± 0.18
1217	isoamyl alcohol	143.67 ± 0.83	206.70 ± 1.22	50.01 ± 3.73	248.63 ± 14.26	31.62 ± 0.34	38.29 ± 3.17	86.99 ± 6.42	60.93 ± 1.02	104.45 ± 15.61	110.83 ± 2.40	58.87 ± 0.14	98.94 ± 2.03	96.80 ± 5.87	85.63 ± 10.53	103.01 ± 10.17	76.91 ± 4.26
1232	1-pentanol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	19.08 ± 0.36	18.27 ± 0.65	nd	nd	17.67 ± 0.01	11.92 ± 0.03
1305	2-ethyl-1-butanol	nd	nd	5.23 ± 0.54	4.63 ± 0.60	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1360	1-hexanol	38.44 ± 3.00	36.20 ± 1.37	30.24 ± 0.39	47.24 ± 3.70	nd	nd	59.52 ± 1.44	49.74 ± 1.01	32.95 ± 0.18	17.19 ± 0.90	20.11 ± 0.47	15.12 ± 1.68	44.89 ± 3.83	40.82 ± 4.42	42.86 ± 0.87	27.63 ± 1.54
1386	3-hexen-1-ol, (Z)-	23.10 ± 0.78	21.37 ± 0.77	33.42 ± 2.63	32.87 ± 0.96	25.11 ± 1.22	24.26 ± 1.96	29.33 ± 4.37	34.12 ± 1.73	11.99 ± 0.54	5.84 ± 0.08	3.97 ± 0.08	2.43 ± 0.12	3.60 ± 0.21	4.05 ± 0.96	3.94 ± 0.46	4.42 ± 0.08
1453	1-octen-3-ol	nd	nd	nd	nd	nd	nd	1.14 ± 0.26	1.22 ± 0.16	2.41 ± 0.07	2.21 ± 0.06	nd	0.67±	4.93 ± 0.25	3.10 ± 0.32	1.07 ± 0.06	1.08 ± 0.05
1489	2 ethyl hexanol	65.31 ± 4.13	45.96 ± 1.89	56.34 ± 2.20	40.68 ± 1.52	40.88 ± 2.66	30.42 ± 0.83	80.60 ± 9.84	54.48 ± 0.32	7.14 ± 0.72	7.94 ± 0.48	4.66 ± 0.47	6.41 ± 0.68	2.61 ± 0.08	3.05 ± 0.26	107.31 ± 9.16	110.77 ± 0.04
1838	benzenemethanol	2.75 ± 0.01	3.21 ± 0.41	3.32 ± 0.23	5.51 ± 0.03	5.65 ± 0.10	3.15 ± 0.15	6.04 ± 0.42	6.36 ± 0.13	1.61 ± 0.10	1.43 ± 0.05	0.65 ± 0.01	0.98 ± 0.01	4.04 ± 0.51	3.63 ± 0.09	2.50 ± 0.16	1.90 ± 0.09
1871	phenylethyl alcohol	10.83 ± 0.27	10.13 ± 0.14	8.07 ± 0.04	12.43 ± 0.85	3.82 ± 0.26	3.19 ± 0.13	3.57 ± 0.11	3.64 ± 0.86	1.81 ± 0.01	0.68 ± 0.01	nd	nd	1.44 ± 0.09	1.70 ± 0.08	1.14 ± 0.02	1.63 ± 0.05
	Total	**3061.18**	**2691.41**	**3441.89**	**2896.02**	**2133.73**	**2246.28**	**2124.59**	**2138.93**	**3367.27**	**2191.20**	**2197.27**	**2240.63**	**3424.30**	**4402.99**	**3447.36**	**4523.96**
Acids																	
1448	acetic acid	905.07 ± 22.68	1265.04 ± 170.48	897.76 ± 30.27	1127.99 ± 0.08	1664.45 ± 128.37	1236.73 ± 11.72	2273.14 ± 130.99	1519.87 ± 191.10	1008.43 ± 85.92	878.44 ± 76.91	550.71 ± 24.32	467.27 ± 37.83	1344.44 ± 80.33	1504.08 ± 188.01	1096.10 ± 70.14	1090.82 ± 140.85
1527	propanoic acid	46.17 ± 1.20	60.01 ± 5.16	34.04 ± 1.02	47.72 ± 1.23	25.50 ± 1.48	32.41 ± 0.33	28.40 ± 3.60	29.08 ± 0.96	16.84 ± 0.05	16.60 ± 1.12	7.08 ± 0.12	11.36 ± 0.03	73.42 ± 14.63	70.80 ± 2.06	460.71 ± 14.52	580.48 ± 11.85
1615	butanoic acid	nd	nd	nd	nd	nd	nd	nd	nd	2.23 ± 0.12	2.13 ± 0.06	1.08 ± 0.02	0.87±	3.46 ± 0.27	3.91 ± 0.03	1.77 ± 0.08	2.89 ± 0.10
1816	hexanoic acid	3.05 ± 0.19	2.78 ± 0.24	2.86 ± 0.05	4.47 ± 0.77	2.39 ± 0.04	2.04 ± 0.24	2.28 ± 0.19	1.32 ± 0.06	1.85 ± 0.14	1.08 ± 0.04	nd	nd	1.38 ± 0.04	1.87 ± 0.05	1.49 ± 0.21	2.09 ± 0.07
1550	isobutanoic acid	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.94 ± 0.06	4.08 ± 0.38	10.72 ± 0.24	13.71 ± 0.78
1724	pentanoic acid	4.18 ± 0.17	5.16 ± 0.44	1.71 ± 0.03	1.34 ± 0.05	nd	nd	nd	nd	nd	nd	nd	nd	0.69 ± 0.01	0.88 ± 0.11	2.02 ± 0.04	1.37 ± 0.05
2034	octanoic Acid	nd	0.32 ± 0.04	0.87 ± 0.17	0.94 ± 0.14	nd	nd	nd	nd	nd	8.39 ± 0.35	5.42 ± 0.05	8.02 ± 0.02	0.88 ± 0.01	0.92 ± 0.07	0.38 ± 0.00	0.59 ± 0.04
	Total	**958.47±**	**1333.31**	**937.24**	**1182.45**	**1692.34**	**1271.18**	**2303.82**	**1550.28**	**1029.35**	**906.64**	**564.29**	**487.52**	**1427.20**	**1586.54**	**1573.18**	**1691.96**
Terpene hydrocarbons																	
1036	α-pinene	162.45 ± 0.97	126.68 ± 4.48	17.43 ± 0.76	14.86 ± 1.26	83.36 ± 3.45	102.62 ± 8.33	15.64 ± 0.38	14.45 ± 0.80	21.03 ± 2.17	17.74 ± 1.47	5.30 ± 0.35	6.01 ± 1.05	13.47 ± 0.49	21.42 ± 1.59	1.39 ± 0.10	0.97 ± 0.04
1127	β-pinene	8.38 ± 0.03	6.96 ± 0.18	nd	nd	nd	nd	nd	nd	31.74 ± 1.69	nd	nd	nd	1.14 ± 0.01	0.75 ± 0.07	nd	nd
1147	δ-3-carene	nd	nd	nd	nd	nd	nd	nd	nd	3.06 ± 0.30	2.10 ± 0.19	nd	nd	0.80 ± 0.01	0.95 ± 0.06	nd	nd
1158	α-phellandrene	1807.19 ± 5.98	1126.48 ± 49.02	16.09 ± 0.19	25.73 ± 1.53	426.22 ± 3.23	273.06 ± 16.37	405.54 ± 19.09	279.22 ± 21.66	nd	0 nd	nd	nd	0.53 ± 0.03	0.43 ± 0.00	nd	1.98 ± 0.10
1166	β- myrcene	197.28 ± 1.46	124.08 ± 3.46	9.58 ± 0.97	8.84 ± 1.10	nd	nd	nd	nd	43.60 ± 3.84	15.60 ± 1.77	3.27 ± 0.32	5.36±	nd	67.93 ± 10.14	nd	nd
1183	α-terpinene	172.16 ± 5.67	101.61 ± 7.08	25.77 ± 0.73	14.83 ± 0.82	68.08 ± 0.63	50.55 ± 2.56	45.93 ± 6.47	36.54 ± 0.44	nd	nd	nd	nd	nd	nd	nd	nd
1191	limonene	3657.60 ± 24.05	4239.55 ± 250.49	24.68 ± 0.97	34.04 ± 0.14	2100.25 ± 97.14	1441.99 ± 9.13	57.27 ± 2.84	52.46 ± 3.36	436.82 ± 27.43	211.98 ± 19.10	10.15 ± 0.16	13.63 ± 0.42	28.70 ± 1.47	31.30 ± 0.70	15.16 ± 0.80	14.85 ± 0.15

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Table 6 (*continued*)

RI	Compounds	Producer 1				Producer 2				Producer 3				Producer 4			
1205	β-phellandrene	567.53 ± 36.62	470.15 ± 1.22	56.02 ± 0.07	28.85 ± 0.84	1.78 ± 0.03	2.72 ± 0.35	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1254	o-cymene	5143.13 ± 101.34	2861.34 ± 266.67	33.58 ± 1.35	39.03 ± 1.88	1965.99 ± 190.00	1275.83 ± 86.32	nd	nd	127.63 ± 8.18	47.94 ± 4.83	nd	nd	46.68 ± 0.76	52.72 ± 1.46	nd	nd
1268	p-cymene	837.21 ± 14.72	1071.49 ± 16.04	85.04 ± 1.79	104.71 ± 6.74	nd	nd	nd	nd	nd	nd	nd	nd	14.35 ± 0.16	14.69 ± 0.43	6.73 ± 0.40	8.57 ± 0.03
1425	dehydro p-cymene	60.44 ± 2.00	35.66 ± 1.30	21.76 ± 1.51	21.16 ± 1.25	nd	nd	nd	nd	5.71 ± 0.08	5.41 ± 0.52	nd	nd	nd	nd	nd	nd
1889	α-ionone	nd	nd	nd	nd	7.27 ± 0.57	5.04 ± 0.18	nd	nd	nd	nd	nd	nd	0.46 ± 0.04	0.54 ± 0.07	0.45 ± 0.02	0.50 ± 0.03
	Total	**12613.36**	**10164.00 ±**	**289.96**	**292.05**	**4652.95**	**3151.81**	**524.37**	**382.66**	**669.59**	**300.78±**	**18.72**	**25.00**	**106.13**	**190.73**	**23.73**	**26.87**
Oxygenated terpenes																	
1203	eucalyptol	nd	nd	nd	nd	nd	nd	41.42 ± 4.47	42.74 ± 4.47	731.98 ± 16.70	246.26 ± 18.65	351.13 ± 11.22	257.81 ± 24.78	66.21 ± 2.86	92.41 ± 6.60	72.05 ± 7.51	63.97 ± 1.20
1537	linalool	198.65 ± 1.35	168.10 ± 3.39	131.57 ± 5.14	182.27 ± 4.00	421.89 ± 21.83	322.84 ± 21.57	223.17 ± 0.36	270.95 ± 1.64	105.07 ± 4.35	87.35 ± 2.41	49.12 ± 1.87	71.56 ± 6.97	46.30 ± 0.02	53.64 ± 0.27	12.07 ± 0.06	16.77 ± 0.83
1546	2-cyclohexen-1-ol, 1-methyl-4-(1-methyl-ethyl)-. trans-	16.23 ± 0.08	13.71 ± 1.67	10.89 ± 0.53	14.20 ± 1.40	34.52 ± 3.94	40.51 ± 3.90	5.84 ± 0.29	6.08 ± 0.15	nd	nd	nd	nd	nd	nd	nd	nd
1592	4-terpineol	4.27 ± 0.09	6.11 ± 0.44	13.36 ± 0.73	12.38 ± 0.62	22.08 ± 0.47	14.25 ± 0.34	27.37 ± 1.33	30.00 ± 3.91	6.89 ± 0.28	5.14 ± 0.70	48.89 ± 0.06	29.35 ± 2.21	5.13 ± 0.03	6.15 ± 0.72	4.36 ± 0.03	3.12 ± 0.10
1613	2-cyclohexen-1-ol, 1-methyl-4-(1-methyl-ethyl)-, cis-	14.95 ± 0.04	17.15 ± 0.17	12.39 ± 0.22	19.25 ± 1.94	36.75 ± 0.15	26.48 ± 0.36	4.79 ± 0.07	4.35 ± 0.24	nd	nd	nd	nd	nd	nd	nd	nd
1669	alpha terpineol	8.45 ± 0.13	9.49 ± 0.26	7.98 ± 0.11	9.38 ± 0.44	37.93 ± 0.96	34.46 ± 0.55	39.79 ± 1.73	40.98 ± 3.26	10.45 ± 1.16	5.48 ± 0.59	16.18 ± 0.52	10.07 ± 0.55	4.75 ± 0.21	5.25 ± 0.13	3.38 ± 0.36	5.53 ± 0.13
1702	cyclohexanol, 2-methyl-5-(1-methylethenyl)	72.28 ± 3.41	80.68 ± 10.57	45.59 ± 0.06	114.54 ± 4.18	6.10 ± 0.75	4.05 ± 0.08	3.73 ± 0.62	3.61 ± 0.35	9.11 ± 0.21	7.70 ± 0.44	3.91 ± 0.13	6.19 ± 0.74	3.29 ± 0.03	3.88 ± 0.31	3.58 ± 0.15	4.23 ± 0.41
1718	carvone	11.28 ± 0.12	13.75 ± 1.71	5.56 ± 0.53	19.75 ± 0.90	371.43 ± 4.24	359.62 ± 23.93	46.35 ± 13.07	37.16 ± 1.19	183.62 ± 6.11	151.20 ± 18.76	50.36 ± 2.97	38.76 ± 2.00	3.85 ± 0.05	4.54 ± 0.27	nd	nd
1730	cis piperitol	4.05 ± 0.01	2.91 ± 0.35	2.36 ± 0.04	4.32 ± 0.68	nd	nd	nd	nd	5.11 ± 0.36	3.03 ± 0.11	1.99 ± 0.04	2.16 ± 0.06	nd	nd	nd	nd
	carveol, dihydro-, cis-	17.21 ± 0.14	13.01 ± 1.56	8.67 ± 0.19	14.02 ± 1.61	22.70 ± 0.19	22.83 ± 0.41	16.54 ± 0.93	nd	nd	nd	nd	nd	0.62 ± 0.03	0.74 ± 0.06	0.35 ± 0.00	0.31 ± 0.03
	isodihydrocarveol	1623.40 ± 7.88	1153.46 ± 130.06	1263.54 ± 12.93	2003.38 ± 104.94	105.63 ± 2.18	83.61 ± 0.21	26.89 ± 3.38	25.37 ± 0.87	11.21 ± 0.19	9.98 ± 0.28	2.58 ± 0.25	2.69 ± 0.03	20.80 ± 2.11	18.31 ± 0.60	6.40 ± 0.18	5.48 ± 0.74
1746	trans carvyl acetate	162.01 ± 5.23	131.97 ± 0.15	131.35 ± 0.91	222.56 ± 9.66	nd	nd	nd	nd	11.77 ± 1.22	8.06 ± 1.10	4.51 ± 0.39	5.97 ± 0.26	2.75 ± 0.24	4.44 ± 0.19	1.29 ± 0.07	1.72 ± 0.16
1806	*trans*-carveol	4.36 ± 0.12	3.20 ± 0.29	2.97 ± 0.00	5.86 ± 0.62	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1594	dihydrocarvone cis 1	208.59 ± 0.33	263.87 ± 31.47	216.31 ± 15.68	190.57 ± 11.74	90.46 ± 1.38	79.62 ± 3.44	68.86 ± 10.05	68.51 ± 1.63	nd	31.16 ± 6.37	17.01 ± 0.95	20.97 ± 2.26	15.89 ± 0.17	17.14 ± 0.72	6.10 ± 0.70	5.22 ± 0.31
1602	trans dihydrocarvone	333.36 ± 3.91	350.51 ± 5.04	33.02 ± 3.96	37.90 ± 0.65	83.41 ± 10.15	45.89 ± 3.83	120.42 ± 13.31	17.33 ± 0.89	29.83 ± 0.77	21.59 ± 1.04	3.21 ± 0.15	4.17 ± 0.10	7.07 ± 0.62	7.30 ± 0.83	2.19 ± 0.06	2.04 ± 0.08
2187	thymol	0.88 ± 0.02	1.19 ± 0.01	nd	nd	1.35 ± 0.05	1.14 ± 0.02	1.02 ± 0.05	1.05 ± 0.02	nd	nd	nd	nd	nd	nd	nd	nd
2219	carvacrol	2.59 ± 0.07	2.61 ± 0.11	0.87	1.69 ± 0.21	8.36 ± 0.68	6.22 ± 0.11	1.08 ± 0.03	0.76 ± 0.02	0.78 ± 0.02	0.56 ± 0.05	0.34±	0.47 ± 0.02	0.46 ± 0.02	0.28 ± 0.03	0.38 ± 0.03	0.43 ± 0.01
2130	eugenol	nd	nd	nd	nd	nd	nd	nd	nd	10.04 ± 0.06	8.39 ± 0.35	5.42 ± 0.05	8.02 ± 0.02	0.62 ± 0.05	nd	0.18 ± 0.00	0.20 ± 0.01
	Total	**2682.57±**	**2231.71**	**1886.41**	**2852.08**	**1242.61**	**1041.52**	**627.28**	**548.90**	**1115.86**	**585.88**	**554.65**	**458.19**	**177.75**	**214.08**	**112.33**	**109.01**
Sesquiterpenes																	

(*continued on next page*)

Table 6 (*continued*)

RI	Compounds	Producer 1				Producer 2				Producer 3				Producer 4			
1573	caryophyllene	nd	nd	nd	nd	5.34 ± 0.72	7.75 ± 0.76	nd	nd	1.09 ± 0.22	0.72 ± 0.01	nd	nd	nd	nd	nd	nd
1635	a-humulene	nd	nd	nd	nd	12.18 ± 0.09	7.09 ± 0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Total	**nd**	**nd**	**nd**	**nd**	**17.52**	**14.84**	**nd**	**nd**	**1.09**	**0.72**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**
Sulfur compounds																	
1107	dimethyldisulfide	140.99 ± 4.62	134.09 ± 1.34	43.34 ± 4.46	57.90 ± 8.36	nd	nd	nd	nd	nd	31.40 ± 0.45	26.17 ± 1.13	33.79 ± 0.05	nd	nd	nd	nd
1150	allyl sulfide	408.99 ± 4.83	551.11 ± 1.09	20.55 ± 0.10	34.34 ± 0.83	nd	nd	nd	nd	69.19 ± 3.93	44.74 ± 0.30	5.49 ± 0.84	6.92 ± 0.57	nd	nd	nd	nd
1261	allyl methyl disulfide	nd	nd	nd	nd	nd	nd	nd	nd	45.68 ± 0.34	32.99 ± 3.22	2.39 ± 0.05	1.76 ± 0.07	nd	nd	nd	nd
1356	allyl isothiocyanate	nd	nd	nd	nd	58.96 ± 8.30	89.08 ± 6.13	10.24 ± 0.06	14.17 ± 1.39	80.93 ± 1.41	30.49 ± 3.12	nd	nd	7.84 ± 0.05	11.76 ± 1.53	nd	nd
1366	dimethyl trisulfide	7.07 ± 0.40	5.22 ± 0.55	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1469	allyl disulfide	208.13 ± 5.23	116.59 ± 13.16	11.75 ± 1.56	17.32 ± 0.23	nd	nd	nd	nd	73.72 ± 5.34	55.48 ± 0.50	5.24 ± 0.33	5.19±	nd	nd	nd	nd
	Total	**765.19±**	**807.00±**	**75.64**	**109.57**	**58.96**	**89.08**	**10.24**	**14.17**	**269.52**	**195.10**	**39.30**	**47.66**	**7.84**	**11.76**	**nd**	**nd**
Other																	
1420	2-methoxy-3-(1-methylethyl)-pyrazine	nd	nd	nd	nd	nd	nd	nd	nd	9.81 ± 0.92	4.68 ± 0.17	3.23 ± 0.28	3.76 ± 0.48	nd	nd	nd	nd
1475	menthofuran	13.59 ± 0.13	21.25 ± 0.19	29.54 ± 3.75	26.78 ± 0.80	13.99 ± 0.50	13.41 ± 0.08	10.63 ± 0.38	7.69 ± 0.25	nd	nd	nd	nd	1.41 ± 0.01	1.40 ± 0.05	2.57 ± 0.14	1.76 ± 0.08
1500	dill ether	2602.20 ± 151.21	1496.81 ± 65.45	1612.58 ± 12.23	1750.21 ± 15.37	654.39 ± 15.03	443.60 ± 43.63	1136.77 ± 83.22	1052.13 ± 5.42	8.92 ± 0.46	6.80 ± 0.30	2.49 ± 0.26	3.10 ± 0.48	99.72 ± 12.82	65.12 ± 1.31	6.93 ± 0.50	6.34 ± 0.54
1611	gamma butyrolactone	nd	nd	nd	nd	nd	nd	2.10 ± 0.05	1.48 ± 0.08	nd	nd	nd	nd	4.06 ± 0.14	4.09 ± 0.34	0.85 ± 0.01	1.26 ± 0.04
2180	3-ethyl-phenol	1.25 ± 0.00	1.67 ± 0.10	1.02 ± 0.06	1.81 ± 0.23	nd	nd	nd	nd	nd	1.33 ± 0.17	nd	nd	nd	nd	nd	nd
	Total	**2617.04±**	**1519.72±**	**1643.14**	**1778.80**	**668.38**	**457.01**	**1149.51**	**1061.30**	**18.74**	**12.81**	**5.71**	**6.86**	**105.19**	**70.60±**	**10.35±**	**9.36**

RI = Retention Index. identification by comparison with RI database (https://www.webbook.nist.gov); nd = not detected.
Results are expressed as RAP = Relative Peak Area (Area Peak compound/ Area Peak Internal Standard)) × 100 (RAP ± SD).

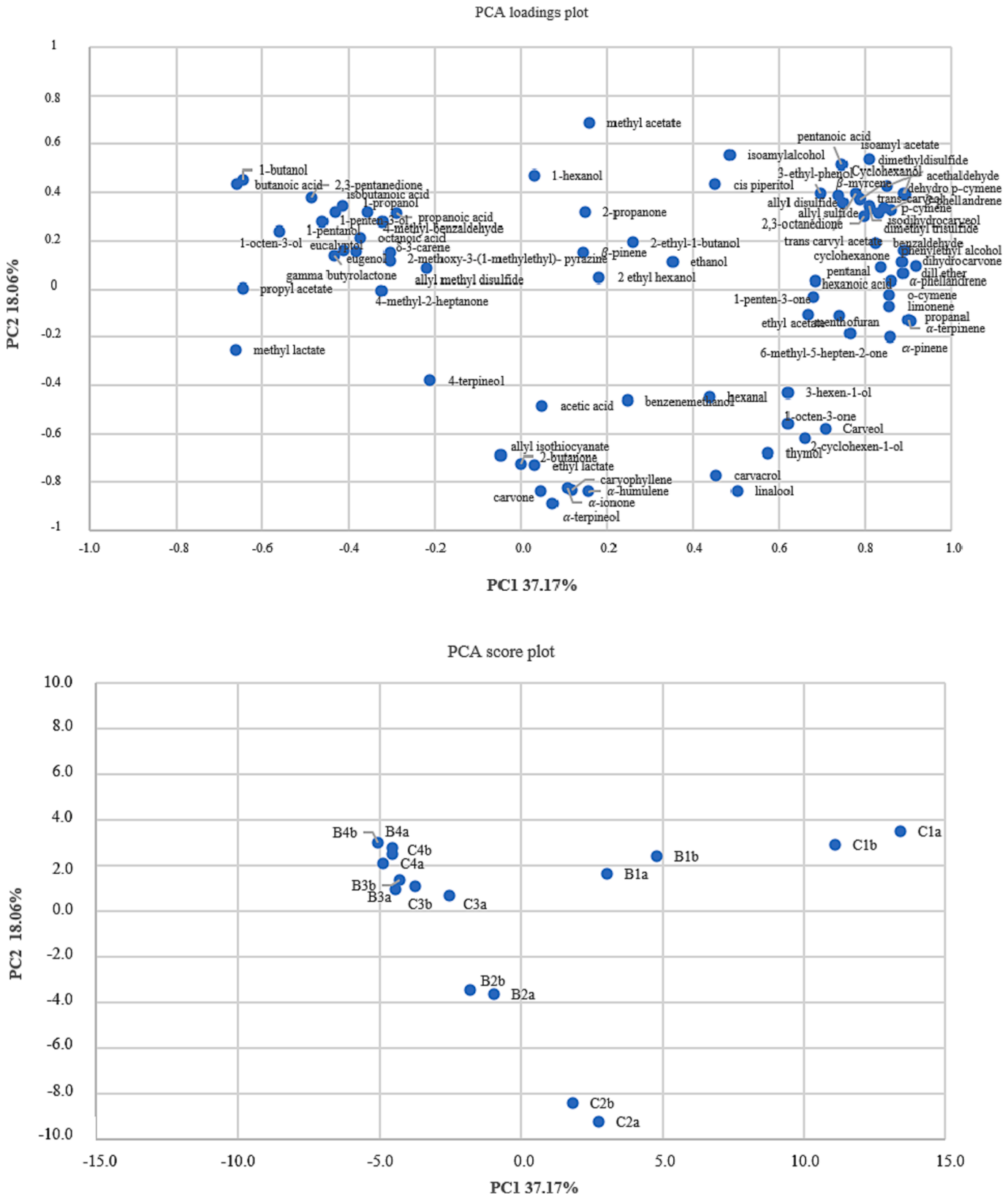

Fig. 8. Principal component analysis (PCA) of volatile organic compounds that mainly differentiated samples of cucumbers (C) and brine (B) from different producers (1, 2, 3, 4) and two different batches (a, b).

et al., 2023). Interestingly, Hussain et al. (1992) reported the bactericidal effect of N-acetyl-β-D-glucosaminidase on some bacterial pathogens such as *S. aureus* and *Pseudomonas aeruginosa*; notwithstanding this activity can only be presumed in the samples examined in the present study.

As for bacteriocins production, no *P. parvulus* isolate was able to inhibit the growth of *L. innocua*. Although there is a paucity of scientific data regarding the production of bacteriocins by *P. parvulus*, the results obtained in the present study are in accordance with those reported by Immerstrand et al. (2010) for a *P. parvulus* strain isolated from cider production, who showed no inhibitory activity against *L. monocytogenes*.

Although the antimicrobial activity of *L. plantarum* group against *L. monocytogenes* has widely been demonstrated (Echegaray et al., 2023), among the studied isolates, no inhibitory activity against the tested *L. innocua* strains was observed, hence, data will no further be discussed.

Among the lactic acid bacteria isolates obtained in the present study, only the closest relatives to *P. parvulus* and *L. plantarum* group were characterized for their acidification activity.

Cucumbers contain about 2 % of fermentable sugars, with glucose and fructose being the two predominant sugars (sucrose is present only in trace amounts) occurring in approximately equal molar concentrations (Lu et al., 2001). Hence, in this vegetable matrix, glucose and fructose are easily fermented by lactic acid bacteria.

In the present study, all the lactic acid bacteria isolates tested for their acid production capacity in the presence of NaCl showed notable performances. The acidification performance of the isolates is the result of their adaptation to the cucumber environment and suggests their suitability as starter or adjunct cultures. Indeed, the final pH values reached by the isolates were in accordance with those detected in the cucumber samples herein analyzed, and with those already reported by several authors for fermented cucumbers, attesting around 3.5 (Ahmed et al., 2021; Stoll et al., 2020; Lu et al., 2001; Pérez-Díaz et al., 2017; Pérez-Díaz et al., 2019). Of note, microbial acidification of cucumbers strongly affects the concentration of γ-aminobutyric acid, isoleucine, leucine, lysine, and ornithine (Moore et al., 2021; 2022), with relevant impact on the nutritional value of the product.

The HS-SPME-GC/MS analysis performed on the brine and cucumber samples revealed the composition of the major and minor volatile components, highlighting the presence of a wide range of volatile organic compounds, which were recognized as being primarily responsible for unique flavor of this fermented food. More than 80 VOCs were recognized as belonging to terpene hydrocarbons, oxygenated terpenes, acids, alcohols, esters, aldehydes, and ketones.

In the samples herein analyzed, the most detected volatile compounds belonged to alcohols, acids and terpenoids, whereas moderate quantities of aldehydes, esters, sulphides and other substances, such as dill ether, were found.

Furthermore, differences were found in the volatile composition of the samples from the different producers. In particular, the samples from producers 1 and 2 had a richer volatile composition than the other samples, whereas the samples from producers 3 and 4 showed similar composition. The results obtained showed that cucumbers differing in origin, type of recipe and processing, contained different types and amounts of VOCs, thus likely resulting in differences in taste.

Samples from producers 1 and 2 differed mainly in the type and quantity of terpenoids. These samples were characterized by a high amount of *α*-phellandrene, limonene, o-cymene, p-cymene, and *β*-phellandrene. These terpenes could derive from spices or herbs included in the production of fermented cucumber, such as dill, garlic, or horseradish. As pointed out by El-Zaeddi et al. (2016), dill (*Anethum graveolens* L.) is an aromatic herb often used as flavoring and seasoning of various foods, such as salads, sauces, and pickled vegetables and is characterized by some main compounds as α-phellandrene, limonene, *β*-phellandrene, *p*-cymene, α-pinene, dill ether, and *trans*-β-ocimene.

At the same time, the presence of different sulphur compounds such as dimethyl disulphide, allyl disulphide, and allyl sulphide in the samples of producers 1 and 3 attests the addition of garlic in the recipe. Sulphides are known as the main volatile components of fermented foods and were often extracted by garlic, cabbage, red pepper, onion, and ginger, suggesting that the use of these spices in cucumber production may strongly influence the aroma profile (Rao et al., 2020, Zhou et al., 2021, Abe et al., 2020).

All samples were also characterized by esters, mainly ethyl acetate, methyl acetate, propyl acetate, and ethyl lactate, which are usually formed by microbial fermentation following the esterification of free fatty acids with alcohol. As also observed by Lin et al. (2023), esters are another important group of volatile compounds characterized by fruity odors whose presence highlights the abundant fermentation activity in the analyzed samples.

Alcohols, of which ethanol was the most represented compound, were found in high amounts in all the samples of cucumbers and brine. As found by other authors (Lin et al., 2023; Xiang et al., 2020), the occurrence of alcohols in fermented cucumbers is strongly correlated with microbial fermentation, especially in the presence of species belonging to *Leuconostoc, Lactobacillus*, and *Weissella* genera. Accordingly, a high relative abundance of *Leuconostoc* and *Lentilactobacillus* was reported in samples herein analyzed, especially in brine that had the highest values of alcohols.

Aldehydes and ketones were found in low amounts, thus poorly contributing to the final aroma of the fermented cucumbers. As observed by Lin et al. (2023), aldehydes are known to be produced by heterofermentative lactic acid bacteria but decrease during fermentation due to ethanol conversion by the microorganisms.

5. Conclusions

The fermented cucumber samples herein studied, collected in 4 different regions of Poland, showed significant nutritional features in terms of polyphenols and antioxidant activity. Of note, the isolation and characterization of the key fermenting species to produce tailored starter of adjunct cultures represented a step forward in product valorization and preservation. The microbiological characterization of cucumber and brine samples allowed the dominant species to be detected, being *Lactococcus* and *Streptococcus* in cucumbers, and *Lactiplantibacillus*, *Leuconostoc*, *Pediococcus*, *Secundilactobacillus*, and *Lentilactobacillus* in brine. The isolation activity offered a clearer picture of the active lactic acid bacteria at the end of fermentation, being *P. parvulus* and *L. plantarum* group. All the studied isolates showed a good attitude in fermenting the cucumber-based broth, thus suggesting their potential application as starter or adjunct cultures for guided cucumber fermentation. Moreover, for the same isolates, strong aminopeptidase activity (due to leucine arylamidase and valine arylamidase) was observed, with potential effect in the definition of the final sensory traits of the product. Further research is needed to confirm the supposed pro-technological traits of the isolates in up-scale trials for the production of fermented cucumbers. Of note, the presence of the *hdcA* gene in some *P. ethanolidurans* isolates also confirmed the need for a thorough characterization of starter candidates to avoid undesired adverse effects on consumer's health. A noteworthy observation is that the substantial presence of pro-technological microorganisms in the analyzed brine samples affirms the potential use of this matrix as a natural source for starter cultures in the fermentation of cucumbers through the back-slopping technique. Based on the results of HS-SPME-GC/MS analysis, a rich and complex volatilome, composed by more than 80 VOCs, was recognized and characterized, thus contributing to depict the olfactive bouquet of the analyzed Polish food delicacy. The data overall collected contributed to reduce the lack of knowledge of microbiological, chemical, and physical factors implicated in cucumber fermentation.

CRediT authorship contribution statement

Federica Cardinali: Investigation, Formal analysis, Writing - Original Draft. **Cristian Botta:** Investigation, Formal analysis, Writing - Original Draft. **Joanna Harasym:** Investigation, Formal analysis, Writing - Original Draft, Resources. **Anna Reale:** Investigation, Formal analysis, Writing - Original Draft. **Ilario Ferrocino:** Investigation, Formal analysis, Writing - Original Draft, Resources. **Agnieszka Orkusz:** Formal analysis. **Floriana Boscaino:** Investigation, Formal analysis. **Vesna Milanović:** Formal analysis, Resources. **Cristiana Garofalo:** Formal analysis, Resources. **Giorgia Rampanti:** Formal analysis. **Lucia Aquilanti:** Review & Editing, Resources. **Andrea Osimani:** Conceptualization, Writing - Review & Editing, Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113851.

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