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Lacto-fermented garlic handcrafted in the Lower Silesia Region (Poland): Microbial diversity, morpho-textural traits, and volatile compounds

Federica Cardinali [a], Cristian Botta [b], Joanna Harasym [c], Ilario Ferrocino [b], Anna Reale [d], Floriana Boscaino [d], Tiziana Di Renzo [d], Vesna Milanović [a], Cristiana Garofalo [a], Giorgia Rampanti [a], Lucia Aquilanti [a], Andrea Osimani [a,d,*]

[a] Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy
[b] Department of Agricultural, Forest, and Food Science, University of Turin, Largo Paolo Braccini 2, Grugliasco, Torino, Italy
[c] Department of Biotechnology and Food Analysis, Wroclaw University of Economics and Business, Komandorska 118/120, 53-345 Wrocław, Poland
[d] Istituto di Scienze dell'Alimentazione, Consiglio Nazionale delle Ricerche, Via Roma 64, 83100 Avellino, Italy

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ABSTRACT

The aim of the present study was to provide a first characterization of lacto-fermented garlic manufactured by local small-scale artisanal producers in the Lower Silesia Region (Poland). The lacto-fermented garlic samples showed high nutritional features in terms of antioxidant activity. A total of 86 compounds, belonging to various chemical classes, were identified by headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC/MS). Most of these compounds belonged to six main classes, being sulfur compounds, esters and acetates, oxygenated monoterpenes, monoterpene hydrocarbons, and alcohols. Aldehydes, acids, ketones, furans, and phenols were also identified. In the analyzed samples, counts up to 8 log cfu g^{-1} were observed for lactic acid bacteria. Metataxonomic analysis revealed the presence of *Levilactobacillus*, *Lactiplantibacillus*, *Latilactobacillus*, *Secundilactobacillus*, *Weissella*, *Leuconostoc*, *Lactococcus*, *Pediococcus*, and *Lacticaseibacillus* among the major taxa. These results were confirmed by the isolation and characterization of viable lactic acid bacteria. Indeed, the presence of the closest relatives to *Lacticaseibacillus casei* group, *Pediococcus parvulus*, *Levilactobacillus brevis*, *Levilactobacillus parabrevis*, and *Lactiplantibacillus plantarum* group was observed. A good acidification performance in salty garlic-based medium was observed for all the isolates that, between 8 and 15 days of fermentation, reached pH values comprised between 4 and 3.5, depending on the tested species. Of note, 15 out of the 37 lactic acid bacteria isolates (*Levilactobacillus parabrevis*, *Pediococcus parvulus*, *Lactiplantibacillus plantarum* group, and *Lacticaseibacillus casei* group) showed the presence of the *hdcA* gene of Gram-positive bacteria encoding for histidine decarboxylase. Furthermore, for 8 out of the 37 isolates the in-vitro exopolysaccharides production was observed. No isolate showed inhibitory activity against the three *Listeria innocua* strains used as surrogate for *Listeria monocytogenes*.

1. Introduction

The use of garlic (*Allium sativum*) as food, condiment, or medicine traces its origins deep within the annals of history. As recently reviewed by Ekşi et al. (2020), the cultivation of this plant began over 4,000 years ago in the ancient Egypt. Moreover, the use of garlic was also popular in ancient Greece and during the Roman and Byzantine Empires (Ekşi et al., 2020).

Garlic is reported to have pharmacological features as antiseptic, antimicrobial, antifungal, and expectorant activities. These functional properties of garlic are due to the presence of some phytoconstituents that include steroids, flavonoids, glycosides, saponins, tannins, alkaloids, and terpenoids (Okoro et al., 2023). Moreover, active organosulfur compounds with bioactive functions in garlic include alliin, s-allylcysteine, s-allyl mercapto cysteineallicin, diallyl sulfides, diallyl disulfides, diallyl trisulfides, and allicin (Okoro et al., 2023). Among these, allicin, which is responsible for the typical spicy aroma and pungent odour, can exert antibacterial action (e.g., against *Streptococcus mutans*,

* Corresponding author at: Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy.
E-mail address: a.osimani@univpm.it (A. Osimani).

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Streptococcus faecalis, *Shigella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterobacter* spp., *Listeria monocytogenes*, and *Clostridium difficile*) and antifungal activity (e.g., against *Candida* spp., *Trichosporon* spp., *Torulopsis* spp., *Trichophyton* spp., *Aspergillus* spp., *Cryptococcus* spp., and *Rhodotorula* spp.) (Okoro et al., 2023).

Of note, the bioactive compounds in garlic also possess anti-inflammatory, antithrombotic, antihypertensive, antidiabetic, anti-obesity, anti-Alzheimer disease, anticancer, and antioxidant activities, thus conferring to this plant health-promoting functions (Okoro et al., 2023).

Garlic can be used as a fresh herb for cooking, or in dried, powdered or extracted (oil) form (Kilic-Buyukkurt et al., 2023). In recent years, black garlic, has become popular among consumers (Kilic-Buyukkurt et al., 2023). Black garlic, erroneously known as fermented garlic (since no microorganisms are involved in its production), is the result of enzymatic and non-enzymatic browning actions (Maillard reaction, caramelization, and oxidation of phenols) that occur during heat treatment of white (raw) garlic (Kilic-Buyukkurt et al., 2023).

The lactic acid fermentation of garlic cloves is another popular practice to obtain a tasty garlic-based food delicacy. At this regard, it is already acknowledged that almost all vegetable products can be fermented by microorganisms, although the fermentation of pure garlic is still rare (Thierry et al., 2023; Torres et al., 2020). The spontaneous fermentation of vegetables is mainly driven by autochthonous lactic acid bacteria, with the genera *Lactiplantibacillus*, *Levilactobacillus*, *Leuconostoc*, *Pediococcus*, and *Weissella* being those mainly detected (Thierry et al., 2023; Torres et al., 2020). Lactic acid bacteria are a very versatile group of microorganisms that produce organic acids (principally lactic acid) as main metabolites during vegetable fermentation, together with numerous compounds (e.g., antimicrobial compounds, viscous exopolysaccharides −EPS, volatile aromatic molecules, etc.) affecting sensory, functional, nutritional, textural, and safety features of the fermented product (de Souza et al., 2023).

To the authors' knowledge, the practice of fermenting garlic cloves is still based on empirical production processes that vary according to geographical area and tradition. According to the most common practices, peeled and raw garlic cloves are usually immersed in a brine containing 3 to 10 % sodium chloride (NaCl) and left to ferment at room temperature for 2 weeks to 1 month, depending on local customs. The lacto-fermented garlic cloves are then stored under refrigerated conditions. Interestingly, Hajar-Azhari et al. (2023) recently exploited the *Lacticaseibacillus casei* ATCC334 strain as starter culture to produce a lacto-fermented garlic sauce with enhanced metabolite production and antioxidant activity. Hajar-Azhari et al. (2023) also observed strong antimicrobial activity of the garlic sauce against *E. coli*, *S. aureus*, and *Salmonella* Typhimurium.

As known from a review of the literature, there is a lack of knowledge of the microbiota naturally occurring in spontaneously lacto-fermented garlic, thus potentially representing an undisclosed source of microbial diversity and a source of beneficial microbes. Moreover, the physico-chemical, morpho-textural, and volatile characteristics of fermented garlic are still poorly studied. Hence, the aim of the present study was to provide the characterization of lacto-fermented raw garlic produced by local small-scale artisanal producers in the Lower Silesia Region (Poland). To this end, culture-dependent and -independent techniques (metataxonomic analysis) were used to study the microbiota. Physico-chemical and morpho-textural analyses were also carried out, together with headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC/MS) to study the volatile organic compounds (VOCs). Moreover, to obtain starter or adjunct cultures with potential pro-technological features, 37 selected lactic acid bacteria isolated from brine and fermented garlic cloves were characterized for: i) acidification performance; ii) presence of the *hdcA* gene of Gram-positive bacteria encoding for histidine decarboxylase; iii) production of sucrose-dependent and -independent EPS. Finally, the candidate starter or adjunct cultures of lactic acid bacteria isolates were also tested for the production of bacteriocins against *Listeria innocua*, utilized as a surrogate for *L. monocytogenes* (ANSES, 2019).

2. Materials and methods

2.1. Collection of samples

Samples of lacto-fermented, unpasteurized, and ready-to-eat garlic were collected from 2 artisanal Polish producers located in the Lower Silesia Region (Poland). For each producer, 2 production batches were collected. For each batch, 6 sample units (glass jars) of 500 g each were analyzed. Neither starter cultures nor preservatives were used in lacto-fermented garlic cloves manufacturing. The lacto-fermented garlic samples were traditionally produced using 70 % raw garlic cloves immersed in a brine containing 2–3 % sodium chloride (NaCl). Moreover, variable amounts of allspice, peppercorns, bay leaves, dried dill flowers and stalks, horseradish root, and mustard were included, depending on the recipe. No further information on the manufacturing process of the samples was provided by the producers.

Samples of fermented garlic cloves and the respective brine were kept under refrigerated conditions (+4 °C) soon after collection and analyzed within 7 days from sampling.

2.2. Physico-chemical analyses of lacto-fermented garlic cloves and brine

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

For titratable acidity, 50 g of lacto-fermented garlic clove samples were added with 50 mL of deionized water and homogenized using a DI 18 Basic blender (IKA, Fazenda Santa Cândida, Campinas, Brazil) for 5 min. The brine was titrated directly. The results were expressed as the amount (mL) of 0.1 M NaOH solution needed to titrate the pH of the homogenates to 8.3 (Cardinali et al., 2024). For each sample, the measurements were performed in duplicate, and the results were expressed as mean ± standard deviation.

NaCl concentration (%) was assessed using a salinity meter (LAQUAtwin salt-22, HORIBA, Ltd., Kyoto, Japan). The device directly measures sodium ion concentration in 0.3 mL sample and converts it into NaCl concentration. For each sample, the measurements were performed in duplicate, and the results were expressed as mean ± standard deviation.

For acetic acid, lactic acid, TPC, DPPH and reducing sugars, 50 g of lacto-fermented garlic clove samples were added with 50 mL of deionized water, homogenized using a DI 18 Basic blender (IKA) for 5 min, and centrifuged at 5,000 × *g* for 10 min, then the supernatant was collected, and sediment washed with 50 mL of water and centrifuged again, whereas brine was used directly. For each sample, the measurements were performed in duplicate, and the results were expressed as mean ± standard deviation.

Acetic acid and lactic acid 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 mean ± standard deviation.

Total polyphenol content (TPC) was measured using the Folin–Ciocalteu colorimetric method previously described by Olędzki and 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 the absorbance of the resulting solution at 765 nm. The TPC results were presented in milligrams of gallic acid equivalent (GAE) per 1 g of raw material used. All the samples were analyzed in duplicate, and results were expressed as mean ± standard deviation.

The antioxidant capacity (DPPH) of the tested extracts was measured according to the method previously described by Olędzki and 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 mg Trolox equivalent (TE) per 1 g of raw material used. All the samples were analyzed in duplicate, and results were expressed as mean ± standard deviation.

Reducing sugars content was measured using a method modified by Olędzki and Harasym (2023), with 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, cooled to room temperature, and the absorbance was measured at 535 nm. The content of monosaccharides was expressed in g of glucose equivalent per 100 g of sample tested. All the samples were analyzed in duplicate and expressed as mean ± standard deviation.

2.3. Morpho-textural analyses of lacto-fermented garlic cloves and brine

Texture measurements were performed on the lacto-fermented garlic cloves using a mesocarp puncture test on a 5.0 mm height slice obtained from the center of the garlic clove. The mesocarp puncture test was conducted on a 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). The peak force of 8 samples per garlic clove was averaged and recorded in Newtons (N) as the firmness value, and expressed as mean ± standard deviation.

The dynamic viscosity of the brine was measured using a MCR 102 rotational oscillatory rheometer (Anton Paar, Stuttgart, Germany) equipped with cylindrical cup-bob geometry. The measuring gap was 1-mm, and the temperature of measurement was 25 °C. Data acquisition, analysis, storage, and retrieval were made with RheoCompass v.1.24.584 (Anton Paar). Samples were measured in quadruplicate, and values expressed as mean ± standard deviation.

2.4. Viable counts

For lacto-fermented garlic cloves, viable counts were determined by mixing 10 g of each sample with 90 mL of sterile peptone water (1 g L^{-1} of bacteriological peptone). The homogenization process was carried out in a Stomacher apparatus (400 Circulator, International PBI, Milan, Italy) for 3 min at 260 rpm. The brine was analyzed directly. Following the homogenization, ten-fold serial dilutions were prepared and the viable counts of presumptive lactococci, presumptive thermophilic cocci, presumptive lactobacilli, coagulase-negative cocci, enterococci, Enterobacteriaceae, and eumycetes were performed as detailed in Table 1.

The results of the viable counts were expressed as mean of log colony-forming units (cfu) per gram or mL of sample ± standard deviation.

2.5. Metataxonomic analysis

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). Library of 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 using 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, USA), according to the manufacturer's instructions. Sequencing was performed using a MiSeq instrument (Illumina) 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 54,380 raw-reads obtained from 16S rRNA amplicon-based sequencing were analysed in R environment (R program version 4.1.1; https://www.r-project.org) using *DADA2* package (Callahan et al., 2016). A total of 31,430 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 shorten than 366 bp were discharged: 79.2 % of the filtered sequences were used to construct the frequency table of Amplicon Sequence Variants (ASVs), with an average value of 6,432 reads/sample. 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#.YObFvhMzZRE). ASVs with unknown Phylum assignment or assigned to mitochondria-chloroplasts were removed from the frequency tables. The samples' taxonomy was displayed to the genus taxonomic rank in relation to sequences' quality.

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

Table 1
Growth media and conditions used for enumeration of culturable bacteria and eumycetes.

Growth media	Microorganisms	Growth conditions				References
		T (°C)	Incubation time (h)	Incubation condition	Plate method	
MRS[a]	Presumptive lactobacilli	37	48–72	Microaerophilia	Pour	De Man et al. (1960)
M17[a]	Presumptive lactococci	22	48–72	Aerobiosis	Pour	Terzaghi and Sandine (1975)
	Presumptive thermophilic cocci	42	48–72	Aerobiosis	Pour	Aquilanti et al. (2012)
MSA	Coagulase-negative cocci	37	48–72	Aerobiosis	Spread	Chapman (1945)Bannerman (2003)
ESA	Enterococci	37	48	Aerobiosis	Spread	Slanetz and Bartley (1957)
VRBGA	Enterobacteriaceae	37	24	Aerobiosis	Pour	Association (1978)
RBA	Eumycetes	25	72–96	Aerobiosis	Spread	American Public Health Association (1978)

MRS: de Man, Rogosa, Sharpe agar; MSA: mannitol salt agar; ESA: Enterococcus Selective Agar; VRBGA: Violet Red Bile Glucose Agar; RBA: Rose Bengal Chloramphenicol Agar.

[a] Added with cycloheximide (250 mg L^{-1}).

absence (1/0) matrix, thus the intersections between the two productions were calculated and displayed with the web-tool Venny v2.0.2 (https://bioinfogp.cnb.csic.es/tools/venny/index2.0.2.html).
Sequencing data were deposited at the Sequence Read Archive of the National Centrer for Biotechnology Information under the bioproject accession number PRJNA1081684.

2.6. GC–MS analysis of volatile components

Headspace volatiles from each sample (garlic cloves and brine) 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 Cardinali et al. (2024) with some modifications.

From each sample, 1 g was collected, shredded, and placed in a 20 mL headspace vial and 5 mL of 3-octanol (internal standard, 100 mg L^{-1} standard solution) was added. 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 75 μm Carboxen/Polydimethylsiloxane (CAR/PDMS (Supelco, Bellefonte, PA, USA) 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 spitless mode. Volatile compounds were separated using a capillary column HP Innowax (Agilent Technologies) (30 m × 0.25 mm id. × 0.50 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: 40 °C (hold 5 min), ramp to 150 °C at 5 °C min^{-1}, ramp to 240 °C at 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 and 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. All the analyses were performed in duplicate, and the results expressed as mean value of two technical replicates ± standard deviation.

2.7. Isolation and characterization of lactic acid bacteria

2.7.1. Isolation and identification

Colonies of lactic acid bacteria cultivated on MRS agar (Merck), supplemented with cycloheximide (Merck), were randomly selected and subsequently sub-cultured to ensure purity. Isolates were collected from all brine and lacto-fermented garlic clove samples, then stored at −80 °C until further analysis. Thawed lactic acid bacteria cultures were sub-cultured twice on MRS agar (Merck) at 30 °C for 48 h and subjected to DNA extraction following the procedure outlined by Osimani et al. (2015). The purity and quantity of the extracted DNA were assessed using a NanoDrop ND 1000 (Thermo Fisher Scientific). DNA extracts were standardized to a final concentration of 100 ng μL^{-1} and utilized for PCR in a MyCycler Thermal Cycler (BioRad), followed by gel-electrophoresis, as detailed by Osimani et al. (2015). Subsequently, the resulting amplicons were sent to Genewiz (Takaley, UK) for purification and sequencing. The Basic Local Alignment Search Tool (BLAST) was employed to compare the obtained sequences with 16S rRNA sequences of type strains from the 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.

2.7.2. Acidification in garlic-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}) before 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 garlic-based growth medium obtained as follows. The garlic-based growth medium was prepared according to Johanningsmeier and McFeeters (2013) with some modifications. In more detail, garlic obtained from a local provider was processed in a mod. CE 11 centrifugal slow juicer (Girmi, Rimini, Italia) at a speed of 55 rpm. After juice extraction, NaCl and water were added to obtain a final concentration of 37 % fresh garlic juice and 57 % water, according to Fleming et al. (1995), and 6 % NaCl. The resulting garlic-based growth medium was sterilized and stored at 4 °C until use.

The pH values of the garlic-based growth medium were measured prior to inoculation (t0) and daily (every 24 h) after incubation at 30 °C for 15 days. Duplicate analyses were conducted for each isolate.

2.7.3. In-vitro EPS production

The isolates were screened for EPS production, following the method previously described by Hilbig et al. (2019) with some modifications. Specifically, the isolates were initially subjected to two consecutive sub-culturing steps in MRS broth (Merck) at 37 °C for 48 h. Subsequently, EPS production was visually assessed by plating 5 μL aliquots of each bacterial culture onto the following solid media: (i) MRS agar (Merck) supplemented with 80 g L^{-1} sucrose (Serva, Heidelberg, Germany) to favour homopolysaccharide (HoPS) synthesis; MRS agar (Merck) supplemented with 10 g L^{-1} yeast extract (VWR Chemicals), 10 g L^{-1} meat extract (VWR Chemicals), 20 g L^{-1} galactose (VWR Chemicals), and 20 g L^{-1} lactose (Carlo Erba Reagents, Cornaredo, Italy) to promote heteropolysaccharide (HePS) synthesis. After incubation for 48 h at 30 °C, colonies were considered positive if they exhibited a mucoid appearance (clearly shiny and slimy appearance) or a ropy consistency (capable of producing visible filaments when probed with a sterile toothpick). Duplicate analyses were conducted for each isolate.

2.7.4. Detection of the hdcA gene in Gram-positive bacteria

The isolates were tested for the presence of the *hdcA* gene by qPCR using a CFX Connect Real-Time System machine (BioRad Laboratories). The cycling conditions and primers utilized in the qPCR reactions were adjusted in accordance with the specifications outlined by Belleggia et al. (2021). *Lactobacillus parabuchneri* DSM 5987 was used as the positive strain for establishing the standard curve. The qPCR analysis was conducted in triplicate for each isolate, along with a blank control and the results were expressed as presence (+) or absence (−) of the target gene.

2.7.5. Assessment of antimicrobial activity

The antimicrobial activity of the isolates was assessed using the agar well diffusion assay, as previously outlined by Osimani et al. (2023). Briefly, *L. innocua* was cultivated at a concentration of 2 % (v v^{-1}) into Brain Heart Infusion (BHI) soft agar (0.75 % agar) (VWR Chemicals). Subsequently, 20 mL of the inoculated medium was transferred into a 90 mm Petri dish (VWR Chemicals) and allowed to solidify. Wells of approximately 50 μL were made in the BHI soft agar (VWR Chemicals) using the cone of a sterile 200 μL tip (VWR Chemicals). The lactic acid bacteria to be tested underwent two rounds of cultivation in MRS broth (Merck) at 37 °C for 48 h. The broth cultures were then adjusted to pH 7.0 by adding a 0.1 N NaOH (AppliChem, Darmstadt, Germany) to neutralize the organic acids produced during bacterial growth. A filtration step using a sterile PES membrane filter with a pore size of

0.22 μm (Laboindustria S.p.A., Padova, Italy) was also conducted.

For each isolate, four wells were created 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 examined for the presence inhibition zones. In the event of positive results (presence of an inhibition halo), three spots of 5 μL each of pepsin (Fluka™, Honeywell, Morristown, USA), trypsin (Fluka™), or Pronase (Merck) were placed along the circumference of the inhibition zone to assess the protein nature of the microbial-derived inhibitory compound. The Petri dishes were further incubated under the same conditions, and the synthesis of bacteriocins by the tested lactic acid bacteria isolates was ultimately confirmed by the formation of crescents.

2.8. Statistical analysis

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

3. Results

3.1. Physico-chemical analyses of lacto-fermented garlic cloves and brine

The results of the physico-chemical analyses of lacto-fermented garlic clove and brine samples are reported in Table 2.

Regarding pH, no differences between the counts detected in garlic or brine were observed.

Titratable acidity in garlic cloves differed between producers, being the lowest in batch 2 of producer 1 (6.79 ± 0.01 mL of 0.1 N NaOH) and the highest in batch 2 of producer 2 (7.07 ± 0.04 mL of 0.1 N NaOH).

Lactic acid was higher in garlic cloves than in brine, and the same was for acetic acid, with no statistical difference between either producers or batches.

The antioxidant activity did not differ between the producers or batches. Whereas, total phenolic content differed significantly between producers, with the highest values for samples from producer 2, batch 2 (56.65 ± 0.57 mg GAE 100 g^{-1}), and the lowest in producer 1, batch 2 (54.74 ± 0.02 mg GAE 100 g^{-1}). In brine, the TPC was the highest for samples of producer 1, batch 1 (21.60 ± 0.00 mg GAE 100 g^{-1}), and the lowest for samples of producer 2, batch 2 (17.15 ± 0.49 mg GAE 100 g^{-1}).

The reducing sugars values were strongly different in garlic cloves comparing to brine. The highest average value in garlic cloves was noted in samples of producer 1, batch 2 (2.84 ± 0.07 g GE 100 g^{-1}), and the lowest in samples of producer 2, batch 2 (2.43 ± 0.03 g GE 100 g^{-1}). For brine samples, the highest reducing sugars content was noted in samples of producer 2, batch 2 (0.98 ± 0.01 g GE 100 g^{-1}), and the lowest in samples of producer 1, batch 2 (0.55 ± 0.01 g GE 100 g^{-1}).

3.2. Morpho-textural analyses of lacto-fermented garlic cloves and brine

The results of the morpho-textural characteristics of lacto-fermented garlic clove and brine samples are reported in Table 3 and Table 4.

In more detail, the color of garlic cloves was slightly different between producers (Table 3). However, for L^* parameter, there was no specific trend observed ($P < 0.05$). Generally, the L^* parameter ranged between 69.93 ± 0.06 (producer 2, batch 2) and 70.94 ± 0.37 (producer 1, batch 2). The a^* parameter was slightly pronounced towards green for all samples, however osculating between −1.50 ± 0.42 (producer 1, batch 2) and −2.25 ± 0.35 (producer 2, batch 1). The yellowness (b^* value) was more pronounced for producer 2 (12.70 ± 0.14 and 12.65 ± 0.07), whereas for producer 1, the samples were less yellow (11.10 ± 0.14 – batch 1; 11.80 ± 0.14 – batch 2).

Regarding viscosity of brine, the lowest value was noted for batch 2 of producer 1 (1.029 ± 0.004 mPa*s), whereas the highest for batch 1 of producer 2 (1.091 ± 0.009).

As for hardness measurements performed on lacto-fermented garlic cloves, the blandest was detected in batch 1 of producer 2 (16.70 ± 0.28

Table 3
Results of color analysis of garlic cloves.

Producer	Batch	L^*	a^*	b^*
Producer 1	1	69.92 ± 0.45^{a}	-1.50 ± 0.42^{b}	11.10 ± 0.14^{a}
	2	70.94 ± 0.37^{a}	-1.65 ± 0.07^{ab}	11.80 ± 0.14^{b}
Producer 2	1	70.28 ± 0.23^{a}	-2.25 ± 0.35^{ab}	12.70 ± 0.14^{c}
	2	69.93 ± 0.06^{a}	-2.35 ± 0.07^{a}	12.65 ± 0.07^{c}

Values are expressed as means ± standard deviation.
For each parameter, values with different superscript letters are significantly different ($P < 0.05$) according to the Tukey–Kramer's (HSD) test.
L^* value describes the lightness; a^* value describes the redness/greenness; b^* describes the blueness/yellowness.

Table 2
Physico-chemical parameters of lacto-fermented garlic cloves (G) and brine (B) samples.

Producer	Batch	Source	pH	TTA (mL of 0.1 N NaOH)	Lactic acid (g 100 g^{-1})	Acetic acid (g 100 g^{-1})	Antioxidant activity(μM TE 100 g^{-1})	Polyphenols(mg GAE 100 g^{-1})	Reducing sugars (g GE 100 $g^{-1)}$	NaCl (g 100 g^{-1})
Producer 1	1	G	4.12 ± 0.60^{a}	6.94 ± 0.07^{b}	0.689 ± 0.012^{a}	0.570 ± 0.029^{a}	976.4 ± 15.9^{a}	54.90 ± 0.84^{a}	2.65 ± 0.06^{b}	2.07 ± 0.12^{a}
		B	3.67 ± 0.09^{A}	7.90 ± 0.01^{B}	0.416 ± 0.007^{A}	0.246 ± 0.021^{A}	477.5 ± 11^{A}	21.60 ± 0.00^{D}	0.65 ± 0.01^{B}	1.00 ± 0.25^{A}
	2	G	4.10 ± 0.31^{a}	6.79 ± 0.01^{a}	0.832 ± 0.065^{a}	0.572 ± 0.004^{a}	976.3 ± 3.8^{a}	54.74 ± 0.02^{a}	2.84 ± 0.07^{c}	2.23 ± 0.15^{a}
		B	3.61 ± 0.31^{A}	7.83 ± 0.02^{B}	0.442 ± 0.013^{A}	0.238 ± 0.007^{A}	468.5 ± 0.7^{A}	20.35 ± 0.07^{C}	0.55 ± 0.01^{A}	1.05 ± 0.05^{A}
Producer 2	1	G	4.00 ± 0.11^{a}	7.06 ± 0.07^{b}	0.720 ± 0.050^{a}	0.528 ± 0.021^{a}	912.8 ± 47.4^{a}	56.65 ± 0.57^{b}	2.55 ± 0.01^{a}	0.46 ± 0.14^{b}
		B	3.82 ± 0.18^{A}	7.62 ± 0.08^{A}	0.377 ± 0.032^{A}	0.255 ± 0.023^{A}	452.4 ± 23.6^{A}	18.80 ± 0.71^{B}	0.77 ± 0.04^{C}	0.34 ± 0.15^{B}
	2	G	4.00 ± 0.13^{a}	7.07 ± 0.04^{b}	0.831 ± 0.066^{a}	0.543 ± 0.028^{a}	955.5 ± 1.2^{a}	56.40 ± 0.57^{b}	2.43 ± 0.03^{ab}	0.36 ± 0.02^{b}
		B	3.84 ± 0.28^{A}	7.53 ± 0.02^{A}	0.420 ± 0.037^{A}	0.249 ± 0.017^{A}	470.5 ± 21.6^{A}	17.15 ± 0.49^{A}	0.98 ± 0.01^{D}	0.27 ± 0.00^{B}

Values are expressed as means ± standard deviation.
For each parameter, means followed by different lowercase letter indicate significant differences among garlic clove samples ($P < 0.05$), whereas means followed by different capital letters indicate significant differences among brine samples ($P < 0.05$) according to the Tukey–Kramer's (HSD) test.

Table 4
Morpho-textural characteristics of lacto-fermented garlic clove (G) and brine (B) samples.

Producer	Batch	Source	Hardness [N]	Source	Viscosity [mPa*s]
Producer 1	1	G	17.90 ± 0.14^{b}	B	1.033 ± 0.005^{ab}
	2	G	18.40 ± 0.28^{a}	B	1.029 ± 0.004^{b}
Producer 2	1	G	16.70 ± 0.28^{c}	B	1.091 ± 0.009^{a}
	2	G	17.50 ± 0.14^{b}	B	1.089 ± 0.005^{a}

Values are expressed as means ± standard deviation.
For each parameter, means followed by different lowercase letter indicate significant differences among samples ($P < 0.05$) according to the Tukey–Kramer's (HSD) test.

N), whereas the hardest one was detected in batch 2 of producer 1 (18.40 ± 0.28 N).

3.3. Viable counts

The results of viable counts are reported in Table 5.

According to ANOVA, for presumptive thermophilic cocci and lactobacilli, no differences between the counts detected in garlic or brine samples were observed. As for coagulase-negative cocci, the highest counts were observed in samples from producer 1.

Finally, counts below the detection limit (1 log cfu g^{-1}) of enterococci, Enterobacteriaceae, and eumycetes were observed in all the samples.

3.4. Microbiota composition

The core microbiota of the samples collected from the two artisan producers encompassed the genera *Levilactobacillus, Lactiplantibacillus, Latilactobacillus, Secundilactobacillus, Weissella, Leuconostoc, Lactococcus,* and *Pediococcus*. The core microbiota did not encompass the genus *Lacticaseibacillus* that was only detected in in the samples of producer 2, in which it represented the most abundant taxa (Fig. 1A and Supplementary Table 1).

Overall, samples of producer 1 harboured a greater number of genera and higher alpha-diversity parameters in comparison to producer 2 (Fig. 1B and Supplementary Table 2).

3.5. Volatilome profile

A total of 86 compounds, belonging to various chemical classes, were identified by SPME-GC/MS (Table 6). Most of these compounds belonged to six main classes, being sulfur compounds (28), esters and acetates (9), oxygenated monoterpenes (10), monoterpene hydrocarbons (10) and alcohols (12). Aldehydes (6), acids (5), ketones (2), furans (2), and phenols (2) were also identified.

Among sulfur compounds, diallyl sulfide, diallyl disulfide, sulfide allyl methyl, methyl 2-propenyldisulfide, and methyl 2-propenyltrisulfide were the most abundant. Among the esters and acetates, ethyl acetate, n-propyl acetate, methyl acetate, 2-butenoic acid ethyl ester (E), were the most abundant. Among the alcohols, ethanol was the most

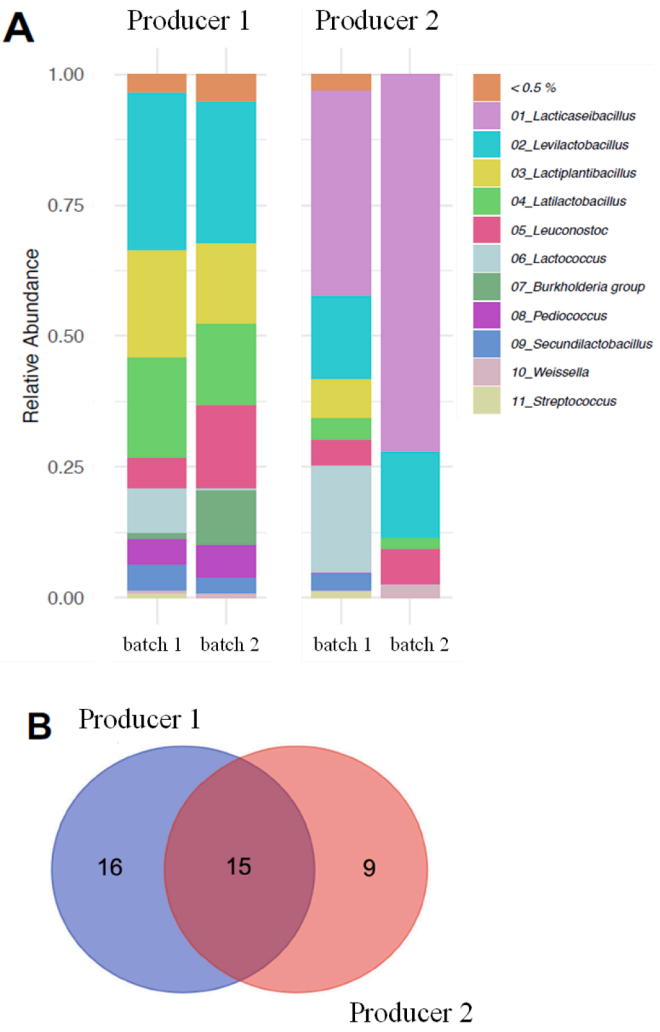

Fig. 1. Microbiota composition occurring in lacto-fermented garlic cloves collected from 2 artisan Polish producers located in the Lower Silesia Region (Poland). Stacked bar plots (A) showing microbiota composition (relative abundance > 0.5 % average) at the genus level and relative color coding key. Venn diagrams (B) displaying the shared genera between producers (producer 1, producer 2). Samples are grouped by producers/batches. Taxa are sorted in the legend from the most to the least abundant.

detected. Aldehydes (2-butenal, hexanal, 4-pentenal 2-methyl, 2-hexenal 2-ethyl, 2-octenal, benzaldehyde) were only identified in the fermented garlic cloves and in the brine of producer 1, where 2-butenal was the aldehyde present in the highest amount. Samples were characterized by monoterpene hydrocarbons such as limonene, α-phellandrene, β-phellandrene, o-cymene, p-cymene, β-myrcene. Samples of producer 2 were characterized for the presence of terpinen-4 ol, α-terpineol, and carvone. Among the ketones, traces of acetone and acetoin were found. Among the acids, acetic acid was found in the greater amount in all the

Table 5
Viable counts detected in fermented garlic clove (G) and brine (B) samples.

Producer	Source	Presumptive lactococci	Presumptive thermophilic cocci	Presumptive lactobacilli	Coagulase-negative cocci	Enterococci	Enterobacteriaceae	Eumycetes
Producer 1	G	7.16 ± 0.01^{a}	7.56 ± 0.06 ^{a}	7.69 ± 0.09 ^{a}	2.96 ± 0.06 ^{a}	<1 ^{a}	<1 ^{a}	<1 ^{a}
	B	8.02 ± 0.02 ^{A}	7.55 ± 0.05 ^{A}	8.31 ± 0.01^{A}	3.93 ± 0.07 ^{A}	<1 ^{A}	<1 ^{A}	<1 ^{A}
Producer 2	G	6.74 ± 0.66 ^{a}	6.93 ± 0.09 ^{a}	6.53 ± 0.07 ^{a}	<1^{b}	<1 ^{a}	<1 ^{a}	<1 ^{a}
	B	7.87 ± 0.02 ^{A}	7.75 ± 0.02 ^{A}	7.68 ± 0.13 ^{A}	2.78 ± 0.04^{B}	<1 ^{A}	<1 ^{A}	<1 ^{A}

Values are expressed as means of log cfu g^{-1} or mL^{-1} ± standard deviation.
For each parameter, means followed by different lowercase letter indicate significant differences among garlic clove samples ($P < 0.05$), whereas means followed by different capital letters indicate significant differences among brine samples ($P < 0.05$) according to the Tukey–Kramer's (HSD) test.

Table 6
Volatile organic compounds (VOCs) identified by solid phase microextraction/gas chromatography-mass spectrometry in fermented garlic and brine samples from 2 different producers.

RI	Compounds	Producer 1		Producer 2	
		G1	B1	G2	B2
Sulfur compounds					
750	Carbon disulfide	Nd	Nd	337.39 ± 14.82	415.42 ± 13.02
850	Sulfur dioxide	Nd	66.91 ± 1.95	22.86 ± 1.67	54.74 ± 1.93
887	Allyl mercaptan	244.25 ± 19.23	Nd	Nd	Nd
924	Thiirane, methyl-	10.60 ± 0.12	202.56 ± 10.21	140.01 ± 3.21	127.88 ± 2.73
948	Sulfide, allyl methyl	1505.15 ± 29.06	3983.93 ± 125.87	1912.17 ± 116.22	1673.67 ± 67.98
1107	Disulfide, dimethyl	51.00 ± 1.61	240.50 ± 11.09	152.73 ± 5.77	140.30 ± 2.78
1150	Diallyl sulfide	11336.29 ± 260.52	4635.97 ± 151.24	9678.48 ± 333.57	6405.39 ± 203.78
1220	Disulfide, methyl propyl	8.26 ± 0.15	Nd	16.53 ± 0.93	10.39 ± 0.25
1332	Methyl propenyl disulfide (E)	22,33 ± 0,73	Nd	140,07 ± 3,89	79,51 ± 3,11
1240	Thiophene, 3,4-dimethyl-	6,81 ± 0,18	Nd	91,59 ± 0,49	65,53 ± 2,12
1272	Methyl 2-propenyldisulfide	3981,44 ± 148,15	935,30 ± 21,60	2712,05 ± 190,99	1693,93 ± 134,41
1308	Methyl 1-propenyl disulfide, (Z)	Nd	Nd	583,69 ± 21,20	305,95 ± 15,83
1285	Benzenethiol	28,14 ± 0,14	Nd	8,47 ± 0,31	5,36 ± 0,16
1345	Acetic acid, (2-propenylthio)-	15,10 ± 0,23	15,93 ± 0,17	261,32 ± 21,06	165,26 ± 15,02
1366	Dimethyl trisulfide	1246.41 ± 9.18	170.31 ± 13.46	213.55 ± 16.23	105.23 ± 2.91
1410	Allyl propyl disulfide	206.00 ± 17.78	Nd	269.96 ± 20.85	143.99 ± 2.93
1464	Allyl-1-propenyldisulfide	327.39 ± 9.90	Nd	758.98 ± 17.07	481.76 ± 27.78
1469	Diallyl disulphide	19946.19 ± 594.88	Nd	9588.68 ± 238.15	6444.77 ± 225.77
1471	(E)-1-Allyl-2-(prop-1-en-1-yl) disulfane	919.76 ± 4.63	32.03 ± 2.58	2183.93 ± 166.34	1293.68 ± 8.83
1494	3H-1,2-Dithiole	609.41 ± 9.85	25.58 ± 0.96	454.97 ± 12.68	399.86 ± 10.95
1585	Methyl 2-propenyltrisulfide	1919.95 ± 74.51	52.73 ± 0.98	687.90 ± 4.12	489.92 ± 5.48
1655	Methyl (methylthio)methyldisulfide	Nd	Nd	2.08 ± 0.15	Nd
1650	4-Penten-2-ol, 5-(methylthio)-, (Z)-	Nd	2.57 ± 0.08	5.13 ± 0.27	9.00 ± 0.28
1770	Trisulfide, di-2-propenyl	Nd	38.46 ± 1.28	8.81 ± 0.19	704.28 ± 14.64
1840	2-Vinyl-4H-1,3-dithiine	184.61 ± 11.38	1.85 ± 0.13	347.06 ± 22.86	289.38 ± 22.33
1825	Thieno[2,3-b]thiophene	5.70 ± 0.20	2.76 ± 0.10	10.35 ± 0.23	8.20 ± 0.27
1841	Disulfide. methyl 1-(methylthio)propyl	3.37 ± 0.29	5.02 ± 0.23	3.68 ± 0.15	2.17 ± 0.11
2230	Disulfide, 1-(1-propenylthio)propyl propyl	4.64 ± 0.10	0.97 ± 0.01	7.00 ± 0.01	Nd
	tot	42582.79 ± 625.89	10413.37 ± 317.22	30599.48 ± 1213.39	21518.50 ± 774.64
Esters and Acetates					
821	Methyl acetate	Nd	102.48 ± 1.29	87.07 ± 2.79	287.95 ± 17.11
870	Ethyl Acetate	Nd	333.65 ± 8.11	1126.68 ± 35.07	1536.68 ± 44.18
953	n-Propyl acetate	Nd	536.97 ± 18.68	Nd	273.52 ± 8.68
1045	Butanoic acid, ethyl ester	Nd	8.87 ± 0.15	6.49 ± 0.21	8.85 ± 0.20
1167	2-Butenoic acid, ethyl ester, (E)-	Nd	Nd	429.38 ± 15.03	381.81 ± 17.24
1228	Hexanoic acid, ethyl ester	8.15 ± 0.13	Nd	Nd	Nd
1311	Methyl lactate	Nd	14.47 ± 0.19	75.98 ± 1.60	4.39 ± 0.21
1339	Ethyl lactate	3.34 ± 0.20	114.61 ± 2.31	Nd	106.89 ± 2.01
1531	Nonanoic acid, ethyl ester	13.10 ± 0.14	32.95 ± 0.96	Nd	Nd
	tot	24.58 ± 0.21	1144.00 ± 13.54	1725.59 ± 54.69	2600.09 ± 89.63
Oxygenated monoterpenes					
1703	Bicyclo[3,1,1]heptan-3-ol, 2,6,6-trimethyl-	5.79 ± 0.19	139.41 ± 1.65	9.04 ± 0.29	Nd
1702	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-	Nd	Nd	Nd	14.18 ± 0.29
1594	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-	27.52 ± 38.92	60.42 ± 0.15	41.36 ± 2.29	53.53 ± 1.73
1613	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-	Nd	Nd	5.56 ± 0.20	8.38 ± 0.23
1537	Linalool	19.85 ± 1.06	1.19 ± 0.02	21.12 ± 1.95	24.64 ± 0.13
1592	Terpinen-4-ol	Nd	Nd	125.85 ± 2.72	217.19 ± 11.07
1669	α-Terpineol	Nd	Nd	19.67 ± 0.88	29.32 ± 1.69
1602	(+)-Dihydrocarvone	168.28 ± 11.71	7.61 ± 0.18	44.06 ± 1.84	49.06 ± 1.53
1718	Carvone	Nd	Nd	163.06 ± 15.36	75.77 ± 1.88
1725	Trans piperitol	Nd	Nd	Nd	1.48 ± 0.03
	tot	221.44 ± 25.95	208.64 ± 1.70	429.74 ± 25.52	473.56 ± 18.32
Monoterpene hydrocarbons					
1036	α-Pinene	12.98 ± 0.21	Nd	54.66 ± 2.05	24.61 ± 1.11
1127	β-Pinene	Nd	Nd	63.80 ± 2.40	33.59 ± 0.54
1147	γ-Terpinene	Nd	Nd	105.34 ± 2.76	100.64 ± 1.29
1158	α-Phellandrene	381.43 ± 16.38	Nd	1788.88 ± 174.77	626.66 ± 11.17
1166	β-Myrcene	114.44 ± 2.49	Nd	229.79 ± 7.74	123.35 ± 1.90
1191	Limonene	427.18 ± 12.81	Nd	1641.44 ± 114.71	1036.16 ± 32.70
1205	β-Phellandrene	278.64 ± 7.46	19.42 ± 0.95	649.15 ± 20.45	302.41 ± 9.31
1229	γ −Terpinene	Nd	Nd	49.37 ± 2.30	38.33 ± 0.66
1254	o-Cymene	428.38 ± 11.17	7.35 ± 0.16	670.80 ± 18.61	500.95 ± 22.35
1268	p-Cymene	531.83 ± 11.85	8.40 ± 0.17	Nd	Nd
	tot	2174.87 ± 62.37	35.17 ± 1.28	5253.23 ± 345.80	2786.70 ± 79.70

(*continued on next page*)

Table 6 (*continued*)

RI	Compounds	Producer 1		Producer 2	
		G1	B1	G2	B2
Alcohols					
910	Methyl alcohol	Nd	155.67 ± 1.15	Nd	Nd
940	Ethanol	660.1 ± 7.9	1739.5 ± 83.1	1679.4 ± 84.6	1966.9 ± 87.2
1053	1-Propanol	Nd	Nd	16.1 ± 1.4	21.0 ± 1.1
1217	1-Butanol, 3-methyl-	Nd	208.15 ± 2.76	0.00	0.00
1221	2-Buten-1-ol, (E)-	Nd	26.69 ± 0.90	32.90 ± 2.42	50.06 ± 2.29
1241	3-Buten-1-ol, 3-methyl-	Nd	1.86 ± 0.14	Nd	Nd
1244	1-Pentanol	Nd	3.85 ± 0.09	Nd	Nd
1291	1-Butanol, 2-ethyl-	Nd	3.66 ± 0.04	2.76 ± 0.13	Nd
1386	3-Hexen-1-ol, (Z)-	Nd	Nd	Nd	1.30 ± 0.02
1390	2-Hexen-1-ol, (E)-	Nd	Nd	Nd	1.30 ± 0.00
1489	1-Hexanol, 2-ethyl-	75.77 ± 2.06	Nd	16.74 ± 1.63	8.45 ± 0.20
1838	Benzyl alcohol	Nd	Nd	Nd	2.91 ± 0.20
	tot	735.91 ± 5.88	2139.35 ± 80.32	1747.98 ± 90.16	2049.03 ± 90.83
Aldehydes					
1051	2-Butenal	1544.41 ± 10.27	400.06 ± 12.22	Nd	nd
1121	Hexanal	25.02 ± 1.09	134.46 ± 2.16	Nd	nd
1141	4-Pentenal, 2-methyl	30.16 ± 1.16	26.39 ± 1.32	Nd	nd
1330	2-Hexenal, 2-ethyl-	26.76 ± 1.13	52.54 ± 1.26	Nd	nd
1420	2-Octenal, (E)-	8.96 ± 0.12	Nd	Nd	nd
1513	Benzaldehyde	35.87 ± 1.18	20.65 ± 1.45	Nd	nd
	tot	1671.19 ± 12.68	634.10 ± 6.03	Nd	nd
Ketones					
814	Acetone	11.07 ± 0.12	80.94 ± 1.51	42.52 ± 2.10	50.40 ± 1.81
1280	Acetoin	Nd	35.74 ± 1.13	Nd	Nd
	tot	11.07 ± 0.12	116.67 ± 2.64	42.52 ± 2.10	50.40 ± 1.81
Acids					
1448	Acetic acid	858.58 ± 15.16	862.18 ± 8.21	804.72 ± 34.88	887.00 ± 14.86
1527	Propanoic acid	Nd	Nd	14.55 ± 0.38	13.88 ± 0.21
1615	Butanoic acid	Nd	2.62 ± 0.12	Nd	Nd
1816	Hexanoic acid	Nd	5.83 ± 0.29	6.41 ± 0.27	5.94 ± 0.26
2130	Nonanoic acid	1.37 ± 0.01	Nd	Nd	Nd
	tot	859.95 ± 16.1	870.63 ± 9.41	825.68 ± 35.54	906.81 ± 15.33
Furans					
1226	2-Pentyl-furan	20.75 ± 1.45	Nd	Nd	Nd
1657	2-Furanmethanol	Nd	Nd	27.58 ± 1.06	27.69 ± 1.63
	tot	20.75 ± 1.45	Nd	27.58 ± 1.06	27.69 ± 1.63
Phenols					
1820	Guaiacol	Nd	Nd	9.86 ± 0.27	9.00 ± 0.27
2225	Carvacrol	1.11 ± 0.01	Nd	1.55 ± 0.05	0.50 ± 0.01
	tot	1.11 ± 0.01	Nd	11.41 ± 0.22	9.51 ± 0.28

Abbreviations: G. garlic clove; B. brine; Nd. not detected; RI = retention index. Identification via comparison with RI database. Results are expressed as RAP = relative peak area (peak area of compound/peak area of internal standard) →| 100 (RAP ± SD). https://www.webbook.nist.gov (Accessed on 15 October 2023).

samples, while traces of butanoic, hexanoic, and nonanoic acids were found. Traces of 2-furanmethanl and 2-pentyl furan were found. Among the phenols, traces of guaiacol and carvacol were found. The fermented garlic cloves were characterized by the highest amount of sulfur compounds, monoterpene hydrocarbons, and aldehydes in respect with the respective brine samples. On the contrary, alcohols, esters and acetates were mainly found in the brine samples compared to the respective garlic clove samples.

3.6. Characterization of lactic acid bacteria isolates

The closest relatives, the percent identities, and the accession numbers of the sequences obtained from the 37 lactic acid bacteria isolated from garlic and brine are reported in Table 7.

In more detail, the closest relatives to *Lacticaseibacillus casei* group represented the most frequently isolated lactic acid bacteria species (18 out of the 37 isolates), followed by *Pediococcus parvulus* (8 out of the 37 isolates), *Levilactobacillus brevis* (6 out of the 37 isolates), *Levilactobacillus parabrevis* (4 out of the 37 isolates), and *Lactiplantibacillus plantarum* group (1 out of the 37 isolates).

As for the presence of the *hdcA* gene (Table 7), 15 out of the 37 isolates were positive for the target gene. In more detail, the positive isolates belonged to the following taxa: *Lacticaseibacillus casei* group (4 out of the 18 isolates), *Pediococcus parvulus* (5 out of the 8 isolates), *Levilactobacillus brevis* (2 out of the 6 isolates), *Levilactobacillus parabrevis* (3 out of the 4 isolates), and *Lactiplantibacillus plantarum* group (1 out of the 1 isolate).

Among the analyzed lactic acid bacteria cultures, a few isolates showed the ability to produce EPS in synthetic medium (Table 7). In more detail, 3 isolates of *P. parvulus* (G16, G20, and G39), 2 isolates of *L. brevis* (G15 and G17), 2 isolates of *L. parabrevis* (G3 and G4), and *L. plantarum* group (G14) produced sucrose-dependent EPS. In addition, 1 isolate of *P. parvulus* (G16), 1 isolate of *L. brevis* (G15), 1 isolates of *L. parabrevis* (G3), and *L. plantarum* group (G14) produced sucrose-

Table 7
Identification and characterization of lactic acid bacteria isolates from lacto-fermented garlic clove (G) and brine (B) samples.

Producer	Isolation source	Isolate code	Species	% Identity[a]	Accession number[b]	*hdcA gene*	Presumptive EPS production		Antimicrobial activity		
							Sucrose-dependent	Sucrose-independent	L1	L2	L3
1	G	G1	*Levilactobacillus brevis*	97.81	NR_116238	−	−	−	−	−	−
	G	G3	*Levilactobacillus parabrevis*	99.44	MT604696	+	+	+	−	−	−
	G	G4	*Levilactobacillus parabrevis*	98.47	MT604696	+	m	−	−	−	−
	G	G5	*Levilactobacillus brevis*	99.08	MT640328	−	−	−	−	−	−
	G	G7	*Pediococcus parvulus*	99.17	MF540542	+	−	−	−	−	−
	G	G8	*Pediococcus parvulus*	100	MK575520	+	−	−	−	−	−
	G	G9	*Pediococcus parvulus*	99.72	MT045969	−	−	−	−	−	−
	B	G11	*Levilactobacillus parabrevis*	99.33	KJ994419	+	−	−	−	−	−
	B	G12	*Levilactobacillus parabrevis*	99.89	MT604696	−	−	−	−	−	−
	B	G13	*Levilactobacillus brevis*	99.71	KU315055	+	−	−	−	−	−
	B	G14	*Lactiplantibacillus plantarum* group	99.10	MT604712	+	+	+	−	−	−
	B	G15	*Levilactobacillus brevis*	99.50	MT613460	+	+	+	−	−	−
	B	G16	*Pediococcus parvulus*	99.41	MF540542	+	+	+	−	−	−
	B	G17	*Levilactobacillus brevis*	98.53	MT640328	−	+	−	−	−	−
	B	G18	*Pediococcus parvulus*	98.94	MK575520	+	−	−	−	−	−
	B	G19	*Levilactobacillus brevis*	98.55	MT604645	−	−	−	−	−	−
	B	G20	*Pediococcus parvulus*	99.90	MF540542	+	+	−	−	−	−
2	G	G21	*Lacticaseibacillus casei* group	99.29	KU366368	+	−	−	−	−	−
	G	G22	*Lacticaseibacillus casei* group	99.91	MT903047	−	−	−	−	−	−
	G	G23	*Lacticaseibacillus casei* group	99.59	MZ930468	+	−	−	−	−	−
	G	G24	*Lacticaseibacillus casei* group	100	OK326465	−	−	−	−	−	−
	G	G25	*Lacticaseibacillus casei* group	98.82	MT903047	−	−	−	−	−	−
	G	G26	*Lacticaseibacillus casei* group	99.03	MT539077	−	−	−	−	−	−
	G	G27	*Lacticaseibacillus casei* group	98.74	CP029536	+	−	−	−	−	−
	G	G28	*Lacticaseibacillus casei* group	99.25	MT903047	−	−	−	−	−	−
	G	G29	*Lacticaseibacillus casei* group	99.10	JN133441	−	−	−	−	−	−
	G	G30	*Lacticaseibacillus casei* group	99.73	MN658813	−	−	−	−	−	−
	B	G31	*Lacticaseibacillus casei* group	99.76	MT539077	−	−	−	−	−	−
	B	G32	*Lacticaseibacillus casei* group	100	OK326465	−	−	−	−	−	−
	B	G33	*Lacticaseibacillus casei* group	99.88	NR_113823	−	−	−	−	−	−
	B	G34	*Lacticaseibacillus casei* group	100	NR_113823	−	−	−	−	−	−
	B	G35	*Lacticaseibacillus casei* group	100	NR_113823	−	−	−	−	−	−
	B	G36	*Lacticaseibacillus casei* group	99.62	NR_113823	−	−	−	−	−	−
	B	G37	*Pediococcus parvulus*	97.18	NR_113922	−	−	−	−	−	−
	B	G38	*Lacticaseibacillus casei* group	99.90	NR_113823	+	−	−	−	−	−
	B	G39	*Pediococcus parvulus*	99.58	NR_029136	−	+	−	−	−	−
	B	G40	*Lacticaseibacillus casei* group	99.62	NR_113823	−	−	−	−	−	−

[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; −, negative; +, positive colonies; m, mucoid colonies; L1, L2, L3, Listeria monocytogenes strain 1, strain 2, and strain 3, respectively. EPS, exopolysaccharides.

independent EPS.

As for the production of bacteriocins, no isolate showed inhibitory activity against the three *L. innocua* strains used as surrogate for *L. monocytogenes* (Table 7).

The result of acidification in the garlic-based medium assayed are showed in Fig. 2. In more detail, the pH value of the unfermented garlic-based medium attested at 5.50 ± 0.04. Regarding *L. brevis* isolates, a neat drop in pH was generally observed for most of the isolates after 4 days of fermentation, with isolate G15 reaching pH 4.24 ± 0.15 after 7 days and, the lowest pH value (3.58 ± 0.03) after 15 days. Moreover, for the isolates G5 and G17 a slower acidification performance was observed. Isolate G5 reached the mean value of 4.14 ± 0.47 after 14 days of fermentation, whereas isolate G17 reached the mean value of 4.25 ± 0.02 after 11 days of fermentation. As for *L. parabrevis* isolates, a neat drop in pH was generally observed for most of the isolates after 7 days of fermentation, with isolate G3 reaching the lowest pH value (3.33 ± 0.02) after 15 days of fermentation. Among the tested isolates, G12 showed a slow acidification performance, reaching pH 4.23 ± 0.06 after 15 days of fermentation. Regarding *P. parvulus* isolates, a neat drop in pH was generally observed for most of the isolates after 9 days of fermentation with isolate G39 reaching the lowest pH value (3.69 ± 0.02) after 15 days of fermentation. Finally, for *L. casei* group isolates, a neat drop in pH was generally observed for most of the isolates after 14 days of fermentation, with isolate G38 reaching the lowest pH value (3.89 ± 0.49) after 15 days of fermentation. Among the tested isolates, G31 reached pH value of 4.22 ± 0.01 after 14 days of fermentation, whereas G28, G30, G33, G34, and G40 reached pH values of 4.15 ± 0.03, 4.08 ± 0.01, 4.04 ± 0.03, 4.00 ± 0.08, and 4.18 ± 0.39 after 15 days of fermentation. A wide variability of the acidification performance among the tested isolates was generally observed (data not shown). pH values of uninoculated control garlic-based medium incubated at the same test conditions did not show pH variation during the 15-day assays.

4. Discussion

In Western countries, fermented vegetables are benefitting of an increase in the consumer's and media's interest (Thierry et al., 2023). However, only a few emblematic fermented vegetable products have massively attracted the interest of the scientific community, being olives, sauerkraut, cucumbers, kimchi, and vegetable juices (Thierry et al., 2023). Surprisingly, despite the wide diffusion of garlic as cultivated plant and its use in culinary practice, lacto-fermented garlic still

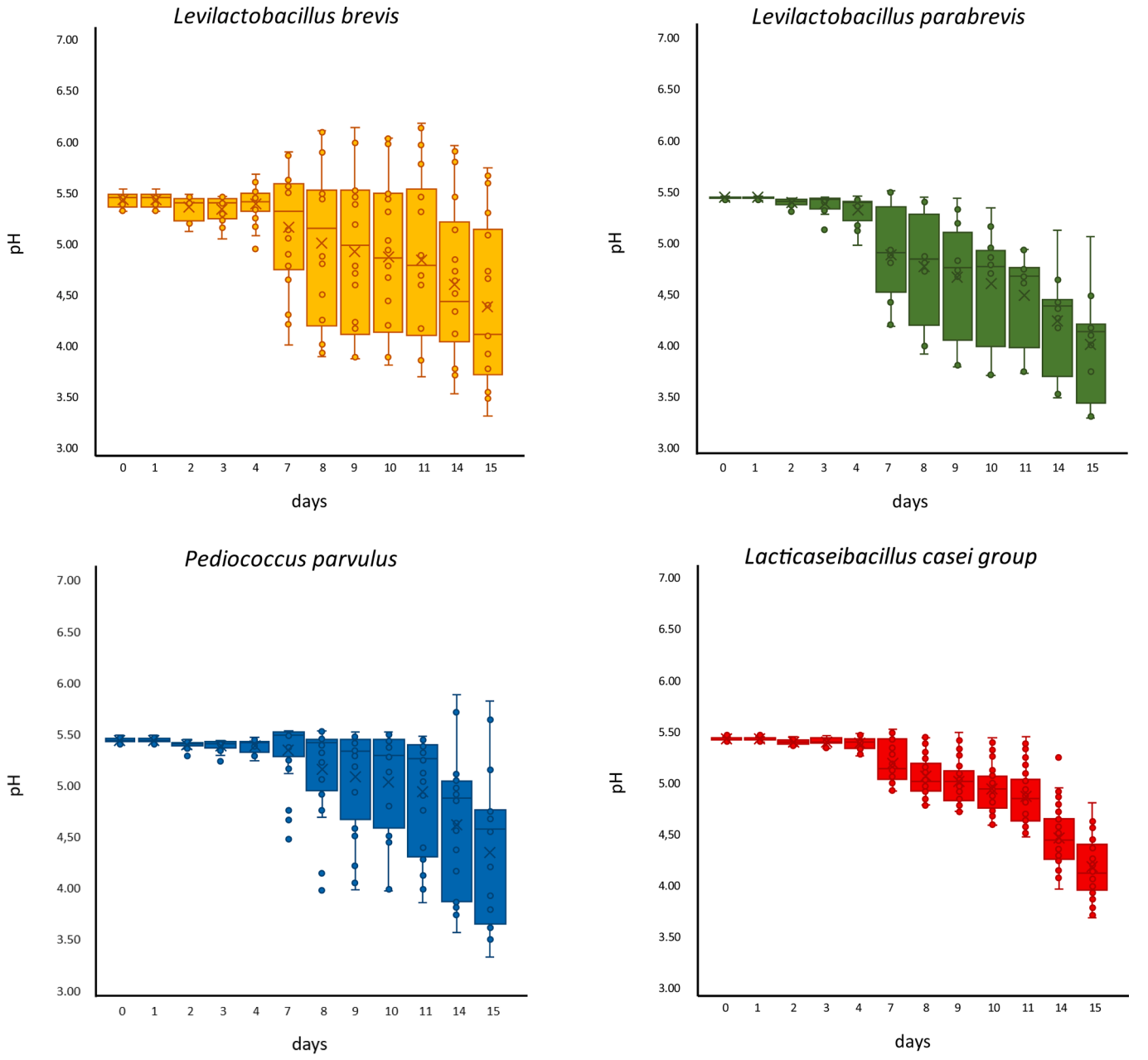

Fig. 2. Box plots summarizing the results of acidification performance of isolated Levilactobacillus brevis, Levilactobacillus parabrevis, Pediococcus parvulus, and Lacticaseibacillus casei group species in garlic-based growth medium from 0 to 15 days of fermentation. For each box, the bottom whisker 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.

represents an almost understudied fermented vegetable. Hence, to fill this gap, the microbial diversity, the physico-chemical and morpho-textural traits, and the volatile compounds of spontaneously lacto-fermented garlic cloves handcrafted in the Lower Silesia Region (Poland) were herein studied for the first time. Of note, given the scarceness of data on lacto-fermented garlic cloves, the results will mainly be discussed in comparison with those obtained for other fermented products of vegetable origin manufactured at artisan level.

The pH values detected in the studied samples of brine and lacto-fermented garlic cloves were consistent with a potent lactic acid fermentation. The results were in accordance with those reported by Hou et al. (2023) in fermented *Allium chinense* bulbs that, after 45 days of fermentation, showed pH values of 3.6.

The titratable acidity differed between batches; however, the levels were in accordance with those reported by Niu et al. (2022) who compared garlic cloves fermented with *Saccharomyces cerevisiae* or *L. plantarum,* thus suggesting the occurrence of a mixed-culture spontaneous fermentation.

Lactic acid was dominant in respect to acetic acid, and most of it was noted in garlic cloves rather than in brine. The levels of both lactic and acetic acids were in accordance with those reported by de Castro et al. (1998) for unblanched fermented garlic.

Reducing sugars were higher in garlic cloves, thus showing a still ongoing fermentation and hydrolysis process; the same trend was also observed for total polyphenol content. Total polyphenol content in fermented garlic cloves was in accordance with Ebrahimi Pure and Ebrahimi Pure (2016), who fermented garlic in different brines. As simple sugars act as prooxidants (Rahal et al., 2014), this could explain the

difference in total polyphenol content and antioxidant activity. Also, antioxidant activity was higher in garlic cloves than in brines, although not differentiated between producers or batches. The obtained results were in accordance with data reported by Boonpeng et al. (2014), who also observed that, for fermented garlic, the antioxidant activity was higher than in the fresh form.

The color of fermented garlic cloves was characterized by high L^* values, showing that the brine fermentation did not change the lightness of garlic cloves. The slight color change to green was visible as a^* was negative for all the samples, whereas b^* was pronounced towards yellow. The color change could also be due to the presence of other ingredients. However, de Castro et al. (1998) noted a similar shift towards green in unblanched fermented garlic.

Regarding viscosity values of brine samples, they were significantly different between the two producers, with product from producer 1 being less viscous. As for texture measurements performed on lacto-fermented garlic cloves, the obtained data were quite in accordance with the results obtained by de Castro et al. (1998), who studied blanched and unblanched fermented garlic obtained using a starter culture of *L. plantarum*. Specifically, de Castro et al. (1998) observed that the hardness values of fermented blanched garlic samples ranged from 16.5 N g^{-1} (after 5 days of storage) to 11.0 N g^{-1} (after 3 months of storage), a range not significantly different from those of fermented unblanched garlic samples (de Castro et al., 1998).

The combination of culture-dependent and −independent analyses allowed the major and minor microbial groups occurring in brine and lacto-fermented garlic cloves to be disclosed.

As for the viable counts, high counts of lactic acid bacteria were observed. The data obtained were in accordance with those detected by Hou et al. (2023) in *A. chinense* bulbs fermented under salt brine (1 % NaCl), showing a stable level of 8 log cfu g^{-1} during 45 days of fermentation. The high counts of lactic acid bacteria detected in the samples herein studied also attested the adaptation of members of this microbial group to the salt brine environment. As recently reviewed by Thierry et al. (2023), the main lactic acid bacteria species found in most traditional fermented vegetables are *L. plantarum*, *L. brevis*, *P. pentosaceus*, and *Leuconostoc mesenteroides*. Moreover, with lesser prevalence, *Limosilactobacillus fermentum*, *Lactococcus lactis*, together with other *Pediococcus* and *Leuconostoc* species were also detected (Thierry et al., 2023). Interestingly, lactic acid bacteria usually constitute only a small fraction of the microbiota naturally occurring in the plant raw material (Gustaw et al., 2021). However, based on their metabolism, lactic acid bacteria are able to rapidly utilize available plant nutrients (e.g., carbohydrates such as amylose and starch) in order to produce organic acids (e.g., lactic and/or acetic acid) that produce a drop in pH, together with the generation of compounds with biological activity (e.g., hydrogen peroxide, bacteriocins, etc.) (Gustaw et al., 2021). It is noteworthy that garlic is the only well-known natural dietary source of hydrogen sulphide (H_2S) donors (Tocmo et al., 2017). H_2S is a volatile molecule with important physiological and pharmacological functions, including regulation of cardiovascular functions and anticancer activities. It is suggested that the biological conversion of organopolysulphides to H_2S may explain the beneficial effects of high garlic consumption (Tocmo et al., 2017). Interestingly, Tocmo et al. (2017) reported that organosulphide profile and hydrogen sulphide-releasing activity in garlic were not negatively affected by fermentation with *L. plantarum*, thus maintaining the beneficial activity of garlic and its preservation.

As reported by Paludan-Müller et al. (1999), garlic has a double effect on microorganisms; on the one hand, it can stimulate the growth of lactic acid bacteria, on the other, the allicin contained in its bulbs inhibits the growth of other microorganisms. This feature could help in explaining the massive presence of lactic acid bacteria and the count < 1 log cfu g^{-1} of Enterobacteriaceae and eumycetes in the samples herein analyzed.

Furthermore, a low (moderate) amount of coagulase negative cocci was found in garlic clove and brine samples.

Coagulase-negative cocci associated with fermented foods usually belong to the genus *Staphylococcus* and are categorized into various groups namely *Staphylococcus epidermidis*-group, *Staphylococcus simulans*-group, *Staphylococcus saprophyticus*-group, and *Staphylococcus sciuri*-group (Khusro & Aarti, 2022). To the authors' knowledge no data regarding the occurrence of coagulase-negative cocci in lacto-fermented garlic are available in the scientific literature for further comparison of data. However, as reviewed by Behera et al. (2020), *Micrococcus* species have already been reported in traditional fermented pickles, thus suggesting that lacto-fermented garlic could represent a biological nice also for these microorganisms.

To the authors' knowledge, the present study represents the first attempt to elucidate the microbial diversity of lacto-fermented garlic cloves through metataxonomic analysis and the obtained data will be discussed considering other spontaneously fermented vegetables. The results of metataxonomic analysis were greatly in accordance with those of culture-dependent methods. Indeed, once again, lactic acid bacteria represented the most abundant group of bacteria detected in all the analyzed samples, with ASVs of *Levilactobacillus*, *Lactiplantibacillus*, *Latilactobacillus*, *Secundilactobacillus*, *Weissella, Leuconostoc*, *Lactococcus*, *Pediococcus*, and *Lacticaseibacillus* being detected.

Among the detected ASVs of lactobacilli, *Levilactobacillus* has already been isolated from salty vegetables (Xu et al., 2024), thus suggesting its adaptation to the osmotic conditions of brine. The genus *Levilactobacillus* encompasses heterofermentative species whose carbohydrate metabolism produces lactic and acetic acid, together with ethanol (Thierry et al., 2023). Strains of *Levilactobacillus brevis* were positively correlated with the synthesis of antioxidant-related compounds in fermented cauliflower stems (Zhang et al., 2024).

As for *Lactiplantibacillus*, Beato et al. (2012) reported a decrease in organosulfur content in garlic cloves fermented with *Lactiplantibacillus pentosus*, whereas Kim et al. (2016) observed an increase in organosulfur content following fermentation with *Lactiplantibacillus plantarum*. Hence, it is likely that different species of *Lactiplantibacillus* could differently interact with this peculiar food matrix, thus affecting nutritional and sensory features of the end product. Of note, similar trends were observed in previous studies on kimchi and pickles (Behera et al., 2020; Torres et al., 2020), thus confirming the adaptation of this genus of lactic acid bacteria to raw and fermented vegetable matrices.

Latilactobacillus species are among those most found in the natural microbiota of many fermented foods. As reviewed by Mota-Gutierrez and Cocolin (2021), *Latilactobacillus* has already been isolated from various plant sources (e.g., food crops, silage, flowers, food items, and compost), and was found among the major genera of fermented vegetables as kimchi and pickles (Daliri et al., 2023; Kim et al., 2022).

Secundilactobacillus comprises 14 validly published species of heterofermentative rods that are able of transforming pentoses into pyruvate to produce lactic acid via transaldolase/transketolase (Zhang et al., 2022). Species of *Secundilactobacillus* have already been isolated from fermented *zha-chili*, a popular traditional fermented food produced in China from grain and fresh chili (Dong et al., 2022; Zhang et al., 2022).

The occurrence of *Weissella* has already been reported by Torres et al. (2020) in fruit (e.g., blackberries, prunes, kiwifruits, and papaya), non-conventional sourdoughs from amaranth and chickpea, and fermented vegetables as kimchi, soybean, and rice (Sturino, 2018). As reviewed by Kavitake et al. (2020), species of *Weissella* are well-known EPS producers, including dextran, glucan, galactan, fructan, levan, and mannan, thus affecting the viscoelastic and rheological properties of fermented foods. In the analyzed brine and lacto-fermented garlic cloves no isolates of *Weissella* were obtained, hence the effect of eventually produced EPS can only be hypothesized.

Lacticaseibacillus has recently been used by Hajar-Azhari et al. (2023) to produce a lacto-fermented garlic sauce to be used in meat marinade. Interestingly, the obtained garlic-based fermented sauce improved the quality and safety of meat products. Indeed, the metabolic activity of

Lacticaseibacillus casei used as starter culture in garlic fermentation enhanced the metabolite production and increased the antioxidant activities of the obtained sauce (Hajar-Azhari et al., 2023).

Among lactococci, *Leuconostoc, Lactococcus,* and *Pediococcus* were detected.

As for *Leuconostoc*, Tong et al. (2021) observed a reduction in the strong pungent odour related with thiosulfinate compounds in *Allium hookeri* fermented by *Leuconostoc mesenteroides*. Thiosulfinate compounds are volatile molecules responsible for the usual pungent flavour associated with plants of the *Allium* genus. Hence, it is likely that the metabolic activity of *Leuconostoc* spp. in the analyzed lacto-fermented garlic samples leads to a depletion in thiosulfinate compounds, thus contributing to a more palatable end product. *Leuconostoc* spp. were also detected among the microbial populations in brine-fermented *A. chinense* (Hou et al., 2023), and as part of the autochthonous microbiota occurring in garlic roots (Janes et al., 1999), thus likely explaining the presence of this taxon in the samples herein analyzed.

As for *Lactococcus*, although species of this genus are typically associated to dairy products, it has recently been detected among the predominant microbial genera occurring in the brine and the bulbs of naturally fermented *A. chinense* samples (Hou et al., 2023). However, to the authors' knowledge, there is a lack of scientific literature regarding *Lactococcus* in fermented garlic for further comparison of data.

A shortage of data is also available regarding the occurrence of *Pediococcus* in lacto-fermented garlic. Hence, the results obtained in the present study represent a contribution in the knowledge of the environmental niches harbouring *Pediococcus* species. However, it is already known that *Pediococcus* species are among those most involved in lactic acid fermentation of vegetables (Torres et al., 2020).

Volatile aroma profile of the analyzed fermented garlic clove and brine samples is the result of the unique aroma associated with garlic due to its sulphur-based compounds, the spices used in the recipe for its preparation, and the fermentative activity of autochthonous microorganisms. Furthermore, the processing conditions, such as pH, temperature, and ageing (storage time) play a crucial role in determining the flavour and aroma of processed garlic.

Usually, raw garlic is characterized by the intense smell and spiciness of allicin, one of the main thiosulfinates in fresh garlic, which is very unstable and easily converted into a variety of volatile compounds during processing ((Yılmaz Oral and Kaban, 2023). As also reported by Abe et al. (2020), allicin can undergo several transformations, including organosulfur compounds, such as diallyl, methyl allyl, and diethyl mono-, di-, tri-, tetra-, penta- and hexasulfides and vinyldithiins, which affect the aroma profile of the final product. However, the pungency of allicin may severely limit the use and consumption of fresh garlic, thus increasing the demand for alternative garlic products, such as black garlic, which does not have an intense odor (Chang et al., 2023), or lacto-fermented garlic.

In the present study, the predominant flavor components occurring in the samples of the two producers were the sulfur compounds. In more detail, diallyl disulfide, diallyl sulfide, methyl 2-propenyldisulfide, and sulfide allyl methyl were found in the highest amount in the garlic cloves. These pungent smelling sulfides are formed by the decomposition of thiosulfinates during the processing of fresh garlic (Abe et al., 2020). Several studies have shown that these sulfur compounds have various health benefits. In particular, diallyl sulfide has been reported to inhibit the cancers of mammary gland, stomach, colon, and lung. Similarly, diallyl disulfide has also been found to be effective against cancers of colon, renal, skin, mammary glands, and esophagus (Bansal et al., 2018). Furthermore, as reviewed by Bayan et al. (2014), the beneficial effect of garlic on diabetes mellitus is mainly attributed to the presence of volatile sulfur compounds, such as alliin, allicin, diallyl disulfide, diallyl trisulfide, diallyl sulfide, S-allyl cysteine, ajoene, and allyl mercaptan. In addition, Nakamoto et al. (2020) observed that the antimicrobial activity of garlic was influenced by the concentration and type of organosulfur compounds present. For example, diallyl disulfide and ajoene have been reported to inhibit the growth of various bacteria, including methicillin-resistant *S. aureus* strains (Nakamoto et al., 2020).

All the samples were also characterized by high levels of alcohols, mainly ethanol, most likely derived from microbial metabolism during fermentation.

In addition, monoterpene hydrocarbons were found in high amount, especially in the samples of producer 2. These compounds are usually present in garlic at lower level than other volatile compounds such as sulfur compounds. The presence of spices such as allspice, peppercorns, bay leaves, dried dill flowers and stalks, horseradish root, and mustard, which were used in the preparation of the analyzed fermented garlic, certainly influenced the component of monoterpene hydrocarbons and oxygenated monoterpenes content. Indeed, discrete amounts of aromatic compounds typical of these plants, such as α-phellandrene, limonene, β-phellandrene, o-cymene, and p-cymene, were found in the aroma profile composition of garlic clove and brine samples.

Esters and acetate, which are responsible for fruity aroma of garlic, were found mainly in the brine and in the sample of producer 2. The most abundant acetate was ethyl acetate, which is a key ester contributing to the overall aroma profile of garlic, providing fruity notes to its aroma (Abe et al., 2020).

In addition, acetic acid was an important component in lacto-fermented garlic, contributing to its qualitative characteristics, and highlighting the important role of the fermentative activity of the naturally occurring microorganisms.

Aldehydes, mainly 2-butenal, were found only in the sample of producer 1. Abe et al. (2020) found that aldehydes are dominant compounds in black garlic, whereas esters are important aroma compounds in aged garlic extract.

In conclusion, the lacto-fermented garlic samples analyzed in this study had a unique flavor profile, combining the characteristics of fresh garlic and the added spices with additional tangy and sour notes from the fermentation process. Hence, the mellowing effect of fermentation can lead to a more nuanced and complex flavor profile.

As for the microbial isolates obtained in the present study, the results were consistent with the main ASVs emerged by the metataxonomic analysis. Indeed, the species *P. parvulus*, *L. brevis*, *L. parabrevis*, and *L. plantarum* group were isolated from samples of producer 1, whereas *L. casei* group represented the most frequently isolated lactic acid bacteria species in samples from producer 2. Interestingly, among the isolates, *L. brevis* and *L. parabrevis* represented the most isolated lactic acid bacteria species during spontaneous leek (*Allium ampeloprasum* var. *porrum*) fermentations, thus confirming their adaptation to the Alliaceae environment (Wouters et al., 2013).

Regarding the presence of the *hdcA* gene detected in *L. brevis* isolates, Ferrante & Mercogliano (2023) already reported that this species of heterofermentative obligate non-starter lactic acid bacteria can be involved in histamine formation in dairy products. To the authors' knowledge, there is a lack of information in the scientific literature concerning the potential histamine formation carried out by *L. parabrevis* in food; hence, the results of the present research could provide a novel insight into this specific feature of *L. parabrevis*. As for *P. parvulus*, this species has already been found capable of producing histamine in wine ((Landete, Ferrer, Polo, & Pardo, 2005)). Among the *L. casei* group isolates, only a few members showed the presence of the *hdcA* gene. Of note, members of the *L. casei* group were generally found able to reduce the content of histamine in fermented foods (e.g., dairy products) (Herrero-Fresno et al., 2012; Klementová et al., 2024); however, Komprda, Sládková, Petirová, Dohnal, & Burdychová (2010) identified histamine-producing *L. casei/paracasei* isolates in fermented sausages. It is noteworthy that the presence of the *hdcA* gene in the lactic acid bacteria isolates herein studied does not necessarily represent a real risk for the consumers, but only a potential threat, since the production of histamine in foods is affected by many process parameters. In more detail, low storage temperature can inhibit the production of histamine, together with the addition of salt (Ferrante & Mercogliano, 2023).

Hence, despite the presence of *hdcA* gene-positive lactic acid bacteria, the application of good manufacturing practices can help in reducing the histamine risk in lacto-fermented garlic.

A very low number of isolates (*L. parabrevis*, *L. plantarum* group, and *P. parvulus*) showed the in-vitro production of EPS. Of note, the production of these biopolymers in foodstuffs can have various effects on their sensory traits. Indeed, on the one hand, EPS can positively modify texture, flavor, and taste of food by affecting rheology, on the other hand, EPS in brined foods can result in ropy appearance of the brine, thus affecting consumers' appreciation of the end product (Magnússon & Möller, 1985). Hence, the use of EPS-producing starter or adjunct cultures should carefully be evaluated.

As for the antimicrobial activity against *L. innocua*, no isolate showed bacteriocin production against any of the tested strains, hence, data will not be further discussed.

Regarding the acidification performance, the lactic acid bacteria herein tested showed good adaptation to the garlic-based growth medium. In more detail, after 4 days of fermentation, the pH of the growth medium progressively decreased to values below 3.50. As reported by Paludan-Müller, Gram, & Rattray (2002), in garlic, carbohydrates are mainly represented by fructans (up to 28 %), together with traces of sucrose, fructose, and glucose (<1%). The fermentation of fructans (e.g., inulin) has already been reported for several lactic acid bacteria including lactobacilli and pediococci (Paludan-Müller, Gram, & Rattray, 2002). Interestingly, Sunu, Sunarti, Mahfudz, & Yunianto (2019) observed a prebiotic effect of garlic on the growth of lactic acid bacteria, thus supporting the results on the acidification performance of the isolates studied in the present research.

5. Conclusions

The analysis of lacto-fermented garlic samples revealed significant nutritional attributes, particularly noteworthy for their antioxidant activity, thus encouraging the popular beliefs regarding the health-promoting qualities of this culinary specialty.

Microbiological profiling of the fermented clove and brine samples allowed for the identification of predominant species, including *Levilactobacillus*, *Lactiplantibacillus*, *Latilactobacillus*, *Secundilactobacillus*, *Weissella*, *Leuconostoc*, *Lactococcus*, *Pediococcus*, and *Lacticaseibacillus*.

The tested lactic acid bacteria isolates exhibited a good acidification performance when inoculated in the salty garlic-based medium, suggesting their potential use as starter or adjunct cultures for controlled garlic lacto-fermentation. The detection of the *hdcA* gene in some isolates underscored the importance of thoroughly characterizing starter cultures to mitigate potential adverse effects on consumer health. Furthermore, the observation of in-vitro EPS production by a few isolates suggests the need for additional investigation to ascertain their impact on brine sensory attributes.

HS-SPME-GC/MS analysis unveiled a diverse and complex volatilome, comprising over 80 volatile organic compounds (VOCs), thus contributing to elucidating the aromatic profile of the studied Polish delicacy.

The abundant presence of pro-technological microorganisms in the analyzed brine suggests the use of this substrate as a natural source for starter cultures in garlic fermentation via the back-slopping technique.

CRediT authorship contribution statement

Federica Cardinali: Writing – original draft, Investigation, Formal analysis. **Cristian Botta:** Investigation, Formal analysis. **Joanna Harasym:** Writing – original draft, Resources, Investigation. **Ilario Ferrocino:** Resources, Investigation, Formal analysis. **Anna Reale:** Resources, Investigation, Formal analysis. **Floriana Boscaino:** Investigation, Formal analysis. **Tiziana di Renzo:** Investigation, Formal analysis. **Vesna Milanović:** Resources, Investigation, Formal analysis. **Cristiana Garofalo:** Resources, Formal analysis. **Giorgia Rampanti:** Investigation, Formal analysis. **Lucia Aquilanti:** Writing – review & editing, Resources. **Andrea Osimani:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.114484.

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