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RESEARCH ARTICLE

Identification of promising lactic acid bacteria for the fermentation of lupine- and faba bean-based substrates to produce refreshing protein-rich beverages—A strain screening

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Abstract

Lupines and faba beans are rising stars among legumes as sources of valuable, vegan plant-based proteins. To enter new application areas like the production of protein-rich refreshing beverages, the typical beany aroma impression has to be overcome, and the sensory appearance has to be improved, as it can be accomplished with lactic acid fermentation. An extensive strain screening of 70 lactic acid bacteria from 16 genera was performed to identify suitable strains to transform substrates made from lupines and faba beans into refreshing beverages and to improve their sensory characteristics. By analyzing carbohydrate utilization, production of organic acids and aroma compounds, and sensory appearance, 22 strains for lupine and eight strains for faba bean were preselected. Subsequently, the most suitable strains (five for lupine and three for faba beans) were identified by a trained sensory panel, and finally their growth kinetics were discussed. Generally, the aroma profile varied highly with the utilized strain. However, by selecting suitable strains, the beany impression can be highly reduced and pleasant aroma impressions (e.g., fruity and buttermilk) can be added. Most strikingly, it was proven that using germinated lupines and faba beans instead of raw ones can bypass the usual growth restriction, and the strain selection can be focused exclusively on sensory aspects. This opens the option to use strains usually excluded for the fermentation of legumes due to their lack of utilization of the legume-typical α-galactosides.

KEYWORDS
aroma analysis, fermentation, lactic acid bacteria, legumes, sensory evaluation, strain screening

1 | INTRODUCTION

Fermentation was used by humankind in the production of food and beverages for thousands of years. The advantages are food preservation (e.g., by reducing the pH value), the introduction of pleasant aroma compounds (e.g., banana-like isoamyl acetate in wheat beers) and organic acids (e.g., lactic acid), the reduction of antinutritive compounds (e.g., flatulence-causing oligosaccharides), the increase of nutraceutical value by microbial metabolites (e.g., γ-aminobutyric acid; Pannerchelvan et al., 2023; Sarasa et al., 2020) or changes of the food matrix (e.g., coagulation of yogurt). While yeast is probably the best-known microorganism employed in the fermentation of beverages (e.g.,

beer, wine), other microorganisms are successfully used traditionally as well as in innovative drinks. Kombucha, a traditional Chinese tea-based beverage, is produced by using a mixed fermentation of yeast, acetic acid, and often lactic acid bacteria (LAB; Baschali et al., 2017; Villarreal-Soto et al., 2018). Other examples are water kefir, which is made by inoculating water, sugar, and sometimes dried fruit with yeast, acetic acid, and LAB (so-called kefir grains; Baschali et al., 2017; Moretti et al., 2022), or kvass, a beverage based on stale bread, malt, or flour and fermented with a mixed starter culture of LAB and yeasts (or traditionally by using the household's sourdough stock; Baschali et al., 2017). Today, we face an increasing demand for functional and healthy refreshing beverages (e.g., kombucha) because consumers in the Western world are becoming more and more aware of sugar consumption, health benefits, and nutritional aspects (Baschali et al., 2017; J. Kim & Adhikari, 2020; Moretti et al., 2022).

In parallel with the advent of functional fermented beverages, the market for plant-based alternatives to substitute dairy or meat products strongly evolved. In recent years, legumes raised attention as a valuable source of plant proteins and besides the long-known soybean, other legumes like peas, chickpeas, or peanuts, and lately lupines and faba beans, have risen to new attention (Nawaz et al., 2020; Tangyu et al., 2019). However, despite this trend to use legumes due to their valuable plant protein and their advantage of being grown in temperate climate zones, their application in beverages is still marginal. Besides several legume-based milk substitutes (Tangyu et al., 2019) and the innovative approach to using faba beans in the production of beer (Black et al., 2019; Viking Malt, 2023), no refreshing beverages based on lupines or faba beans were marketed so far.

LAB are a promising microorganism for fermenting such protein-rich legume-based beverages. LAB can reduce the unpleasant beany aroma of legumes (Singh & Vij, 2018; Tangyu et al., 2019; Wang et al., 2021), which is often regarded as a major hindrance in the use of legumes in food and beverage applications (Singh & Vij, 2018; Tangyu et al., 2019). This is achieved by reducing compounds with a negatively associated odor (e.g., hexanal, contributes to the "beany" aroma) as well as by adding pleasant aroma compounds (e.g., β-damascenone, evokes a fruity aroma), and therefore highly improves the character of a beverage. Moreover, LAB produce lactic acid, which prolongs shelf life and adds a very refreshing character to a beverage. Additionally, LAB are known to possess a wide variety of intra- and extracellularly active proteolytic enzymes (Kieliszek et al., 2021; Kuerman et al., 2024; Lim et al., 2019; Savijoki et al., 2006; Singh & Vij, 2018; Xie & Gänzle, 2024), which can help to decompose proteins in the substrate and improve solubility once the acidification reaches the isoelectric point of plant proteins (usually around pH 4–4.5; Ritter et al., 2023; Schlegel, Sontheimer, Eisner, & Schweiggert-Weisz, 2019; Schlegel, Sontheimer, Hickisch, et al., 2019; Vogelsang-O'Dwyer, Bez, et al., 2020; Vogelsang-O'Dwyer, Petersen, et al., 2020), which coincides with the pH value of refreshing beverages. Last but not least, food-related LAB are well known and accepted in food products by consumers.

Besides the applied process design and the type of microorganism employed for the fermentation (e.g., yeast or LAB), the aroma of a final beverage varies highly by the choice of the individual strain. It might seem natural to use strains, which are successfully employed for other food products, as those are accepted as safe for human consumption and known to produce pleasant flavors. However, as the substrate changes, so does the strain's growth performance and flavor production (Tangyu et al., 2023). This can be explained by the variety of plant-based substrates and their individual composition (e.g., phytochemical content).

Whereas mono- and disaccharides (e.g., glucose, fructose, saccharose) could be metabolized by several LAB, those bacteria are often very specific regarding their use of oligosaccharides (Gänzle & Follador, 2012). The lack of required enzymes (e.g., α-galactosidase) disables certain strains to grow on the legume-typical storage carbohydrates raffinose, stachyose, and verbascose. This can be seen clearly in the strain screening from Harlé et al. (2020) in soy juice, where only 6% out of 276 strains could grow on stachyose. Moreover, strains might also grow but retain considerable amounts of α-galactosides after fermentation (Champagne et al., 2009; Donkor et al., 2007; Singh & Vij, 2018; Tangyu et al., 2021), which trigger flatulence and would reduce consumer acceptance for beverages.

Comparably, the structure of the proteins as nitrogen source needs to be considered. For some LAB, the complex protein structure is not hindering, as they express extracellular peptidases to decompose proteins and transport amino acids and peptides into the cell to be metabolized (Kieliszek et al., 2021; Savijoki et al., 2006). However, for the sourdough fermentation of wheat, it is known that LAB reduce disulfide bonds, which leads to an increased accessibility of proteins to proteolysis by cereal enzymes (Gänzle et al., 2008). To our best knowledge, comparable mechanisms were not reported for legumes, so far. However, even as legumes are described as poor in sulfuric amino acids, lupines and faba beans contain considerable amounts of disulfide bonds forming cysteine, while lacking methionine (Kaczmarek et al., 2016; Labba et al., 2021; Roman et al., 2023; Siegert et al., 2021; Sujak et al., 2006). Therefore, the combination of germination, addition of technical enzymes, and LAB fermentation might result in a combination of proteolytic events, which lead to the strong degradation of complex legume proteins. Additionally, intracellular peptidases are released into the substrate due to cell lysis and decompose high molecular proteins extracellularly. Nevertheless, the production of short peptides might also increase the bitter perception of a beverage (Kohl et al., 2013; Maehashi & Huang, 2009; Schlegel, Sontheimer, Eisner, & Schweiggert-Weisz, 2019; Schlegel, Sontheimer, Hickisch, et al., 2019). Moreover, the amino acid composition highly affects the aroma. While branched-chain amino acids like isoleucine and leucine lead to 2- and 3-methylbutanal (Ardö, 2006; Serrazanetti et al., 2011) with sweet and malty odors (Kreissl et al., 2022), methionine can be metabolized to methional (Amárita et al., 2001; Ardö, 2006), which is described as unpleasantly cooked-potato-like (Kreissl et al., 2022). Among different legumes, the nutritive and antinutritive compounds can vary strongly. Starch is not measurable in lupines (Torres et al., 2005), whereas it is the main carbohydrate storage compound in most legumes (e.g., faba beans; Ambigaipalan et al., 2011; Duc et al., 1999; Hood-Niefer et al., 2011; Setia et al., 2019). Knowing that a particular strain grows well and produces a pleasant flavor in one legume-based substrate does

not imply that it can be used in the substrates from a different legume (Tangyu et al., 2023). Consequently, a structured and extensive strain screening is required to find suitable strains for the specific legume. In addition to the ability to grow in the individual legume substrates, the produced aroma compounds are of utmost importance, as they are crucial for consumer acceptance and, therefore, decisive for the success or failure of a new product.

Germination proved to be a feasible process step to entirely decompose the flatulence-causing oligosaccharides while releasing fermentable glucose, fructose, and saccharose. Moreover, plant protein is enzymatically broken down to lower molecule peptides and amino acids, increasing protein solubility significantly (Ritter et al., 2023). Therefore, we hypothesize that using germinated legumes instead of raw legumes for substrate preparation is very promising to highly increase the number of possible strains for fermentation. This would allow to focus the strain screening on the resulting strain-specific aroma and taste character of the final product and therefore on its overall sensory profile instead of being strongly limited by the carbohydrate utilization and growth performance.

The aim of this study was to identify LAB strains that introduce an appealing aroma and taste in refreshing, protein-rich lupine- and faba bean-based beverages. Besides the flavor, proper acidification and protein solubility in the acid pH range were of importance to accomplish a refreshing character and maintain a high protein content. Therefore, 70 strains from 16 LAB genera were used to ferment lupine- and faba bean-based substrates. Those substrates were prepared from germinated legumes, using a mashing step (adapted from the substrate production in the brewing process) to facilitate microbial growth and therefore focus on the flavor aspects. After considering changes in the analytically observed volatile, non-volatile, and sensory profiles of the substrates, unsuitable strains were excluded, and the number of strains was narrowed down to the promising candidates. In the second part, the growth kinetics of several selected strains in the two legume-based substrates are discussed, and based on an extended sensory analysis, the most promising strains are recommended for producing lupine- and faba bean-based refreshing beverages.

2 | MATERIAL AND METHODS

2.1 | Chemicals

All calibrations and identifications using gas and liquid chromatography were performed with commercially available analytical standard compounds. The following aroma standards were purchased from Sigma-Aldrich: 3-methylbutanal (CAS 590-86-3), 2-methylbutanal (CAS 96-17-3), hexanal (CAS 66-25-1), benzaldehyde (CAS 100-52-7), octanal (CAS 124-13-0), octan-1-ol (CAS 111-87-5), 2-methylbutan-1-ol (CAS 137-32-6), 3-methylbutan-1-ol (CAS 123-51-3), hexan-1-ol (CAS 111-27-3), (*E*)-oct-3-en-2-one (CAS 1669-44-9), oct-1-en-3-ol (CAS 3391-86-4), 2-ethylhexan-1-ol (CAS 104-76-7), 5-pentyloxolan-2-one (*γ*-nonalactone) (CAS 104-61-0), ethyl hexanoate (CAS 123-66-0), and (*E*)−4-(2,6,6-trimethylcyclohexen-1-yl)but-3-en-2-one (*β*-damascenone) (CAS 23696-85-7). The internal standards, ethyl-2-methylpentanoat (CAS 39255-32-8), 4-fluorobenzaldehyde (CAS 459-57-4), and 5-methyl-2-propan-2-ylcyclohexan-1-ol (menthol) (CAS 2216-51-5) were supplied by Sigma-Aldrich. For the carbohydrate analysis, glucose (CAS 50-99-7), fructose (CAS 57-48-7), saccharose (CAS 57-50-1), maltose (CAS 6363-53-7), and the internal standard 2-deoxy-D-glucose (CAS 154-17-6) were purchased from Sigma-Aldrich. 2-hydroxypropanoic acid (lactic acid) (CAS 79-33-4), acetic acid (CAS 64-18-7), and phosphoric acid (CAS 7664-38-2) for analyzing the organic acids were purchased from Sigma-Aldrich. For the various eluents and sample dilutions, sodium hydroxide (CAS 1310-73-2) and methanol (CAS 67-56-1), sodium dihydrogen phosphate dihydrate (CAS 13472-35-0), and acetonitrile (CAS 75-05-8) were acquired from VWR International. The total nitrogen analysis was performed using Kjeltabs Cu-3.5 from FOSS Analytical; sulfuric acid 95% (CAS 7664-93-9) and hydrogen peroxide (CAS 7722-84-1) from VWR International; sulfuric acid 0.05 M (CAS 7664-93-9) and sodium hydroxide 32% (CAS 1310-73-2) from neoFroxx; and 2-[[4-(dimethylamino)phenyl]diazenyl]benzoic acid (methyl red) (CAS 493-52-7) and 2,6-dibromo-4-[3-(3,5-dibromo-4-hydroxy-2-methylphenyl)−1,1-dioxo-2,1*λ*6-benzoxathiol-3-yl]−3-methylphenol (bromocresol green) (CAS 76-60-8) from Merck. The free amino nitrogen (FAN) analysis was performed with disodium hydrogen phosphate dehydrate (CAS 10028-24-7) from VWR International; potassium dihydrogen phosphate (CAS 7778-77-0), fructose (CAS 57-48-7), and potassium iodate (CAS 7758-05-6) from Merck; 2,2-dihydroxyindene-1,3-dione (ninhydrin) from AppliChem; and ethanol from Supelco. deMan, Rogosa and Sharpe (MRS) agar ISO and MRS broth for microbial cultivations were purchased from Th. Geyer.

2.2 | LAB

The strains screened in this study were obtained from the strain selections of the DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany), the Chair of Microbiology (Technical University of Munich, Freising, Germany), and the Chair of Brewing and Beverage Technology (Technical University of Munich, Freising, Germany). The 70 strains from 16 genera (one *Agrilactobacillus*, eight companilactobacilli, 10 lacticaseibacilli, eight lactiplantibacilli, 14 lactobacilli, one *Lapidilactobacillus*, one *Laticaseibacillus*, three latilactobacilli, one *Lentilactobacillus*, one *Leuconostoc*, two levilactobacilli, five liquorilactobacilli, five loigolactobacilli, six pediococci, three schleiferilactobacilli, and one *Streptococcus*) are listed together with information regarding their isolation background in Table S1 in the Supporting Information. No preselection was performed to ensure growth on legume-based substrates. Strains used in this study are named according to Zheng et al. (2020). When references are addressed in the discussion, species names were translated into the current nomenclature using the database (http://lactobacillus.ualberta.ca) based on the taxonomic note of Zheng et al. (2020).

2.3 | Samples

The low-vicine/covicine faba beans *Vicia faba* var. TIFFANY were obtained from Norddeutsche Pflanzenzucht Hans-Georg Lembke KG. Sweet lupines *Lupinus angustifolius* var. BOREGINE were provided by Saatzucht Steinach GmbH & Co KG.

2.4 | Substrate production

The germination of lupines and faba beans was performed according to Ritter et al. (2023). Briefly, seeds were soaked in water at 20°C for 4 h (faba beans) or 3.5 h (lupines). Afterward, the seeds were placed in trays (approx. 2.4 kg per tray) and kept in a germination chamber (Viessmann) at 20°C. To reach a water content of 52% and 62% for faba beans and lupines, respectively, further soakings for 10 min were performed for 4 to 5 days. On the seventh day, germs were dried in a pilot malting plant for 24 h at 50°C to obtain a storable malt. The malt was ground to pass a 1–mm mash in a laboratory mill ZM200 (Retsch). To transfer proteins, sugars, and so forth into the liquid substrate for the fermentations with different strains, the flour was mashed in a laboratory mashing device BMW12/CPU (Dinkelberg Analytics) to further enzymatically decompose the major storage compounds.

After mashing in at 45°C, the proteolytic enzyme Neutrase 0.8 L (Novozymes) was added, and the temperature was held for 30 min to facilitate enzymatic proteolysis. Subsequently, the temperature was increased (heating rate: 1 K/min) to 60°C, and the phytic acid cleaving enzyme Phytase L and the amylolytic enzyme Ceremix Flex (both Novozymes) were added. After 30 min, the temperature was increased to 70°C (for 20 min) and afterwards to 80°C (for 20 min). The temperature rests were chosen according to the temperature optima of the enzymes and the gelatinization temperature of faba bean starch (68°C) determined in pre-experiments (data not shown). Afterward, the mash was centrifuged (10 min, 4000 g, ambient temperature) in a Multifuge 4 KR centrifuge (Thermo Electron LED) and subsequently filtered to remove malt particles using folded filters (Macherey-Nagel). The liquid substrate was frozen until used in fermentation experiments.

2.5 | Preparation of inocula

Strains were taken from cyro storage and cultivated in MRS broth one week before the screening experiments. Faba bean- and lupine-based substrates were inoculated 1:50 (v/v) with MRS culture to adapt the strains to the legume substrates. After 24 h at 28°C, pH (FiveEasy F20 pH meter, Mettler-Toledo) and optical density (600 nm) were measured (Genesys 10 S UV-Vis photometer, Thermo Scientific), to ensure proper growth. This adapted culture was then used as inoculum for the screening experiments.

2.6 | Fermentation

Lupine and faba bean substrates were inoculated with log8 cells per mL of the adapted pre-cultured strains. After approx. 24 h at 28°C, samples were taken to analyze volatile compounds, carbohydrates, organic acids, and total and free nitrogen. To remove cells and to avoid further changes in the samples due to LAB metabolism, the samples were centrifuged using a Universal 320 R centrifuge (Hettich Zentrifugen; 5 min, 2800 g, ambient temperature) and filtered through 0.4-μm dead end filters (Macherey-Nagel). The internal standard mixture (see section 2.10 gas chromatography-mass spectrometry [GC-MS] analysis) was added for the aroma analysis before centrifuging and filtering. All samples were frozen and stored at −20°C until further analysis. Additionally, samples for the sensory evaluation were taken and stored at approx. 7°C. Furthermore, pH and optical density (600 nm) were measured using a pH meter FiveEasy F20 pH meter (Mettler-Toledo) and Genesys 10 S UV-Vis photometer (Thermo Scientific), respectively. All fermentations were performed in biological triplicates.

2.7 | Carbohydrate analysis

Soluble carbohydrates were analyzed based on MEBAK online (2020c), using high-performance anion exchange chromatography with a pulse amperometric detector on a Dionex ICS-5000 system (Thermo Fisher Scientific) with a Dionex CarboPak PA10 analytical (2 × 250 mm) analytical column and Dionex CarboPak PA10 guard (2 × 50 mm) guard column (both Thermo Fisher Scientific). The mobile phases were (A) 0.250 M sodium hydroxide and (B) high-performance liquid chromatography (HPLC)-grade water. The gradient was 20% A at 0 min, 20% A at 10 min, 90% A at 11 min, and 90% A at 19 min, followed by 10 min of equilibration at 20% A. The flow was set to 0.25 mL/min, and 1 μL was injected. The method was calibrated for the analytes glucose, fructose, saccharose, and maltose, obtaining regression coefficients of 0.99 or higher. Spiking experiments confirmed recovery rates of 89%–109%. Detailed information regarding the validation is given in Table S2 in the Supporting Information. Samples were prepared by defreezing, diluting with methanol (50%), and mixing with the internal standard 2-deoxy-glucose. All analyses were performed in technical duplicates.

2.8 | Organic acid analysis

The organic acids were measured based on MEBAK online (2020d) by HPLC-UV detector on an Ultimate U3000 system (Thermo Fisher Scientific Inc.). The mobile phase consisted of a Synergi 4 μm Hydro-RP column (4.6 × 250 mm) with a C18 SecurityGuard Cartridge (4 × 3 mm) (Phenomenex). As mobile phase (A) 0.01 M sodium dihydrogen phosphate solution adjusted to pH 2.2 with phosphoric acid (85%) and (B) methanol was utilized. The gradient was 100% A at 0 min, 100% A at 10 min, 20% A at 14 min, and 20% A at 18 min, followed by 5 min

of equilibration at 100% A. The flow was set to 0.7 mL/min, and the injection volume was 30 μL. All analytes were measured at 210 nm. Calibration curves were obtained for lactic acid and acetic acid with regression coefficients of 0.99 or higher, and the recovery rates were 98%–103%. Detailed information regarding the validation is given in Table S3 in the Supporting Information. All analyses were performed in technical duplicates.

2.9 | Total and free nitrogen analysis

The total nitrogen in the supernatant was measured according to MEBAK online (2020a) with a Kjeldahl method, a Kyeltec 8460 Analyze Unit with Sampler 8460, a Tecator Digestor Auto, and a Tecator Scrubber (all FOSS Analytical, Höganös). The conversion factor 5.4 was used for both legumes as recommended for edible legumes (Mariotti et al., 2008). FAN was measured in the supernatant of the samples using the ninhydrin method MEBAK online (2020b). The measurement was performed photometrically at 570 nm in a Synergy H1 multiplate reader (BioTek Instruments). Proline was used as a reference for calibration. The calibration curve showed high linearity ($R^2 = 0.999$) in the 0–400 mg/L linear range. Total nitrogen was analyzed in technical duplicates and FAN in technical quadruplicates.

2.10 | Headspace solid phase microextraction (SPME) GC-MS (headspace-SPME arrow-GC-MS)

The frozen samples containing the internal standard mixture (menthol, ethyl-2-methylpentanoate, 4-fluorobenzaldehyd) were defrozen, and 5 mL were transferred into a 20 mL glass vial and immediately sealed airtightly using an aluminum cap. The 1.1-mm SPME arrow fiber 110 μm 20 mm (Thermo Scientific) used for the aroma extraction was coated with divinylbenzene/carbon wide range/polydimethylsiloxane and conditioned for 1 min at 40°C, before analysis. Samples were positioned in the autosampler (thermostable at 17°C), then individually preheated for 0.5 min at 40°C, and exposed to the SPME for 30 min at 40°C while shaking vigorously. Afterward, the volatiles adsorbed at the phase were desorbed thermally at 250°C for 1 min within the GC system Trace 1310 (Thermo-Scientific). The sample was injected with a split ratio of 1:5 onto the low-polar TG-5MS column (length 60 m, inner diameter 0.25 mm, film thickness 0.25 μm; Thermo Scientific). The GC oven was initially set to 60°C. After 4 min, the temperature was increased at a heating rate of 5 K/min to 225°C and finally with 10 K/min to 250°C and held for 4 min. The compounds were detected in an ISQ OD mass spectrometer (Thermo Scientific) in electron ionization (EI) mode adjusted to full scan (m/z 35–350) with a dwell time of 0.2 s. All aroma analyses were performed in technical duplicates.

The method was calibrated for all analyzed aroma compounds in both, fermented lupine- and faba bean-substrates with pooled samples from several different strains to compensate for matrix effects and to include strain-dependent variations. The calibration curves for the individual aroma compounds showed all correlation coefficients of 0.97 or higher (see Table S4 in the Supporting Information).

2.11 | Observation of growth kinetics

Growth kinetics were observed in fermentations in the Cytation 5 Cell Imaging Multimode Reader (Agilent). Therefore, after inoculating lupine- and faba bean-based substrates in Erlenmeyer flasks, small amounts (600 μL) were transferred sterile into a Cellstar 48 Well Suspension Culture Plate (Greiner bio-one). The device was set to 28°C, and the absorbance was measured every 2 min at 600 nm with double orbital shaking (2 mm, 365 cpm) between the measurements. Each measurement was performed in quadruplicate.

The changes in the specific growth rates were identified by deriving the growth curve numerically. The growth rates were calculated using the following formula (according to Takors, 2014), modified by transforming the differential quotient by the difference quotient as required for numerical evaluation):

$$\mu = \frac{\ln(X_2) - \ln(X_1)}{\Delta t}.$$

Thereby, X_2 and X_1 are the data points at the maximum of the first deviation of the growth curve and the data points prior the maximum, respectively. Δt represents the time difference between the two data points. To avoid falsifications in the calculations due to data fluctuations, the average of five measured points (e.g., X_1, point at the time of the maximum of the first deviation and two data points prior and after the maximum of the first deviation) were used for the calculation.

2.12 | Sensory analysis

Two different levels of sensory analyses were performed. In the first step, two to three test persons evaluated the sensory impression of all 140 fermented samples in parallel with the strain screening experiments. In the second step, the pre-selected strains were used to produce a higher volume of samples, which were presented to a trained sensory panel. The fermentations were always performed in biological triplicates and united prior to the sensory evaluations.

In the parallel sensory evaluations, a descriptive "check all that applies" (CATA) test was performed, which included the attributes "sour," "sweet," "fruity," "citrus," "fungal," "bread-like," "beany," "vegetable-like," "potato-like," "buttermilk-like," "earthy," "cheesy," "stinging," "vomit-like," and "drain-like." Furthermore, the overall odor and taste were evaluated on a scale from 1 (*not acceptable*) to 3 (*very acceptable*). All test persons had previous experience with sensory evaluations, were mostly certified by the German Agricultural Society as sensory experts, and were trained explicitly on legume-based beverages' sensory impressions.

For the panel-based sensory evaluations, the sessions included three parts. First, in a "rate all that apply" approach, the panel was asked to rate the descriptors from 0 (*not perceivable*) to 5 (*highly perceivable*).

Therefore, the descriptors for the odor impressions were clustered to "fruity, citrus," "buttermilk," "beany," "cooked vegetables," "potato-like, sulfuric," and "musty, earthy, fungus" due to the experience in the CATA evaluations. For the taste, the descriptors "acidic," "sweet," "bitter," "salty," and "umami" were used. Second, the hedonic acceptance of the taste, the odor, and the acidic character were asked to be rated on a scale from 1 (*not acceptable*) to 5 (*highly acceptable*). Third, the panelists had to sort the samples according to their preferences. These sensory evaluations were performed in four different sessions, where the panel consisted of 14 and 18 evaluators for lupine-based beverages and 12 and 15 evaluators for faba bean-based beverages.

2.13 | Statistical analysis

Concentrations are given as mean values (± standard deviation) if not indicated otherwise. One-way analysis of variance and Tukey's honestly significant difference post hoc test were used to statistically analyze data ($\alpha = 5\%$). Fisher's exact test was used to statistically analyze differences between sample sets ($\alpha = 5\%$). The Grubb's test ($\alpha = 5\%$) was used to identify outliers. All statistical analyses were performed using Origin 2018b (OriginLab Corporation).

3 | RESULTS AND DISCUSSION

Fermentation had a very profound impact on both the volatile and non-volatile compounds. After evaluating these analytically observed changes, the resulting sensory profile of the different fermented samples is discussed. Based on these attributes, a strain selection scheme is proposed and applied. For a number of elected strains, their growth kinetics were evaluated, and the specific growth rates were calculated and discussed in context to the analytical results. Finally, recommendations for the most applicable strains are presented based on the evaluations of a sensory panel.

3.1 | Impact of fermentation on non-volatile compounds

Probably the most important result is already perceivable from the pH changes during fermentation (Tables 1 and 2). In lupine, the final pH values after fermentation ranged from 3.42 to 4.86, while 68 out of 70 strains reduced the pH by more than 1.0, compared with the control (pH 5.65; as it was the criterion chosen to preselect LAB in the strain screening of Harlé et al., 2020), and 32 strains even reached a pH ≤ 4. Even as the faba bean-based substrate showed a slightly higher pH value of 6.14, in the fermentation, the pH drop was generally more intense, and final pH values ranged from 3.54 to 4.03, whereas 67 strains reduced the pH to values < 4. These findings are supported by the intense production of organic acids in the fermentation. No lactic or acetic acid was measurable in unfermented lupine- or faba bean-based substrates. However, after fermentation 1.45–10.95 g/L lactic acid and none to 1.97 g/L acetic acid were measured in lupine, which led to acid ratios ($g_{acetic\ acid}/g_{lactic\ acid}$) of 0–0.46. In faba beans, higher acid production of 2.62–8.56 g/L lactic acid and 0.13-4.94 g/L acetic acid corresponds to the greater pH drop. Therefore, the acid ratio was 0.03–0.97 g/L and, thus, higher than in lupines. Interestingly, the descriptor "sour" was more often used for lupines than for faba beans, and its intensity was generally higher rated (compare Table S5 in the Supporting Information). Most likely, the sour perception in faba beans was attenuated by the sweetness of remaining maltose in the fermented beverages.

The majority of strains used in this study are categorized as homofermentive LAB. Therefore, the production of high acetic acid might seem to be questionable at first. However, the results of this study substantiate formerly reported shifts from lactic to acetic acid in LAB fermented soy cheese analogues (Xie & Gänzle, 2024; Xie et al., 2024) and remain to be explained in future research.

Interestingly, many strains (51 out of 70) produced more acetic acid in faba bean-based than in lupine-based substrate, and 23 strains reached acid ratios more than three times higher when fermenting faba bean-based substrates (e.g., *Companilactobacillus alimentarius* L894 produced an acid ratio of 0.94 in faba beans and 0.27 in lupines). The strains *Agrilactobacillus composti* L1275, *Schleiferilactobacillus perolens* L50, *Pediococcus pentosaceus* Pd94, *Lactobacillus delbrueckii* subsp. *indicus* DSM 15996, and *Streptococcus thermophilus* DSM 20617 produced exclusively lactic acid when fermenting lupine-based substrates. However, considerable amounts of acetic acid were found, after the fermentation of faba bean-based substrates by those strains. This indicates that the composition of the substrate needs to be considered as highly impacting the strain's metabolic activity. The main difference in carbohydrates between the lupine- and faba bean-based substrates in this study was the absence of maltose in lupines in combination with higher concentrations of saccharose, glucose, and fructose. Both the total nitrogen and the FAN were higher in the lupine-based substrate. The maltose level in faba beans was excessive, and no strain depleted or highly reduced it until the end of fermentation. In some samples, the final maltose concentration even exceeded the initial one. This might be explained by the amylolytic activity of some strains to cleave starch-borne dextrins (Kanpiengjai et al. 2015). However, most likely the highly potent technical amylolytic enzymes used in the substrate production remained a certain degree of activity as no excessive heating step was applied to prevent protein losses. It needs to be mentioned that the faba bean-based substrate did not contain starch in its native granular form, as it was already gelatinized and enzymatically cleaved to maltose and higher molecular dextrins in the substrate preparation. Therefore, the starch degradation products in the substrate were available even for strains that are not known to ferment native starch itself. According to Gänzle and Follador (2012), LAB split complex maltodextrines extracellularly, transport fragments of different molecular sizes into the cell, and further decompose them to obtain carbohydrates for the metabolism.

Out of the 39 strains with an acid ratio > 0.2 in faba beans, 33 strains reduced the saccharose very strongly (remaining concentration of 0.05 g/L or below; compare Table 3). In contrast, out of the

TABLE 1 Changes of the pH, carbohydrates, organic acids, and nitrogen in lupine-based substrate by Lactic acid bacteria (LAB) fermentation with 70 different strains after 24 h of fermentation ($n = 3$).

				Carbohydrates (g/L)						Organic acids (g/L)					Nitrogen (g/L)				
Species	Strain	pH		Glucose		Fructose		Saccharose		Lactic acid		Acetic acid		Acid ratio	Free amino nitrogen (FAN)		Total nitrogen		N ratio
Agrilactobacillus composti	L1275	3.55	± 0.0	2.32	±0.06	0.50	±0.02	0.04	±0.00	6.38	±0.12	0.00	±0.00	0.00	0.39	±0.08	1.92	±0.31	0.20
Companilactobacillus alimentarius	L894	4.61	± 0.0	0.57	±0.02	0.00	±0.00	0.03	±0.00	3.78	±0.12	1.03	±0.02	0.27	0.54	±0.01	1.94	±0.22	0.28
Companilactobacillus bobalius	L1274	4.64	± 0.1	0.40	±0.10	0.00	±0.00	0.01	±0.00	2.81	±0.16	0.78	±0.04	0.28	0.45	±0.02	2.10	±0.41	0.21
Companilactobacillus farciminis	L1259	3.96	± 0.0	0.50	±0.01	0.21	±0.02	0.00	±0.00	4.50	±0.23	0.98	±0.06	0.22	0.60	±0.01	2.37	±0.05	0.25
Companilactobacillus furfuricola	L1254	4.05	± 0.0	0.92	±0.01	0.00	±0.00	0.02	±0.00	4.63	±0.16	1.22	±0.04	0.26	0.53	±0.03	2.36	±0.05	0.22
Companilactobacillus ginsenosidimutans	L1251	4.14	± 0.0	0.93	±0.00	0.00	±0.00	0.02	±0.00	3.10	±0.03	1.11	±0.02	0.36	0.47	±0.04	2.13	±0.23	0.22
Companilactobacillus heilongjiangensis	L1258	3.95	± 0.0	0.38	±0.03	0.00	±0.00	0.00	±0.00	4.45	±0.02	1.18	±0.01	0.26	0.52	±0.06	2.14	±0.27	0.25
Companilactobacillus kimchiensis	L1270	4.75	± 0.3	0.27	±0.38	0.00	±0.00	0.00	±0.00	2.60	±0.41	0.67	±0.18	0.26	0.35	±0.05	1.54	±0.27	0.23
Companilactobacillus kimchii	L1250	3.95	± 0.0	0.90	±0.03	0.00	±0.00	0.00	±0.00	4.24	±0.03	1.19	±0.01	0.28	0.56	±0.01	2.44	±0.04	0.23
Lacticaseibacillus camelliae	L1266	4.19	± 0.0	2.23	±0.39	0.00	±0.00	0.00	±0.00	4.33	±0.61	1.97	±0.31	0.46	0.99	±0.15	1.83	±0.26	0.54
Lacticaseibacillus casei	L1227	4.61	± 0.0	0.08	±0.01	0.00	±0.00	3.55	±0.17	3.51	±0.14	0.44	±0.03	0.13	0.52	±0.13	2.44	±0.02	0.21
Lacticaseibacillus manihotivorans	L1249	3.68	± 0.0	0.62	±0.06	0.20	±0.05	0.59	±0.06	7.32	±0.26	0.93	±0.04	0.13	0.54	±0.02	2.41	±0.06	0.22
Lacticaseibacillus paracasei	L1140	3.93	± 0.0	0.05	±0.00	0.02	±0.00	0.33	±0.02	4.04	±0.18	0.21	±0.02	0.05	0.27	±0.02	2.02	±0.10	0.13
Lacticaseibacillus paracasei	L1154	3.88	± 0.0	0.13	±0.00	0.05	±0.01	2.02	±0.18	5.05	±0.17	0.34	±0.03	0.07	0.54	±0.01	2.39	±0.00	0.23
Lacticaseibacillus paracasei	L1172	3.60	± 0.0	0.16	±0.01	0.02	±0.00	1.37	±0.08	8.21	±0.24	0.80	±0.02	0.10	0.54	±0.01	2.51	±0.02	0.21
Lacticaseibacillus paracasei	L1212	3.88	± 0.0	0.10	±0.00	0.04	±0.00	3.70	±0.03	7.01	±0.44	1.09	±0.03	0.16	0.60	±0.01	2.48	±0.06	0.24
Lacticaseibacillus paracasei	L1145	3.56	± 0.1	0.10	±0.01	0.00	±0.00	0.16	±0.02	8.44	±0.74	0.32	±0.02	0.04	0.43	±0.02	2.17	±0.03	0.20
Lacticaseibacillus paracasei	L1150	4.04	± 0.0	0.19	±0.02	1.78	±0.09	4.03	±0.11	4.33	±0.06	0.30	±0.01	0.07	0.83	±0.01	3.07	±0.04	0.27
Lacticaseibacillus rhamnosus	L1264	4.03	± 0.0	0.13	±0.00	0.00	±0.00	4.31	±0.20	5.25	±0.21	0.63	±0.01	0.12	0.42	±0.18	2.30	±0.11	0.18
Lactiplantibacillus argentoratensis	L1276	3.59	± 0.0	0.13	±0.02	0.00	±0.00	0.03	±0.00	6.89	±0.17	0.62	±0.09	0.09	0.47	±0.03	2.01	±0.11	0.23
Lactiplantibacillus paraplantarum	L1257	3.92	± 0.0	0.62	±0.07	0.00	±0.00	0.16	±0.00	5.04	±0.13	1.07	±0.05	0.21	0.52	±0.04	2.33	±0.10	0.22
Lactiplantibacillus plantarum	L758	3.44	± 0.0	0.49	±0.01	0.44	±0.00	1.89	±0.07	10.75	±0.12	0.75	±0.01	0.07	0.52	±0.02	2.40	±0.09	0.22
Lactiplantibacillus plantarum	L1045	3.58	± 0.0	0.08	±0.00	0.00	±0.00	0.16	±0.00	8.24	±0.15	0.05	±0.01	0.01	0.45	±0.01	2.20	±0.02	0.20
Lactiplantibacillus plantarum	L628	3.87	± 0.0	0.78	±0.02	0.05	±0.00	1.76	±0.05	6.59	±0.12	0.60	±0.03	0.09	0.60	±0.01	2.46	±0.02	0.24
Lactiplantibacillus plantarum	L736	4.14	± 0.0	0.89	±0.04	0.64	±0.04	1.70	±0.17	4.87	±0.13	0.71	±0.04	0.15	0.59	±0.00	2.50	±0.02	0.24
Lactiplantibacillus plantarum	L762	3.48	± 0.0	0.89	±0.03	0.00	±0.00	2.21	±0.43	10.95	±0.65	0.70	±0.02	0.06	0.55	±0.01	2.48	±0.05	0.22
Lactiplantibacillus plantarum	L879	4.09	± 0.2	0.07	±0.04	0.00	±0.00	0.00	±0.00	4.82	±0.27	0.51	±0.20	0.11	0.37	±0.04	1.49	±0.25	0.25
Lactiplantibacillus plantarum	L911	3.42	± 0.0	0.36	±0.02	0.00	±0.00	2.70	±0.23	10.41	±0.31	0.39	±0.02	0.04	0.54	±0.01	2.38	±0.01	0.23

(Continues)

TABLE 1 (Continued)

Species	Strain	pH		Carbohydrates (g/L)						Organic acids (g/L)					Nitrogen (g/L)				
				Glucose		Fructose		Saccharose		Lactic acid		Acetic acid		Acid ratio	Free amino nitrogen (FAN)		Total nitrogen		N ratio
Lactobacillus acetotolerans	L940	4.58	± 0.0	0.03	±0.00	0.00	±0.00	0.00	±0.00	3.64	±0.15	0.68	±0.02	0.19	0.38	±0.03	1.68	±0.01	0.23
Lactobacillus acidophilus	TL65	3.96	± 0.0	1.05	±0.01	0.19	±0.02	2.69	±0.24	5.65	±0.05	0.80	±0.03	0.14	0.74	±0.01	2.69	±0.05	0.28
Lactobacillus amylolyticus	TL5	4.19	± 0.0	0.80	±0.02	0.31	±0.02	0.00	±0.00	5.01	±0.47	0.65	±0.08	0.13	0.63	±0.05	2.49	±0.03	0.25
Lactobacillus amylolyticus	TL9	4.30	± 0.0	1.02	±0.06	0.00	±0.00	0.00	±0.00	3.76	±0.08	1.13	±0.07	0.30	0.72	±0.03	2.88	±0.03	0.25
Lactobacillus crispatus	TL62	4.09	± 0.0	0.07	±0.00	0.00	±0.00	1.93	±0.10	3.25	±0.01	0.47	±0.02	0.15	0.60	±0.04	2.36	±0.06	0.25
Lactobacillus delbrueckii ssp. *bulgaricus*	L1261	3.69	± 0.0	0.14	±0.00	0.00	±0.00	2.17	±0.06	8.18	±0.20	0.76	±0.03	0.09	0.52	±0.02	2.11	±0.25	0.25
Lactobacillus delbrueckii ssp. *delbrueckii*	L1262	3.92	± 0.0	0.62	±0.06	0.02	±0.00	1.02	±0.03	5.55	±0.26	0.82	±0.05	0.15	0.56	±0.06	2.19	±0.32	0.26
Lactobacillus delbrueckii subsp. *indicus*	15996	4.23	± 0.0	0.95	±0.03	0.00	±0.00	2.03	±0.17	2.80	±0.23	0.00	±0.00	0.00	0.63	±0.03	2.23	±0.06	0.28
Lactobacillus delbrueckii subsp. *jakobsenii*	L1265	4.10	± 0.0	0.76	±0.05	0.00	±0.00	0.00	±0.00	1.86	±0.11	0.63	±0.06	0.34	0.44	±0.01	1.26	±0.13	0.35
Lactobacillus delbrueckii subsp. *sunkii*	L1269	4.03	± 0.0	1.44	±0.60	0.00	±0.00	0.27	±0.23	7.38	±1.24	1.40	±0.02	0.19	0.88	±0.27	2.56	±0.32	0.34
Lactobacillus helsingborgensis	L1263	3.71	± 0.0	0.20	±0.04	0.26	±0.01	1.25	±0.07	8.34	±0.33	0.78	±0.10	0.09	0.50	±0.02	2.22	±0.14	0.23
Lactobacillus helveticus	TL64	3.96	± 0.0	0.77	±0.04	0.12	±0.01	2.17	±0.30	5.58	±0.20	0.78	±0.01	0.14	0.73	±0.00	2.72	±0.02	0.27
Lactobacillus kefirano-faciens ssp. *kefiranofaciens*	L1256	4.03	± 0.0	0.44	±0.13	0.00	±0.00	0.00	±0.00	5.21	±0.45	1.19	±0.15	0.23	0.52	±0.01	2.35	±0.08	0.22
Lactobacillus kefiranofaciens subsp. *kefirgranum*	L1272	4.32	± 0.0	0.04	±0.00	0.13	±0.01	0.01	±0.00	3.70	±0.09	0.53	±0.03	0.14	0.40	±0.03	1.60	±0.02	0.25
Lapidilactobacillus dextrinicus	L1244	4.10	± 0.0	0.97	±0.01	0.19	±0.01	0.01	±0.00	4.15	±0.02	1.41	±0.03	0.34	0.56	±0.02	2.47	±0.03	0.23
Laticaseibacillus casei	L1047	3.94	± 0.0	0.61	±0.01	0.46	±0.01	1.17	±0.16	5.57	±0.40	0.87	±0.06	0.16	0.72	±0.04	2.57	±0.06	0.28
Latilactobacillus curvatus	L477	3.99	± 0.1	1.11	±0.26	0.00	±0.00	4.30	±0.31	4.82	±0.09	0.25	±0.00	0.05	0.61	±0.02	2.20	±0.21	0.28
Latilactobacillus sakei	L497	4.17	± 0.0	1.14	±0.13	0.00	±0.00	4.01	±0.23	4.52	±0.04	0.29	±0.03	0.06	0.50	±0.07	2.45	±0.01	0.20
Latilactobacillus sakei	L651	4.08	± 0.0	0.96	±0.13	0.00	±0.00	4.92	±0.24	4.24	±0.06	0.29	±0.02	0.07	0.81	±0.02	3.09	±0.01	0.26
Lentilactobacillus parabuchneri	L690	3.87	± 0.0	0.70	±0.02	0.04	±0.00	1.97	±0.03	5.42	±0.09	0.48	±0.01	0.09	0.62	±0.01	2.40	±0.02	0.26
Leuconostoc mesenteroides	Ln56	4.14	± 0.0	1.03	±0.03	0.08	±0.01	0.22	±0.01	4.14	±0.15	0.76	±0.02	0.18	0.57	±0.01	2.52	±0.04	0.23
Levilactobacillus brevis	L1223	4.18	± 0.0	0.69	±0.03	0.00	±0.00	5.10	±0.49	4.52	±0.14	0.19	±0.05	0.04	0.58	±0.03	2.44	±0.03	0.24
Levilactobacillus brevis	L982	4.07	± 0.1	0.71	±0.34	0.00	±0.00	4.28	±0.35	4.56	±0.35	0.24	±0.02	0.05	0.66	±0.11	2.66	±0.41	0.25
Liquorilactobacillus cacaonum	L1273	4.61	± 0.4	0.15	±0.10	0.00	±0.00	0.00	±0.00	1.45	±0.26	0.50	±0.06	0.34	0.36	±0.04	1.12	±0.08	0.32
Liquorilactobacillus hordei	L1255	3.70	± 0.0	0.96	±0.10	0.06	±0.00	1.97	±0.06	7.14	±0.14	0.65	±0.03	0.09	0.55	±0.02	2.26	±0.09	0.24
Liquorilactobacillus mali	L1271	4.86	± 0.0	0.37	±0.07	0.00	±0.00	0.02	±0.00	2.63	±0.23	0.63	±0.07	0.24	0.44	±0.06	1.89	±0.20	0.24

(Continues)

TABLE 1 (Continued)

Species	Strain	pH		Carbohydrates (g/L)						Organic acids (g/L)					Nitrogen (g/L)				
				Glucose		Fructose		Saccharose		Lactic acid		Acetic acid		Acid ratio	Free amino nitrogen (FAN)		Total nitrogen		N ratio
Liquorilactobacillus nagelii	L1260	3.92	± 0.0	0.20	±0.01	0.03	±0.00	0.03	±0.00	4.84	±0.07	1.14	±0.05	0.24	0.54	±0.01	2.37	±0.09	0.23
Liquorilactobacillus satsumensis	L1268	4.43	± 0.0	0.32	±0.02	0.00	±0.00	0.01	±0.00	3.57	±0.18	0.44	±0.05	0.12	0.41	±0.10	1.48	±0.08	0.28
Loigolactobacillus backii	L1253	4.07	± 0.0	0.93	±0.00	0.00	±0.00	0.02	±0.00	4.20	±0.04	1.08	±0.02	0.26	0.56	±0.02	2.29	±0.04	0.25
Loigolactobacillus backii	L456	4.48	± 0.0	0.07	±0.01	0.00	±0.00	1.54	±0.19	2.72	±0.15	0.40	±0.03	0.15	0.65	±0.03	1.38	±0.09	0.47
Loigolactobacillus coryni-formis ssp. *coryniformis*	L1252	3.72	± 0.0	0.53	±0.04	0.28	±0.02	0.36	±0.14	8.10	±0.38	0.89	±0.01	0.11	0.47	±0.10	2.45	±0.08	0.19
Loigolactobacillus coryni-formis subsp. *torquens*	L1267	3.88	± 0.0	0.28	±0.01	0.05	±0.00	0.01	±0.00	3.74	±0.12	0.90	±0.04	0.24	0.45	±0.00	1.88	±0.04	0.24
Pediococcus acidilactici	Pd75	4.03	± 0.0	0.08	±0.00	0.00	±0.00	3.68	±0.13	5.26	±0.07	0.60	±0.01	0.11	0.70	±0.00	2.45	±0.02	0.29
Pediococcus damnosus	Pd100	4.37	± 0.0	0.08	±0.01	0.00	±0.00	0.02	±0.00	3.39	±0.02	1.05	±0.16	0.31	0.73	±0.03	2.86	±0.03	0.26
Pediococcus damnosus	Pd59	3.94	± 0.0	1.22	±0.04	0.00	±0.00	2.76	±0.08	6.62	±0.14	1.08	±0.01	0.16	0.68	±0.01	2.55	±0.03	0.27
Pediococcus damnosus	Pd60	3.92	± 0.0	1.17	±0.02	0.00	±0.00	2.60	±0.09	5.42	±0.18	0.76	±0.01	0.14	0.66	±0.02	2.58	±0.01	0.26
Pediococcus pentosaceus	Pd94	4.18	± 0.0	0.09	±0.00	0.00	±0.00	3.80	±0.13	5.61	±0.13	0.00	±0.01	0.00	0.45	±0.01	1.86	±0.01	0.24
Pediococcus pentosaceus	Pd71	4.65	± 0.0	0.07	±0.01	0.00	±0.00	3.64	±0.07	3.06	±0.09	0.59	±0.00	0.19	0.36	±0.06	1.36	±0.10	0.26
Schleiferilactobacillus harbinensis	L77	3.72	± 0.1	0.69	±0.05	0.06	±0.00	3.39	±0.07	7.72	±0.75	0.74	±0.02	0.10	0.62	±0.03	2.45	±0.02	0.25
Schleiferilactobacillus perolens	L50	4.19	± 0.0	0.12	±0.01	0.00	±0.00	2.86	±0.21	3.45	±0.16	0.00	±0.00	0.00	0.53	±0.02	2.38	±0.01	0.22
Schleiferilactobacillus perolens	L532	4.29	± 0.0	0.14	±0.01	2.50	±0.01	3.78	±0.18	4.15	±0.00	0.57	±0.13	0.14	0.86	±0.04	3.11	±0.00	0.28
Streptococcus salivarius subsp. *thermophilus*	20617	4.29	± 0.0	0.87	±0.03	0.00	±0.00	2.13	±0.12	3.30	±0.21	0.00	±0.00	0.00	0.62	±0.01	2.20	±0.03	0.28
Control		5.65	± 0.0	2.48	±0.06	0.59	±0.01	4.89	±0.01	0.00	±0.00	0.00	±0.00		0.45	±0.09	2.67	±0.04	0.17

TABLE 2 Changes of the pH, carbohydrates, organic acids, and nitrogen in faba bean-based substrate by LAB fermentation with 70 different strains after 24 h of fermentation ($n = 3$).

				Carbohydrates (g/L)								Organic acids (g/L)					Nitrogen (g/L)				
Species	Strain	pH		Glucose		Fructose		Saccharose		Maltose		Lactic acid		Acetic acid		Acid ratio	FAN		Total nitrogen		N ratio
Agrilactobacillus composti	L1275	3.78	±0.0	0.17	±0.02	0.37	±0.01	0.03	±0.00	8.10	±0.50	4.74	±0.04	3.42	±0.11	0.72	0.26	±0.03	1.18	±0.12	0.22
Companilactobacillus alimentarius	L894	3.92	±0.0	0.28	±0.02	0.71	±0.03	0.00	±0.00	8.60	±0.49	4.91	±0.11	4.62	±0.06	0.94	0.40	±0.06	1.68	±0.27	0.24
Companilactobacillus bobalius	L1274	3.86	±0.0	0.30	±0.04	0.31	±0.05	0.00	±0.00	15.47	±1.01	4.48	±0.30	3.70	±0.24	0.83	0.37	±0.09	1.15	±0.32	0.32
Companilactobacillus farciminis	L1259	3.81	±0.0	0.34	±0.10	0.00	±0.00	0.11	±0.01	10.07	±0.69	5.07	±0.13	0.69	±0.01	0.14	0.24	±0.04	0.93	±0.09	0.26
Companilactobacillus furfuricola	L1254	3.73	±0.0	0.28	±0.01	0.00	±0.00	0.00	±0.00	11.64	±0.96	5.63	±0.18	4.40	±0.18	0.78	0.32	±0.03	1.30	±0.11	0.25
Companilactobacillus ginsenosidimutans	L1251	3.88	±0.0	0.17	±0.02	0.14	±0.01	0.00	±0.00	14.73	±0.33	4.72	±0.03	1.46	±0.11	0.31	0.26	±0.07	1.02	±0.25	0.25
Companilactobacillus heilongjiangensis	L1258	3.80	±0.0	0.23	±0.05	0.32	±0.01	0.03	±0.00	7.38	±0.26	5.03	±0.23	0.71	±0.06	0.14	0.25	±0.06	1.03	±0.25	0.24
Companilactobacillus kimchiensis	L1270	3.84	±0.0	0.13	±0.01	0.31	±0.01	0.00	±0.00	8.52	±0.31	3.99	±0.03	2.88	±0.06	0.72	0.27	±0.05	1.17	±0.18	0.23
Companilactobacillus kimchii	L1250	3.89	±0.0	0.46	±0.02	0.15	±0.01	0.00	±0.00	14.73	±0.33	3.85	±0.05	0.71	±0.03	0.18	0.26	±0.02	1.06	±0.12	0.24
Lacticaseibacillus camelliae	L1266	3.91	±0.0	0.35	±0.01	0.13	±0.00	0.00	±0.00	14.82	±0.82	3.62	±0.09	2.34	±0.14	0.65	0.31	±0.04	1.27	±0.07	0.25
Lacticaseibacillus casei	L1227	3.98	±0.1	0.93	±0.29	0.41	±0.05	0.33	±0.05	18.98	±0.50	4.44	±0.15	0.41	±0.05	0.09	0.43	±0.14	1.33	±0.15	0.32
Lacticaseibacillus manihotivorans	L1249	3.62	±0.0	0.52	±0.02	0.37	±0.02	0.00	±0.00	12.76	±0.16	6.75	±0.26	4.30	±0.18	0.64	0.28	±0.02	1.38	±0.11	0.21
Lacticaseibacillus paracasei	L1140	3.92	±0.0	0.32	±0.02	0.51	±0.10	0.00	±0.00	10.02	±0.37	5.02	±0.20	4.59	±0.29	0.91	0.41	±0.03	1.01	±0.02	0.41
Lacticaseibacillus paracasei	L1154	3.87	±0.0	0.26	±0.01	0.36	±0.15	0.06	±0.00	14.91	±0.75	4.88	±0.06	0.81	±0.06	0.17	0.31	±0.04	1.50	±0.16	0.21
Lacticaseibacillus paracasei	L1172	3.63	±0.0	0.21	±0.04	0.21	±0.02	0.07	±0.00	10.92	±0.93	5.77	±0.17	0.96	±0.07	0.17	0.30	±0.02	1.26	±0.04	0.24
Lacticaseibacillus paracasei	L1212	3.75	±0.0	0.47	±0.01	0.07	±0.00	3.77	±0.11	21.66	±0.24	4.40	±0.10	0.14	±0.01	0.03	0.29	±0.01	1.26	±0.04	0.23
Lacticaseibacillus paracasei	L1145	3.77	±0.0	0.27	±0.04	0.00	±0.00	0.08	±0.02	20.88	±0.34	5.89	±0.48	3.45	±0.02	0.59	0.34	±0.01	1.45	±0.06	0.23
Lacticaseibacillus paracasei	L1150	3.95	±0.0	2.39	±0.06	0.50	±0.02	0.00	±0.00	19.80	±1.98	2.62	±0.25	0.60	±0.02	0.23	0.29	±0.01	1.27	±0.09	0.23
Lacticaseibacillus rhamnosus	L1264	3.61	±0.0	0.23	±0.00	0.19	±0.00	0.69	±0.05	13.28	±0.50	6.96	±0.23	4.26	±0.11	0.61	0.30	±0.02	1.23	±0.07	0.25
Lactiplantibacillus argentoratensis	L1276	3.83	±0.0	0.24	±0.01	0.00	±0.00	0.10	±0.00	9.38	±0.31	3.84	±0.08	2.82	±0.17	0.73	0.26	±0.04	1.14	±0.06	0.22
Lactiplantibacillus paraplantarum	L1257	3.88	±0.0	0.49	±0.01	0.00	±0.00	0.00	±0.00	9.83	±0.15	4.69	±0.05	1.45	±0.01	0.31	0.26	±0.07	1.09	±0.28	0.24
Lactiplantibacillus plantarum	L758	3.59	±0.0	0.31	±0.01	0.28	±0.01	0.08	±0.00	9.96	±1.05	6.82	±0.14	0.72	±0.02	0.10	0.28	±0.01	1.27	±0.03	0.22
Lactiplantibacillus plantarum	L1045	3.66	±0.0	0.47	±0.01	0.24	±0.01	0.00	±0.00	14.78	±1.13	8.56	±1.59	3.13	±0.04	0.37	0.32	±0.01	1.48	±0.02	0.22
Lactiplantibacillus plantarum	L628	3.79	±0.0	0.69	±0.06	0.00	±0.00	0.05	±0.00	19.72	±1.00	4.83	±0.13	0.75	±0.02	0.15	0.27	±0.03	1.17	±0.10	0.23
Lactiplantibacillus plantarum	L736	3.70	±0.0	0.94	±0.06	0.00	±0.00	0.05	±0.00	20.37	±2.01	5.62	±0.15	0.73	±0.01	0.13	0.28	±0.02	1.21	±0.08	0.23
Lactiplantibacillus plantarum	L762	3.54	±0.0	1.30	±0.03	0.10	±0.00	3.43	±0.04	21.88	±1.42	6.77	±1.29	0.20	±0.01	0.03	0.28	±0.01	1.19	±0.07	0.23
Lactiplantibacillus plantarum	L879	3.68	±0.0	0.26	±0.02	0.23	±0.02	0.07	±0.01	12.53	±0.71	5.83	±0.17	3.49	±0.11	0.60	0.26	±0.05	1.09	±0.23	0.24
Lactiplantibacillus plantarum	L911	3.83	±0.0	0.24	±0.01	0.33	±0.01	0.00	±0.00	7.74	±0.55	4.39	±0.30	2.44	±0.30	0.56	0.29	±0.02	1.29	N/A	0.22

(Continues)

TABLE 2 (Continued)

				Carbohydrates (g/L)								Organic acids (g/L)					Nitrogen (g/L)				
Species	Strain	pH		Glucose		Fructose		Saccharose		Maltose		Lactic acid		Acetic acid		Acid ratio	FAN		Total nitrogen		N ratio
Lactobacillus acetotolerans	L940	3.89	±0.0	0.36	±0.02	0.70	±0.01	0.03	±0.00	9.02	±0.30	4.73	±0.14	3.42	±0.02	0.72	0.30	±0.07	1.29	±0.16	0.23
Lactobacillus acidophilus	TL65	3.83	±0.0	0.00	±0.00	3.06	±0.28	0.00	±0.00	21.42	±0.08	4.27	±0.05	0.94	±0.02	0.22	0.28	±0.05	1.23	±0.22	0.23
Lactobacillus amylolyticus	TL5	3.84	±0.0	0.03	±0.00	3.61	±0.17	0.00	±0.00	21.28	±1.18	4.17	±0.09	0.56	±0.01	0.13	0.33	±0.02	1.46	±0.05	0.22
Lactobacillus amylolyticus	TL9	3.90	±0.0	0.47	±0.05	0.66	±0.04	0.00	±0.00	9.16	±0.20	4.05	±0.09	2.86	±0.19	0.71	0.35	±0.05	1.45	±0.20	0.24
Lactobacillus crispatus	TL62	3.79	±0.0	1.58	±0.01	0.06	±0.00	0.03	±0.00	11.10	±1.19	4.65	±0.38	0.74	±0.07	0.16	0.29	±0.04	1.21	±0.12	0.24
Lactobacillus delbrueckii ssp. *bulgaricus*	L1261	3.61	±0.0	0.15	±0.01	0.00	±0.00	0.07	±0.01	10.15	±1.20	6.18	±0.48	2.96	±0.53	0.48	0.27	±0.01	0.98	±0.07	0.27
Lactobacillus delbrueckii ssp. *delbrueckii*	L1262	3.81	±0.0	0.45	±0.03	0.59	±0.01	0.00	±0.00	9.50	±0.27	5.28	±0.13	4.36	±0.11	0.83	0.31	±0.02	1.28	±0.06	0.24
Lactobacillus delbrueckii subsp. *indicus*	15996	4.02	±0.0	1.30	±0.38	0.23	±0.02	0.00	±0.00	8.44	±0.47	3.41	±0.06	3.32	±0.22	0.97	0.35	±0.05	1.93	±0.43	0.18
Lactobacillus delbrueckii subsp. *jakobsenii*	L1265	3.88	±0.0	0.34	±0.07	0.12	±0.05	0.00	±0.00	13.27	±1.16	3.53	±0.33	2.66	±0.42	0.75	0.26	±0.03	1.15	±0.28	0.23
Lactobacillus delbrueckii subsp. *sunkii*	L1269	3.87	±0.0	0.22	±0.01	0.30	±0.04	0.00	±0.00	15.00	±0.17	4.29	±0.25	3.27	±0.25	0.76	0.45	±0.03	1.44	±0.01	0.31
Lactobacillus helsingborgensis	L1263	3.68	±0.0	0.17	±0.02	0.25	±0.02	0.00	±0.00	11.96	±0.70	6.24	±0.25	3.71	±0.04	0.60	0.30	±0.06	1.31	±0.22	0.23
Lactobacillus helveticus	TL64	4.01	±0.0	0.40	±0.17	0.39	±0.01	1.60	±1.19	20.78	±7.55	3.34	±0.10	0.19	±0.00	0.06	0.30	±0.04	1.21	±0.19	0.25
Lactobacillus kefirano-faciens ssp. *kefiranofaciens*	L1256	3.77	±0.0	0.21	±0.01	0.23	±0.01	0.00	±0.00	12.19	±0.26	5.48	±0.46	4.22	±0.37	0.77	0.28	±0.01	1.16	±0.11	0.24
Lactobacillus kefiranofaciens subsp. *kefirgranum*	L1272	3.90	±0.0	0.24	±0.02	0.39	±0.02	0.00	±0.00	14.26	±0.23	5.01	±0.10	1.45	±0.13	0.29	0.39	±0.05	1.55	±0.21	0.25
Lapidilactobacillus dextrinicus	L1244	3.72	±0.0	0.39	±0.01	0.43	±0.01	1.21	±0.05	13.79	±0.43	5.48	±0.03	1.06	±0.01	0.19	0.29	±0.03	1.26	±0.11	0.23
Laticaseibacillus casei	L1047	3.86	±0.0	1.24	±0.04	0.47	±0.13	1.17	±0.51	13.16	±0.34	4.03	±0.11	0.47	±0.02	0.12	0.29	±0.01	1.17	±0.01	0.25
Latilactobacillus curvatus	L477	3.86	±0.0	0.33	±0.03	0.20	±0.00	0.05	±0.00	19.40	±0.37	5.47	±0.10	0.98	±0.03	0.18	0.31	±0.03	1.28	±0.09	0.24
Latilactobacillus sakei	L497	3.84	±0.0	1.05	±0.28	0.29	±0.04	0.00	±0.00	18.94	±0.52	3.87	±0.05	0.89	±0.05	0.23	0.29	±0.06	1.35	±0.17	0.22
Latilactobacillus sakei	L651	4.03	±0.0	1.90	±0.04	0.36	±0.03	0.00	±0.00	17.58	±0.36	2.87	±0.09	0.60	±0.02	0.21	0.27	±0.04	1.31	±0.05	0.21
Lentilactobacillus parabuchneri	L690	3.97	±0.0	0.34	±0.01	0.32	±0.02	0.00	±0.00	15.14	±2.97	3.83	±0.13	1.05	±0.07	0.27	0.34	±0.02	1.45	±0.01	0.23
Leuconostoc mesenteroides	Ln56	3.81	±0.0	1.43	±0.40	0.00	±0.00	0.32	±0.07	15.57	±0.19	6.28	±0.46	0.19	±0.03	0.03	0.23	±0.01	0.95	±0.04	0.24
Levilactobacillus brevis	L1223	3.88	±0.0	0.33	±0.03	0.52	±0.02	1.71	±0.09	24.86	±1.04	4.81	±0.16	0.57	±0.02	0.12	0.34	±0.04	1.42	±0.09	0.24
Levilactobacillus brevis	L982	3.85	±0.0	0.39	±0.07	0.38	±0.02	0.46	±0.04	21.43	±0.25	5.45	±0.02	0.84	±0.08	0.15	0.33	±0.02	1.08	±0.21	0.31
Liquorilactobacillus cacaonum	L1273	3.79	±0.0	0.21	±0.07	0.09	±0.00	0.00	±0.00	9.76	±0.43	3.21	±0.17	1.62	±0.07	0.51	0.30	±0.02	0.78	±0.16	0.39
Liquorilactobacillus hordei	L1255	3.82	±0.0	0.45	±0.02	0.30	±0.05	0.00	±0.00	12.22	±0.64	4.44	±0.15	3.47	±0.02	0.78	0.22	±0.00	1.20	±0.11	0.18

(Continues)

TABLE 2 (Continued)

				Carbohydrates (g/L)								Organic acids (g/L)					Nitrogen (g/L)				
Species	Strain	pH		Glucose		Fructose		Saccharose		Maltose		Lactic acid		Acetic acid		Acid ratio	FAN		Total nitrogen		N ratio
Liquorilactobacillus mali	L1271	3.82	±0.0	0.26	±0.01	0.18	±0.02	0.00	±0.00	12.99	±0.91	3.96	±0.33	2.66	±0.34	0.67	0.38	±0.02	1.08	±0.11	0.35
Liquorilactobacillus nagelii	L1260	3.79	±0.0	0.17	±0.02	0.38	±0.03	0.03	±0.00	7.44	±0.08	5.20	±0.11	0.75	±0.02	0.14	0.28	±0.03	1.01	±0.14	0.27
Liquorilactobacillus satsumensis	L1268	3.93	±0.0	0.23	±0.02	0.20	±0.00	0.00	±0.00	15.23	±0.20	4.94	±0.14	1.18	±0.07	0.24	0.38	±0.07	1.48	±0.26	0.26
Loigolactobacillus backii	L1253	3.86	±0.0	0.47	±0.02	0.18	±0.01	0.00	±0.00	14.17	±0.17	4.57	±0.05	0.71	±0.03	0.15	0.27	±0.05	1.09	±0.18	0.25
Loigolactobacillus backii	L456	3.84	±0.0	0.31	±0.02	0.53	±0.04	0.03	±0.00	6.91	±0.69	3.96	±0.18	2.67	±0.20	0.68	0.26	±0.03	0.93	±0.06	0.28
Loigolactobacillus coryni-formis ssp. *coryniformis*	L1252	3.88	±0.0	1.27	±0.06	0.57	±0.05	0.10	±0.01	10.28	±0.84	6.21	±0.35	4.94	±0.28	0.80	0.37	±0.06	1.77	±0.15	0.21
Loigolactobacillus coryni-formis subsp. *torquens*	L1267	3.95	±0.0	0.23	±0.00	0.24	±0.01	0.00	±0.00	17.60	±0.32	4.74	±0.05	1.27	±0.13	0.27	0.40	±0.05	1.52	±0.26	0.26
Pediococcus acidilactici	Pd75	3.91	±0.0	0.10	±0.01	0.00	±0.00	2.81	±0.07	12.73	±0.23	7.17	±0.70	0.20	±0.03	0.03	0.35	±0.01	1.32	±0.01	0.27
Pediococcus damnosus	Pd100	3.81	±0.0	0.12	±0.01	0.26	±0.04	1.99	±0.01	12.46	±0.31	2.92	±0.04	0.13	±0.03	0.04	0.31	±0.02	1.32	±0.07	0.23
Pediococcus damnosus	Pd59	3.80	±0.0	0.71	±0.00	0.00	±0.00	0.14	±0.01	14.31	±0.34	6.67	±0.01	0.19	±0.00	0.03	0.33	±0.04	1.30	±0.02	0.25
Pediococcus damnosus	Pd60	3.88	±0.0	0.88	±0.01	0.00	±0.00	0.39	±0.03	12.48	±0.18	7.10	±0.15	0.20	±0.04	0.03	0.31	±0.02	1.28	±0.08	0.24
Pediococcus pentosaceus	Pd94	3.90	±0.0	0.88	±0.03	0.00	±0.00	0.37	±0.01	13.11	±0.28	6.67	±0.08	0.20	±0.01	0.03	0.35	±0.03	1.39	±0.10	0.25
Pediococcus pentosaceus	Pd71	3.73	±0.0	1.30	±0.80	0.00	±0.00	0.54	±0.29	18.17	±8.25	6.99	±0.24	0.23	±0.01	0.03	0.17	±0.03	0.63	±0.11	0.27
Schleiferilactobacillus harbinensis	L77	3.69	±0.0	0.33	±0.02	0.28	±0.02	0.96	±0.24	13.07	±0.78	5.47	±0.06	0.55	±0.02	0.10	0.32	±0.01	1.26	±0.04	0.26
Schleiferilactobacillus perolens	L50	3.88	±0.0	0.60	±0.06	0.00	±0.00	0.06	±0.00	21.35	±0.95	4.94	±0.13	0.90	±0.06	0.18	0.31	±0.01	1.33	±0.01	0.23
Schleiferilactobacillus perolens	L532	3.78	±0.0	1.05	±0.02	0.44	±0.01	0.00	±0.00	15.24	±0.48	3.66	±0.20	0.59	±0.02	0.16	0.28	±0.03	1.30	±0.04	0.22
Streptococcus salivarius subsp. *thermophilus*	L20617	3.95	±0.0	0.81	±0.11	0.34	±0.01	0.00	±0.00	8.62	±0.89	4.40	±0.10	4.08	±0.21	0.93	0.39	±0.06	1.91	±0.28	0.20
Control		6.14	±0.0	4.35	±0.14	0.28	±0.01	3.73	±0.14	17.03	±0.64	0.00	±0.00	0.00	±0.00		0.36	±0.03	1.62	±0.11	0.22

TABLE 3 Utilization of saccharose and fructose depending on the final acid ratio (acetic acid/lactic acid).

		Faba beans		Lupines	
		Acid ratio <0.2	Acid ratio >0.2	Acid ratio <0.2	Acid ratio >0.2
Saccharose	> 0.05 g/L	24^{Aa}	6^{Ba}	43^{Aa}	1^{Ba}
	< 0.05 g/L	7^{Ab}	33^{Bb}	6^{Ab}	19^{Bb}
Fructose	> 0.05 g/L	21^{Aa}	34^{Ba}	19^{Aa}	2^{Ba}
	< 0.05 g/L	10^{Ab}	5^{Ab}	32^{Ab}	18^{Bb}

Note: The numbers represent the sum of strains fitting into the matrix according to Tables 1 and 2. Capital letters indicate statistical differences in the rows and smaller letters for the column ($p = .05$).

31 strains with an acid ratio < 0.2, only the four strains, *C. kimchii* L1250, *L. amylolyticus* TL5, *Loigolactobacillus backii* L1253, and *S. perolens* L532, depleted saccharose, and seven strains reduced saccharose beneath 0.05 g/L. The different saccharose consumption behaviors of the two groups of high and low acid ratio forming strains were highly statistically significant ($p < .001$). In the fermentation of lupine-based substrates, out of 20 strains with an acid ratio >0.2, 19 strains reduced the saccharose concentration to <0.05 g/L. Among those 20 strains, 16 additionally depleted fructose entirely, while the remaining glucose was measured at the end of fermentation for all of them. Lu et al. (2001) studied the carbohydrate utilization of glucose and fructose in *Lactiplantibacillus plantarum* and found that glucose is metabolized faster, but the depletion is incomplete, while fructose is depleted entirely. They assumed that the different transporters for fructose and glucose into the cell might show different pH optima and are therefore influenced in a different manner by the lactic acid production in the fermentation. Out of the 49 strains (acid ratio <0.2), only six strains reduced saccharose to concentrations of <0.05 g/L. A total of 60 strains reduced fructose to beneath 0.05 g/L, whereas some remaining glucose was measured in all samples after fermentation. Again, the difference in the saccharose consumption of the two groups is highly statistically significant ($p < .001$). Donkor et al. (2007) reported a substantial shift from lactic acid to acetic acid production in six LAB and two bifidobacteria when grown on raffinose instead of glucose or a glucose-raffinose mixture. As raffinose consists of galactose bound to saccharose via an α–1,6-glycosidic bond and all strains were proven to show the required α-galactosidase activity to cleave raffinose, the lactic to acetic acid shift might be due to the availability of saccharose. Environmental conditions influence the metabolic pathways utilized by LAB, and the presence of electron acceptors like oxygen, fructose (only heterofermentative LAB), aldehydes, or phenolic compounds usually leads to changes in the metabolic direction. The formation of acetic acid from pyruvate in the presence of oxygen by pyruvate oxidase and acetate kinase, in combination with the pH stabilizing production of acetoin and usually diacetyl, is an energetically favorable metabolic pathway (Gänzle, 2015). More likely, as no forced aeriation was performed in this study, pyruvate dehydrogenase might have been involved as proposed by Xie and Gänzle (2024). The latter also reported shifts of the final acid concentration from lactic to acid acetic acid, when germinated soy was used instead of ungerminated one and assumed that further electron acceptors are released during the germination. This also might explain the strong production of acetic acid by the homofermentative LAB in this study. Such required electron acceptors might be hydroxycinnamic acids (Filannino et al., 2014) or isoflavones (Shimada et al., 2010).

Lorquet et al. (2004) reported that under aerobic conditions, the acetic acid production in *L. plantarum* is increased in substrates containing maltose in comparison with glucose-containing ones. Therefore, they found that the final acetic acid concentration after 30 h was more than doubled in media containing excessive amounts of maltose. This might explain that, generally, more acetic acid was found in the faba bean-based substrates in this study than in the ones made from starch-free lupines. However, there was no forced aeration in the experimental setup in this study. Consequently, the available oxygen was minimal, and it remains in doubt whether a pyruvate oxidase activity had a substantial impact on the acetic acid production at all. Nevertheless, high levels of acetate formation were also observed in soy-based cheese alternatives fermented with homofermentative LAB (Xie et al., 2024). Generally, it remains unknown, which electron acceptor was utilized by the LAB and should be addressed in further research activities. Moreover, the possible impact of high saccharose concentrations on the pyruvate oxidase or dehydrogenase in particular and on the acetate production in general needs to be examined in future studies.

Regarding the nitrogen compounds, the FAN, the total nitrogen, and their ratio are very useful parameters for evaluating the fermentation process. Increasing FAN-values and nitrogen (N ratios indicate high proteolytic activities as proteins and peptides are decomposed). This can be seen especially by comparing the N ratios of the fermented samples with the control. Thereby, 69 strains (out of 70) in lupine-based substrates and 56 in faba bean-based substrates increased the N ratio. The total nitrogen in the supernatant should remain at a high level to guarantee a high protein content in the final beverage. However, as the isoelectric point of lupines and faba beans ranges between pH 4.0 and 4.5 (Ritter et al., 2023), higher molecular protein would coagulate and lose its solubility and sediment due to the ongoing acidification. Furthermore, nitrogen can be lost by uptake into the LAB cells and their sedimentation. Further research might elucidate the impact and interaction of other factors influencing the impact of the fermentation on the protein content in the supernatant (e.g., substrate-derived proteases, acidification kinetics).

The average of the measured total nitrogen after fermentation was 2.26 g/L for lupine-based beverages and 1.27 g/L for faba bean-based ones. Comparing those values to the unfermented substrates, on average, 84.5% and 78.1% were present after the fermentation in lupines and faba beans, respectively. By using the conversion factor 5.4 $g_{Protein}/g_{nitrogen}$ recommended for legumes (Mariotti et al., 2008), this means that lupine-based beverages contained on average 12.2 $g_{Protein}$/L and faba bean-based ones 6.8 $g_{Protein}$/L. In the lupine-based substrate, the fermented samples of 13 strains contained lower FAN than the substrate, and 18 strains resulted in less than 80% of the initial total nitrogen. These two negative criteria coincided in only 11 cases, meaning that most likely, the proteolytic activity in the majority of strains did prevent substantial losses of protein due to isoelectric precipitation. For faba beans, the results are generally worse, and decreased FAN values were obtained after fermentation with 58 strains. At the same time, 43 strains showed total nitrogen contents of 80% or lower than the original ones. These high losses are exceptionally inconvenient as faba beans generally contain less protein than lupines (8.75 $g_{Protein}$/L, compared with 14.4 $g_{Protein}$/L in the respective substrates). The remaining nitrogen after the fermentation is a vital criterion for the selection of strains as it quantifies the remaining protein in the liquid phase.

The strong acidification and the consequently low pH values show that all 70 strains were able to grow on lupine and faba bean-based substrates. It needs to be mentioned that the strains were not preselected by growth tests. Therefore, this proves that germination is a beneficial step in the preparation of legume-based substrate production. Ritter et al. (2023) studied the changes in germinating lupines and faba beans and reported that the α-galactosides raffinose, stachyose, and verbascose are enzymatically decomposed into saccharose and galactose. While the growing seedling metabolizes galactose, saccharose accumulates and is partially cleaved into glucose and fructose. Harlé et al. (2020) performed a strain screening for the fermentation of soy juice and reported that out of 273 strains, only 36% were able to grow well on raffinose and as few as 6% on stachyose. By including germination in the substrate preparation, the ability of the LAB to express α-galactosidase is no longer required for the growth in legume-based substrates. Therefore, this major restriction in the strain screening for legume-based substrates vanishes, and the selection of a strain can be exclusively based on the acidification, protein content, aroma profile, and sensory attributes.

The fermentation of lupine- and faba bean-based substrates introduced profound changes in the non-volatile composition of both substrates. Even as the same 70 strains were utilized for the fermentation, the evaluation of the resulting beverages shows no overlapping in the principal component analysis, and a clear differentiation is observable (Figure 1). Lupine-based beverages are characterized by higher amounts of nitrogen compounds (FAN and total nitrogen) and higher concentrations of remaining saccharose, while in faba beans, maltose is the main remaining carbohydrate, and more acetic acid is produced in the fermentation. The monosaccharides fructose and glucose do not influence the separation of the fermented samples highly. However, the glucose concentration and the apparent pH value separate the fermented and non-fermented samples. The clear separation between substrate and fermented samples substantiates the finding that all 70 strains were able to grow on lupine- and faba bean-based substrates. As those strains were chosen without pre-selecting for specific α-galactosidase activity, this highlights the critical impact of germination for the accessibility of legume-based substrates for the LAB fermentation.

3.2 | Volatile compounds

Fermentation did not only change the sugar, acid, and nitrogen profiles of the legume-based substrates but also highly influenced the aroma profile. Thereby, several of the aldehydes associated with negative odors were removed and compounds with positive aroma descriptions (e.g., β-damascenone) were newly introduced or increased in concentration (compare Figure 2). Aldehydes that are strongly formed in the germination (like hexanal, 2- and 3-methylbutanal) decreased considerably in the fermentations with almost all strains, whereas their corresponding alcohols hexanol, 2- and 3-methyl butanol increased up to high concentrations. The removal of aldehydes derived from the oxidation of fatty acids (e.g., hexanal) is very beneficial for a refreshing beverage, as it might remove the typical "beany" aroma impression, which hinders consumer acceptance. Such a shift in the aroma profile was expectable, as LAB are known to reduce aldehydes to their corresponding alcohols to regenerate 1,4-dihydronicotinamide adenine dinucleotide (NADH) to nicotinamid adenine dinucleotide (NAD^+) (Gänzle, 2015). Sugahara et al. (2022) studied the ability of several LAB species to reduce aldehydes (e.g., hexanal) and stated that heterofermentative LAB reduce aldehydes considerably stronger than homofermentative LAB. However, statistically significant reductions were also found for several homofermentative LAB (e.g., *L. plantarum*) as primarily utilized in this study.

Comparable reductions of hexanal to non-measurable amounts were already reported by Blagden and Gilliland (2005) in the fermentation of soymilk with several strains of *S. thermophilus*, *L. acidophilus*, and *Lacticaseibacillus casei*. The total depletion of hexanal was also stated for a mixed LAB fermentation of soy whey with *L. rhamnosus* and *L. paracasei* (Zhu et al., 2019). In the fermentation of pea protein with a mixture of *L. acidophilus*, *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and the bifidobacterium *Bifidobacterium lactis*, El Youssef et al. (2020) stated strong hexanal decreases. Harlé et al. (2020) reported partially contradictive results as they screened 46 LAB and found that some *S. thermophilus*, increased the hexanal concentration, but all *Lactobacillaceae* (*L. delbrueckii* subsp. *delbrueckii*, *L. delbrueckii* subsp. *lactis*, *L. plantarum*, *L. coryniformis*, *L. pentosus*) reduced the hexanal concentration comparable to this study. Further studies with lupine protein and pea protein reported no changes in the hexanal content with *L. plantarum* (Schindler et al., 2011, 2012). However, the latter two studies reported the concentration ranges instead of definitive numbers, and therefore only considerable changes are distinguishable, and less profound changes remain unknown. Hexanol, the corresponding alcohol to hexanal, was reported to decrease in soy (Zhu et al., 2019) and

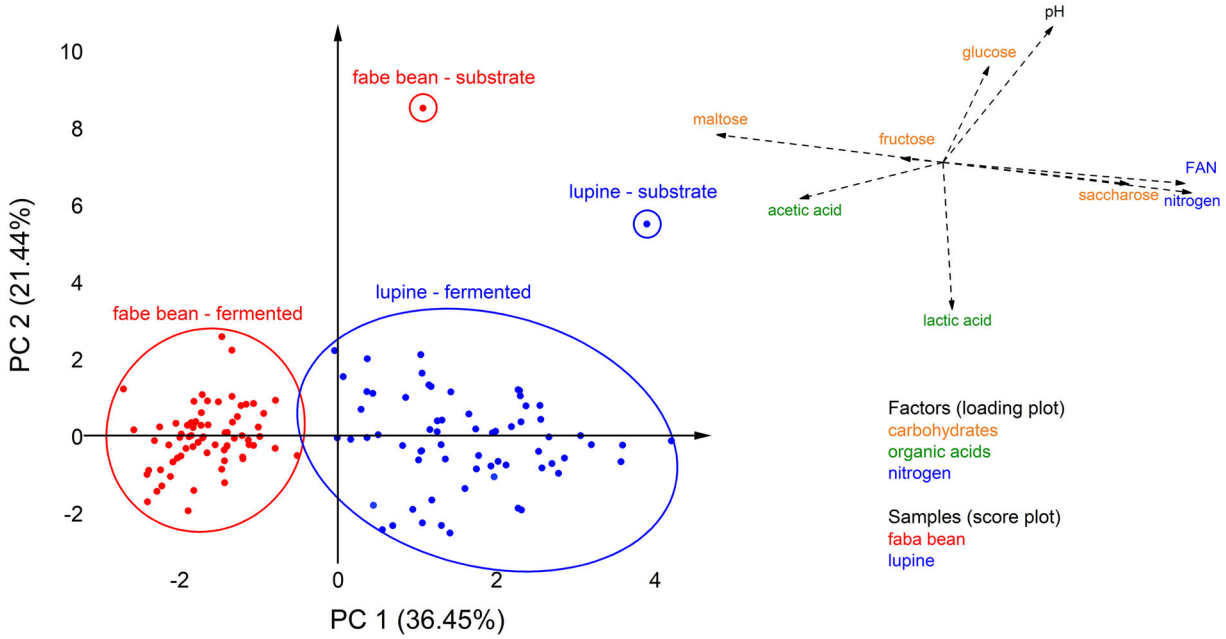

FIGURE 1 Impact of lactic acid bacteria (LAB) fermentation on non-volatile compounds in lupine- and faba bean-based substrate. The score plot (left) shows a clear separation of fermented lupine and faba bean beverages without any intermingling due to the different LAB strains. Moreover, all fermented samples are strongly separated from the unfermented substrates. The loading plot related to the score plot (right) depicts that the separation between lupine and faba bean is mainly due to maltose (high in faba beans) and saccharose, free amino nitrogen (FAN), and nitrogen (high in lupines). Glucose, pH, and organic acids mainly separate the substrates from the fermented samples, whereas a strong tendency of acetic acid toward the fermented faba bean samples is observable. Principal Component 1 and 2 explain 57.9% of the total variation. Values are the mean of three biological replications.

lupine protein (Schindler et al., 2011), whereas it remained constant or decreased in pea (El Youssef et al., 2020; Schindler et al., 2012). As hexanal can react to hexanol but likewise to hexanoic acid, a decreasing hexanal concentration does not necessarily require an increasing hexanol concentration, which is substantiated by Zhu et al. (2019), who reported an increase of hexanoic acid in addition to decreases in hexanol and hexanal.

Slightly different developments were reported for 3-methylbutanal. While in soy juice, the *Lactobacillaceae* highly reduced the concentration, it was increased, unaffected, or only reduced in a minor way by the *S. thermophilus* (Harlé et al., 2020). However, reductions were stated in lupine protein (Schindler et al., 2011) and pea protein (El Youssef et al., 2020; Schindler et al., 2012). Like hexanal, 3-methylbutanal is reduced to its corresponding alcohol. However, LAB can form 3-methylbutanal newly from leucine (Ardö, 2006) and in a strain and substrate-dependent manner, the formation and degradation reactions might compensate each other. For benzaldehyde, Zhu et al. (2019) reported decreases in soy, and Harlé et al. (2020) found an increasing trend with the *S. thermophilus* strains and reductions with the *Lactobacillaceae*. However, in peas, both Schindler et al. (2012) and El Youssef et al. (2020) found increased concentrations after the LAB fermentation. Similar mechanisms for hexanal exist for the amino acid-derived compounds 2- and 3-methylbutanal, -butanol, and -butanoic acid.

In unfermented substrates, the ester ethyl hexanoate is present in traces only or not at all. However, the ester increases in almost all strains during fermentation. The formation of ethyl hexanoate is related to the degradation of hexanal, as the aldehydes can be oxidized by aldehyde dehydrogenase to the corresponding carboxylic acid (e.g., hexanoic acid; Fischer et al., 2022). In a subsequent step, those carboxylic acids can react with alcohols to esters. The lactone γ-nonalactone, described to have a sweet and coconut-like odor impression (Kreissl et al., 2022), increased in the fermentations with almost all strains in faba beans but only 36 out of 70 strains in lupines. As reviewed by Romero-Guido et al. (2011), the formation of γ-nonalactone is connected to the oxidation of fatty acids, for example, linoleic acid that is available in both legumes. The ketone β-damascenone was present only in trace amounts in the unfermented substrates but increased considerably in both substrates with all strains. β-damascenone can be formed from the carotenoid neoxanthin enzymatically, thermally induced, or by acidic oxidation (reviewed by Yang et al., 2013). Notably, the required precursors for β-damascenone can be found in both legumes (El-Qudah, 2014; Estivi et al., 2022). β-damascenone was reported to remain at only trace amounts throughout the fermentation of lupine protein and pea protein with *L. plantarum* (Schindler et al., 2011; Schindler et al., 2012). However, both studies conducted olfactory analysis in addition to the analytical measurements and stated increased odor impressions for β-damascenone in the fermented samples. The formation of this carotenoid-derived aroma compound is particularly positive as its aroma is described as fruity and cooked apple-like (Kreissl et al., 2022), and it might therefore introduce an appealing aroma aspect into the beverage.

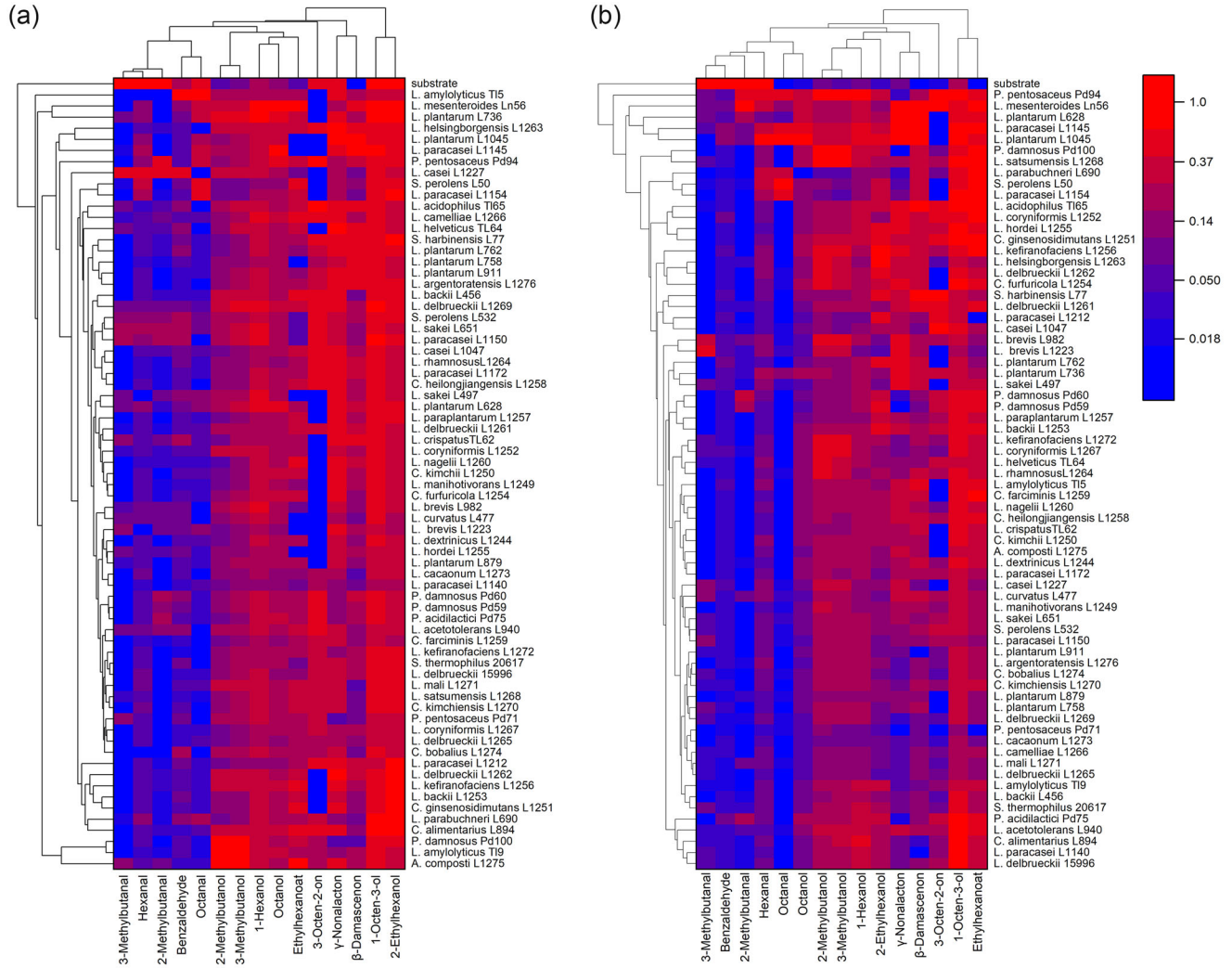

FIGURE 2 Impact of LAB fermentation on volatile compounds in lupine-based substrates (a) and faba beans-based substrate (b). Data normalized by dividing through maximum of the respective aroma compound. Values are the mean of three biological replications.

However, distinct differences between the two legumes are perceivable even as the strains used for the fermentations were the same. The hexanal reduction is more intense in lupine, which can also be seen for benzaldehyde. In lupines, also more alcohols (hexanol, 2- and 3-methyl butanol) are present in the fermented samples, which originate from the reduction of aldehydes. Such a different outcome from the fermentation of different legumes with the same strain is not very surprising, as the substrates differ profoundly (different sugar and amino acid composition, different amounts of secondary plant metabolites like tannins), and comparable results were formerly reported for the fermentation of four different plant-based milk substitutions with several LAB (Tangyu et al., 2023).

The hierarchical cluster analysis supports the differences in the changes. In both substrates, aldehydes and alcohols form separate clusters, which shows the contrary development of the two chemical groups of aroma compounds.

The multivariate analysis showed that lupine- and faba bean-based beverages are clearly separated from each other but cluster together within the respective group (Figure 3). The separation is mainly due to higher concentrations of the aldehydes hexanal, 2- and 3-methylbutanal as well as the alcohol 1-octen-3-ol in faba beans and the alcohol hexanol, as well as the pleasant aroma compounds β-damascenone and γ-nonalactone in lupines. The latter also gradually differentiates the samples within the lupine cluster. In the loading plot, the orientation of the vectors indicates the interactions of the represented factors. Vectors pointing into the same direction are usually correlated, vectors pointing in opposing directions depict negative correlations, and vectors orthogonally orientated to each other show no correlation. In Figure 3, this can be clearly seen in the negative correlation of the aldehydes to their corresponding alcohols, which is explained with the reduction of aldehydes to regenerate NADH to NAD^+, while forming the corresponding alcohols (Gänzle, 2015). The formation pathways of β-damascenone and γ-nonalactone are not connected with the aldehydes, which can be seen from the orthogonal orientation of their vectors. For both legumes, the unfermented substrates are clearly separated from the fermented ones due to the high

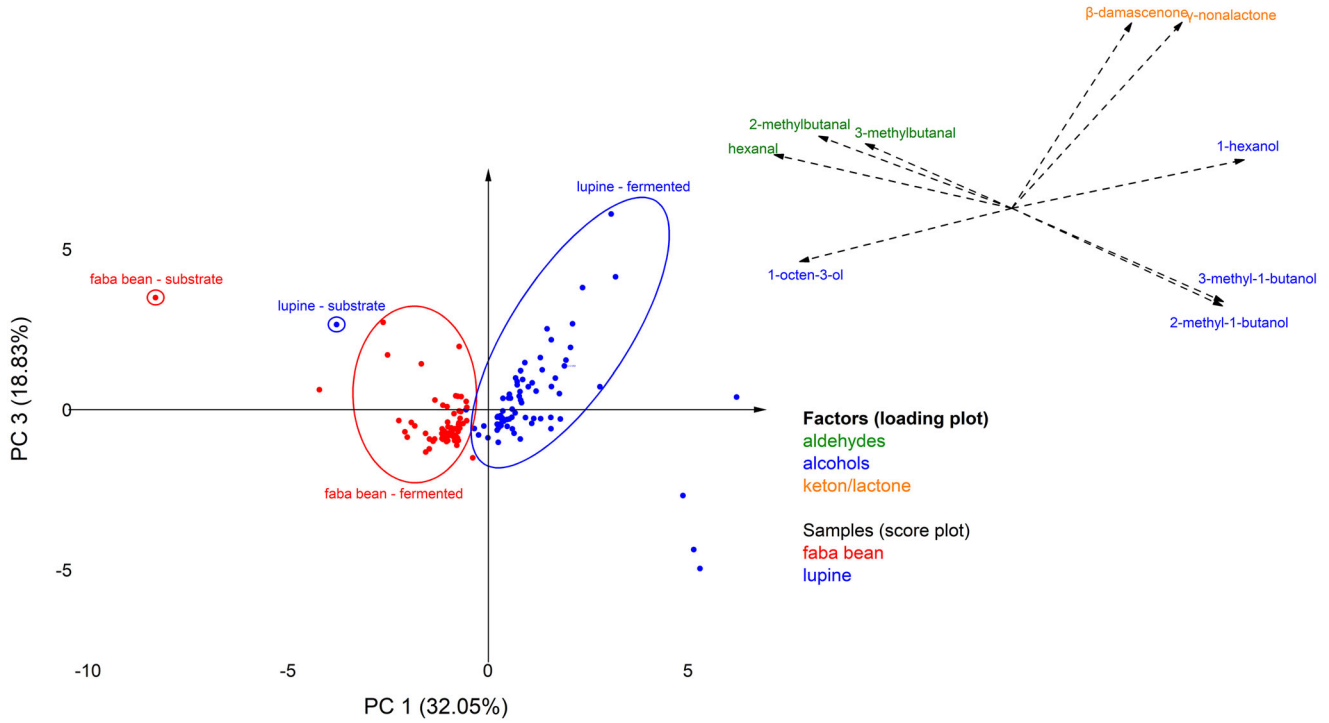

FIGURE 3 Impact of LAB fermentation on volatile compounds in lupine- and faba bean-based substrates. The score plot (left) shows a clear separation of lupine and faba bean beverages fermented with 70 different LAB strains each. Only a few data points are outside the main area of lupines (blue oval) and faba beans (red oval). Moreover, all fermented samples are strongly separated from the unfermented substrates. The loading plot (right) explains that the separation is mainly due to the aldehydes hexanal, 3-methylbutanal, and 2-methylbutanal in the substrates and higher remaining concentrations after fermentation in faba beans. At the same time, more alcohols (1-hexanol, 3-methyl-1-butanol, 2-methyl-1-butanol) are formed in the fermentation of lupines. Only the alcohol 1-octen-3-ol is clearly more dominant in fermented faba beans. Within the fermented lupine beverages, β-damascenone and γ-nonalactone separate the different LAB strains. The two axes of the principal component analysis (PCA) explain 50.9% of the total variation. Values are the mean of three biological replications.

concentrations of aldehydes and lower concentrations of alcohols. This proves that the aroma spectrum of both legume-based beverages is highly different, even if fermented with the same strains.

3.3 | Sensory pre-evaluation

From all 140 fermentations, sensory evaluations were performed to gain a first impression of the odor and taste of the samples and therefore pre-evaluate the sensory performance of the strains in the respective substrate (see also Table S5 in the Supporting Information). Figure 4 depicts the summed rating for lupine- and faba bean-based beverages, which is the sum of the ratings for taste and odor from 1 (*not acceptable*) to 3 (*very acceptable*), respectively. The general acceptance was rated significantly less in faba bean-based samples than in lupine-based samples. While in lupine samples, 34 out of 70 strains resulted in a summed rating of ≥4, and even 12 strains were rated with 5 or higher, in faba bean samples, only 17 out of 70 strains were rated with ≥4 and as less as six strains with ≥5.

In lupine-based substrates, sour impressions and beany and buttermilk-like flavors were often recognized in general. However, there is a trend perceivable that the upper third of the strains (ranked according to the summed rating of taste and odor) is dominated by sour and buttermilk-like impressions and often by additional fruity aromas, while no or only slight beany impressions were named (compare Table S5 in the Supporting Information). In the lower third, this trend is shifted to stronger beany and cooked vegetable-like odors, while less sour and fruity impressions were named. Moreover, very unpleasant odors described as a musty and sewer- or even vomit-like were named more frequently in the lower third. The taste was generally described as sour and even as intensely sour in a number of samples. Especially in the lower third, samples appeared to be less sour but bitter instead. This might indicate an unsuitable proteolytic performance, as too many bitter-tasting peptides were released in the fermentation (Maehashi & Huang, 2009). These bitter impressions were not mentioned in the more suitable strains, which might result from a more appropriate proteolytic activity that resulted in less remaining bitter peptides. Thereby it needs to be mentioned that it is known that bitter peptides are perceived as more bitter than the sum of their amino acids (Maehashi & Huang, 2009). Additionally, these well-performing strains might have further metabolized the resulting bitter-tasting amino acids. Moreover, the intense sour impressions might have masked the remaining bitter impressions (Keast & Breslin, 2002). The bitter perception might additionally originate from phytochemicals (Yan & Tong, 2023), which are also known to be degraded in the lactic acid fermentation (Gänzle, 2020; Gaur & Gänzle, 2023).

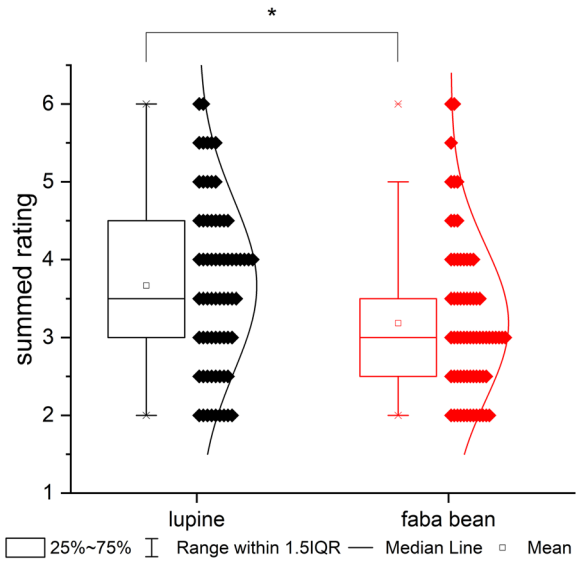

FIGURE 4 Sensory pre-evaluation of lupine- and faba bean-based substrates, individually fermented with all 70 strains. The box plots show that the median is shifted 0.5 points toward higher ratings in lupine-based samples. Especially in the distribution, it is clearly perceivable that considerably more high-rated samples (≥4) were reported for lupine-based samples. These sensory perceptions of the fermented lupine- and faba bean-based substrates are statistically different ($p = .00764$). The degree of freedom for the analysis of variance analysis was 139.

Generally, the faba bean-based samples were rated as less sour. Buttermilk-like, fruity, or citrus odors were less frequently named. In contrast to lupine-based samples, the rather unpleasant fungal and earthy impressions were recognized in many cases. While the majority of strains did not return acceptable results, there are some strains in the first third of the samples that were able to produce refreshing fruity and buttermilk-like odors without the often mentioned strong fungal impressions. However, in the last third, fungal odors were very dominant and repeatedly named in combination with other unpleasant impressions like earthy, cooked vegetable-like, potato peel-like, and musty. In the taste, sourness was as often mentioned as in lupines, whereas the intensity was rated less. A very ambiguous, sweet impression further characterized the taste. In low intensities, it was perceived as pleasant and nicely balancing the sour character. However, in many samples, it appeared as impure or even spoiled and rotten.

For the strain selection, this first sensory impression is vital as the strains can be roughly grouped in promising strains (summed rating ≥ 4) and less suitable strains (summed rating < 4). Especially, the fruity, citrus, and buttermilk-like odors are very advantageous for a refreshing beverage. Comparing the aroma descriptors with the volatile compounds (Section 3.2), certain parallels are observable. Exemplary, *L. casei* L1227 was described as strongly beany and vegetable-like as well as sulfuric, and the volatile profile for this strain showed an insufficient reduction of hexanal and other aldehydes. Moreover, the fruity compound β-damascenone was only negligibly formed by this strain, which was insufficient to counterbalance the negatively associated odors, resulting in the lowest summed rating. On the opposite end of the rating scale, *L. plantarum* L628 was described as very fruity, citrus, and not "beany" at all. The volatile profile showed that aldehydes were strongly reduced and high amounts of the fruity, cooked apple-like β-damascenone and the fruity, coconut-like γ-nonalactone were introduced.

3.4 | Pre-selecting of LAB strains

All 70 strains grew well on both legume-based substrates as the germination step in the substrate preparation modified the nutritional character of lupines and faba beans. This highly improved the accessibility of vital nutrients like carbohydrates and amino acids. Therefore, the screening did not exclude a high number of strains and channel the strain selection toward α-galactoside utilizing strains as in other studies like Harlé et al. (2020) or Tangyu et al. (2021). However, several analytical parameters were selected to narrow down the number of strains suitable for fermentation. First, the pH should be lowered at least to pH 4.0 to ensure good fermentation performance and minimum microbial safety (the growth of bacteria like *Listeria* or *Salmonella* is stopped or highly delayed; Lorenzo et al., 2018). Second, the remaining protein content (expressed as total nitrogen) should be at least two-thirds (66.7%) in legume-based beverages, compared with the unfermented substrates. This criterion should reduce the protein lost in the fermentation and ensure that the majority of the valuable plant protein is transferred into the final beverage. The acid ratio was considered as a further criterion. However, the sour characteristic of a beverage is often mediated or highly masked by taste interactions (e.g., by sweet or umami tastes; Keast & Breslin, 2002). The sensory data in this study strongly suggest such a taste interaction as the faba bean samples were generally perceived as less sour, compared with lupine samples, even as the acid ratio data suggest otherwise. Comparably to the acid ratio, the concentrations of the aroma compounds might be misleading. Almost all strains strongly reduced the negatively associated aldehydes hexanal, 2- and 3-methylbutanal. Solely comparing the final concentrations with their respective aroma thresholds might be too shortsighted as the aroma thresholds stated in the literature are usually reported for water, water-ethanol mixtures, or oil. Putting the complex legume-based samples on a level with water or a simple model mixture would be oversimplifying. The aroma thresholds are valuable for comparing the impact of different aroma compounds with each other and indicate an idea of the magnitude at which an aroma compound might induce an aroma perception. However, in a complex beverage, the matrix interacts with aroma compounds and leads to retention or expulsion effects (Paravisini & Guichard, 2017). Moreover, the interaction of different aroma compounds can trigger an intensified perception or mask the perception of volatiles (Thomas-Danguin et al., 2017). Therefore, the application of the acid ratio or the concentrations of aroma compounds in combination with their threshold values seems misleading or at least not adequate. To avoid excluding strains preliminarily due to wrongly assumed impacts on the odor or taste, the sensory impression was chosen instead, thereby ensuring the

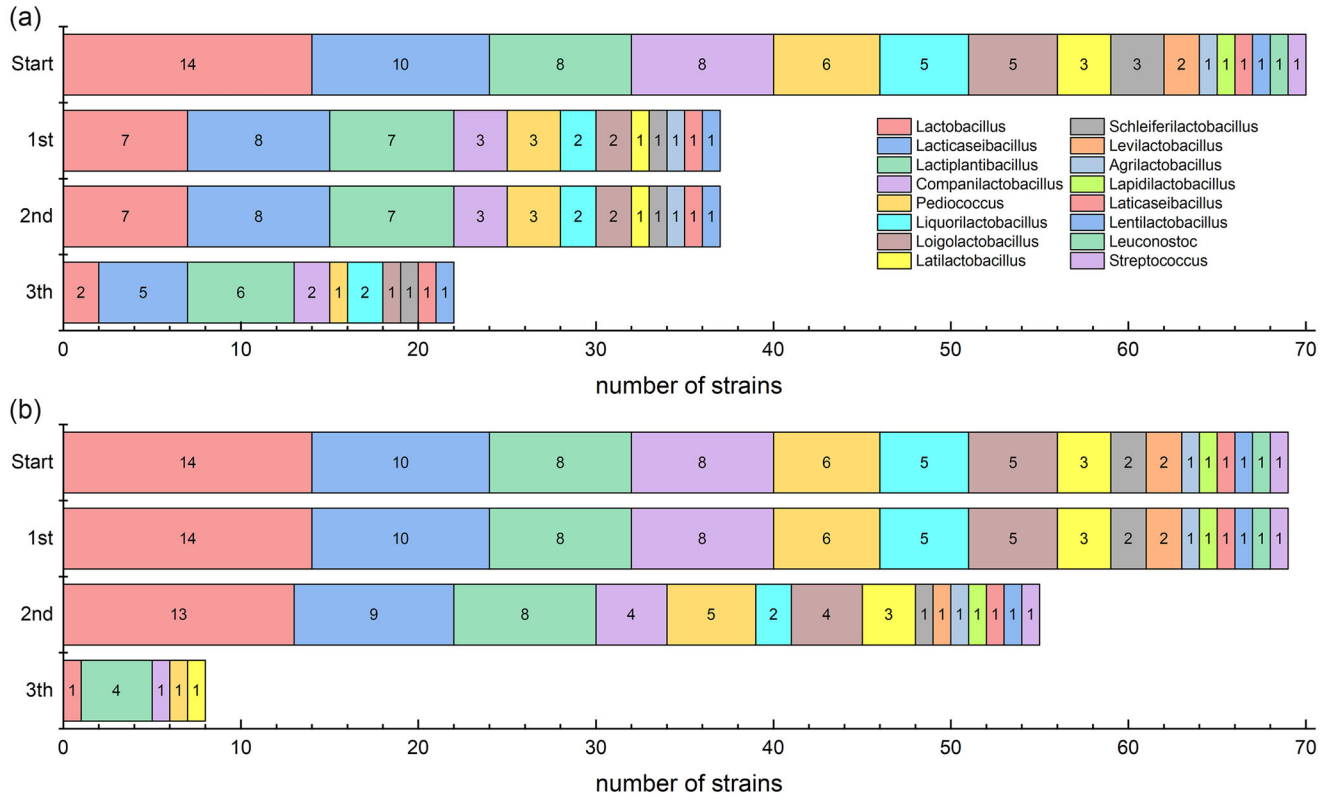

FIGURE 5 Stepwise reduction of LAB strains for lupine- (a) and faba bean-based substrates (b). The reduction is according to the criteria: 1st, pH value ≤4.0; 2nd, remaining protein ≥66.7%; 3rd, sensory attributes. In lupines, the number of eligible strains decreases strongly with the 1st criterion (pH) from 70 to 37, while the 2nd criterion (protein) had no impact on the strain number. After considering the 3rd criterion (sensory), 22 strains remain. For faba beans, the first criterion (pH) had no impact on the number of eligible strains, while the 2nd criterion (protein) reduced the number to 57. The strongest reduction is perceivable for the 3rd criterion (sensory), where only eight strains turned out to be promising for the production of beverages.

true impact of taste and odor-active compounds was not distorted. To pass the sensory criterion, strains had to be rated at least 2 out of 3 points in the odor and taste perception, leading to a minimum summed rating of ≥4.

As Figure 5 shows, this preselection strategy led to the exclusion of 33 strains due to pH (1st criterion) in lupine-based beverages. As all strains preserved at least the required 66.7% protein, none was excluded because of the 2nd criterion. Further, 15 strains were excluded as they failed to produce an acceptable taste and aroma impression or due to off-flavors (e.g., vomit-like odors in *A. composti* L1275). However, in total, 22 strains were considered promising for the production of lupine-based beverages. In faba bean-based beverages, all strains reached pH values of ≤4.0, whereas 13 strains failed to maintain 66.7% of the initial protein content. Most strikingly and in contrast to the lupine-based substrate, a very high number of 49 strains was unable to produce acceptable sensory impressions. Many strains produced an unpleasant slimy mouthfeel, pungent vegetable, and/or blood-like odors. Consequently, for faba bean-based beverages, only eight strains can be named as promising.

To summarize, the application of the three criteria enabled to pre-select suitable strains for the production of refreshing (pH criterion), protein-rich (protein criterion), and appealing (sensory criterion) lupine- and faba bean-based beverages. This helped to narrow down the number of strains for the final evaluations and exclude a total of 48 (lupine) and 62 stains (faba bean) that were not able to meet the requirements for the beverage production.

3.5 | Growth kinetics

The growth kinetics were recorded in fermentations for a number of selected LAB strains and both legume-based substrates (Figure 6). Generally, the kinetics proved that all strains grew well in the two substrates. However, the individual growth was characterized by some variations in the specific growth rate or diauxic growth behavior.

In lupine-based substrates, the onset of growth varied strongly and ranged from almost instantly after inoculation (*L. plantarum* L628) to an adaption phase of approx. 3.5 h (*Liquorilactobacillus nagelii* L1260). The maximum specific growth rates were between 0.247 h^{-1} (*L. nagelii* L1260) and 0.494 h^{-1} (*L. rhamnosus* L1264). For three out of five strains in lupine, a second minor growth rate was identified, and the stationary phase was reached after approx. 5.0–13.5 h (compare *L. plantarum* L628 and *Liquorilactibacillus nagelii* L1260). A different growth

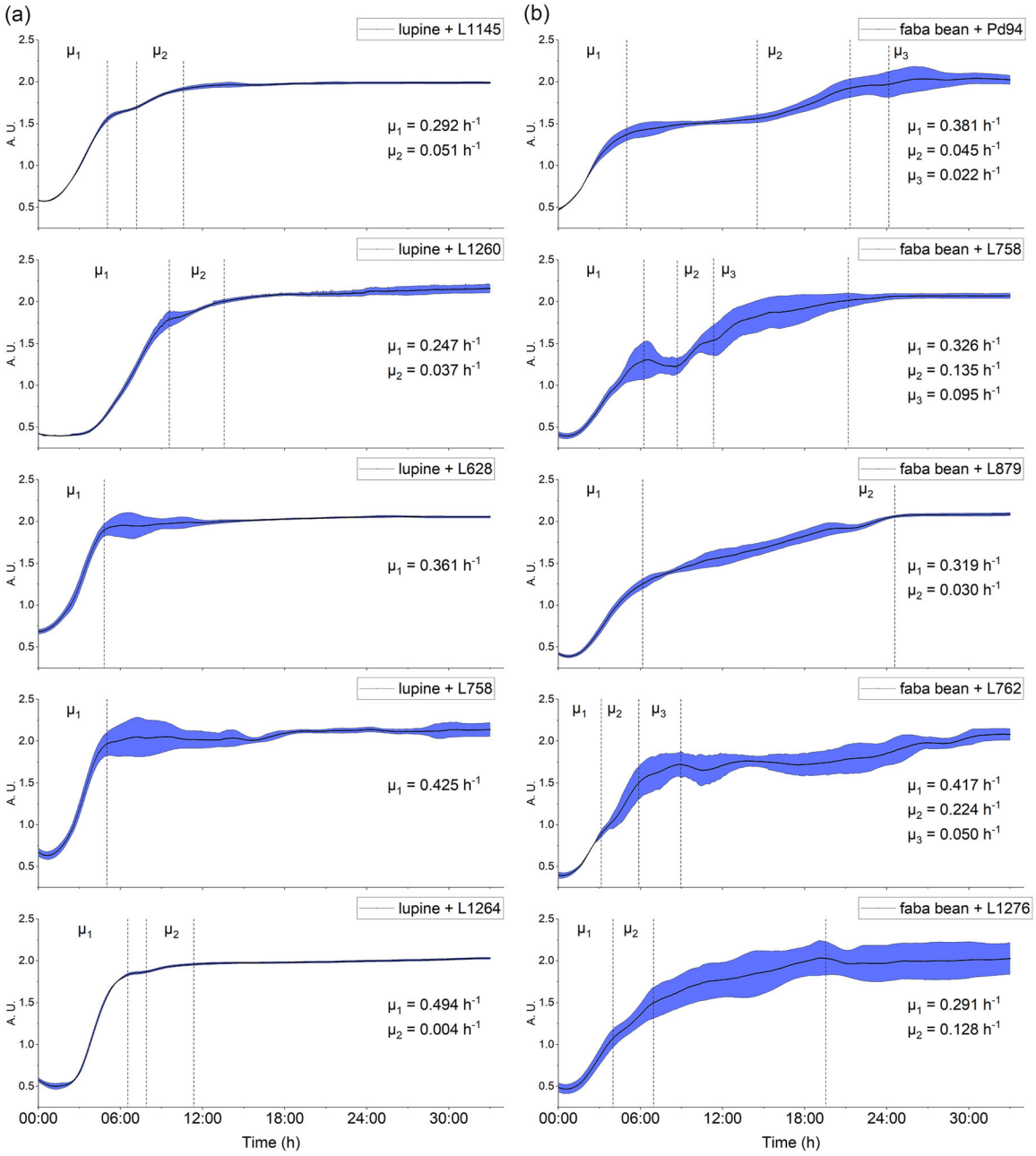

FIGURE 6 Growth kinetics of selected LAB in lupine-based substrate (a) and faba bean-based substrates (b). The dotted vertical lines roughly indicate changes in the growth phases in the individual curves. The growth kinetics are depicted as average values from respectively four biological replicates (black line) with standard deviation (blue area). Specific growth rates (μ) are given for the different growth phases and are indicated above the curve.

behavior was observed in faba bean-based substrates. There, growth set in almost immediately (*L. plantarum* L879 and *P. pentosaceus* Pd94) up to approx. 1 h after inoculation (*L. argentoratensis* L1276). The maximum growth rates were between 0.291 h^{-1} (*L. argentoratensis* L1276) and 0.417 h^{-1} (*L. plantarum* L762) and for each strain two growth phases, and even a third for three of the strains were distinguishable. The last growth phase set in as late as approx. 24 h after inoculation for *P. pentosaceus* Pd94 and *L. plantarum* L879. The growth ended with the beginning stationary phase after approx. 9–25 h (marked by the two *L. plantarum* L762 and L879).

Comparing both substrates, it sticks out that the fermentation was finished considerably faster in lupine-based substrates, even as the adaption phase was slightly longer (especially in *L. nagelii* L1260 and *L. rhamnosus* L1264). Furthermore, in faba bean-based substrates, the variation among the biological replicates was generally higher. The strain *L. plantarum* L758 was used in both substrates, and it is perceivable that the adaption needed slightly longer in faba bean-based substrate (even as the strains were pre-cultured in the same substrates for 24 h prior to inoculation in the kinetic trials). Also, the maximum specific growth rate was with 0.425 h^{-1} higher in lupine- than in the

faba bean-based substrates with 0.326 h^{-1}. Furthermore, it can be seen that the growth in the lupine-based substrate ends considerably earlier than in the faba bean-based substrate. This might be due to the additional sources of carbohydrates (maltose and higher molecular starch degradation products). In faba bean-based substrates, all strains showed two growth phases, and three strains, even a third one. This substantiates the utilization of additional nutrients, especially as in lupine-based substrates, where only three strains showed a secondary growth phase, and no third one was identified at all. However, the existence of secondary plant metabolites (e.g., tannins) impeding the growth in faba bean-based substrates should not be excluded prematurely.

The maximum specific growth rates ranged from 0.292 to 0.494 h^{-1} in lupine-based substrates and from 0.291 to 0.417 h^{-1} in faba bean-based substrates. These values are in accordance with formerly reported ones for LAB. Smetanková et al. (2012) studied the influence of oxygen on the growth of *L. plantarum* and found maximum specific growth rates of approx. 0.2–0.65 h^{-1} (whereas the majority of results were in the range of 0.2–0.4 h^{-1}) for three different strains under varying temperatures in anaerobic and aerobic cultures. In a study with *L. rhamnosus*, *L. paracasei*, *Limosilactobacillus reuteri*, *L. plantarum*, and *L. pentosus* growing on MRS as well as on cereal-based semi-solid state medium, comparable maximum specific growth rates of 0.12–0.37 h^{-1} were reported (Śliżewska & Chlebicz-Wójcik, 2020). Youssef et al. (2005) used kinetic modeling to explain the growth, glucose consumption, and lactic acid production behavior of *L. casei* subsp. *rhamnosus* and found a good fit with a maximum specific growth rate of 0.45 h^{-1} for various media modifications.

Knowing the growth kinetics allows to approximate the required process duration for the latter beverage production. As the stationary phase was reached faster in lupine- than in faba bean-based substrates, the production process should also be faster. The choice of strain might also reduce the fermentation time considerably. Utilizing *L. plantarum* L762 instead of *L. plantarum* L879 would reduce the fermentation time to a third. As a reduced fermentation time means that the same equipment can be used more often in the same time period, the production increases considerably. However, the growth kinetics are limited to the growth of the microbial culture alone and do not indicate the development of the aroma compounds. A prediction of the fermentation time to produce an appealing beverage is not feasible. To include the formation and degradation of aroma compounds over the fermentation time, further studies should observe the timely development of key aroma compounds.

3.6 | Final sensory evaluation

The final selection of the preselected strains was performed with a trained sensory panel. Thereby, the unfermented substrates of both legumes were described as unpleasant, as they show strong beany and vegetable-like odors. Moreover, while the lupine-based substrate was intensely bitter, for the faba bean-based substrate, sweet tastes were also reported (Figure 7).

The striking difference is that lupines contain no or only negligible amounts of starch, which accounts for up to 45% in faba beans (Ritter et al., 2022). In the substrate production, it was broken down enzymatically and added sweet-tasting mono-, di- and oligosaccharides to the substrate. The bitterness was most likely due to bitter peptides originating from the decomposition of high molecular protein in the germination and proteolytic treatment in the substrate production (Ritter et al., 2023), . The released amino acids and low-molecular peptides are known to trigger such a bitter taste impression (Kohl et al., 2013; Maehashi & Huang, 2009).

In addition to this amino acids (e.g., lysine) and peptides, phenolic compounds (e.g., flavonoids), terpenoids (e.g., monoterpene glycosides), saponins, glucosinolates, and alkaloids are typical bitter inducing compounds in plant-derived products (reviewed by Yan and Tong, 2023). For lupines, flavones, isoflavones (primarily genistein), and hydroxycinnamic acid are named as main phytochemicals, besides, for example, alkaloids, tocopherols, and phytosterols (Khan et al., 2015). Moreover, while quinolizidine alkaloids are degraded in the germination of lupines (Ritter et al., 2023), Dueñas et al. (2009) showed that germination strongly increases the concentrations of hydrocinnamics, flavones, and isoflavones. The utilized faba beans were low in the alkaloid glycosides vicine and covicine but contained tannins (Bundessortenamt, 2022). Phytochemicals, like tannins (mainly in faba beans) or isoflavones might also trigger a bitter taste perception in the unfermented substrates and are decomposed in the lactic acid fermentation, reducing the bitter perception. However, further research in the area of enzymatic degradation of phytochemicals by LAB is required (reviewed by Gaur & Gänzle, 2023; Gänzle, 2020). Gaur and Gänzle (2023) compared sequences of enzymes for several LAB genus-type strains. Thereby, the genus *Lactiplantibacillus*—to which six out of eight strains recommended in this study belong— was identified as equipped with a very broad enzymatic system for the conversion of phenolic compounds. Additionally, *L. plantarum* is equipped with an intracellular tannase and some strains even with an additional extracellular tannase (Jiménez et al., 2014). Specifically, Dymarska et al. (2024) studied the ability of different LAB to convert glycosylated phytochemicals and found that phytochemicals are utilized as substrates by, for example, plant-associated LAB (e.g., *L. plantarum*). Moreover, Filannino et al. (2015) found that LAB are suitable to metabolize phenolic compounds, identified *L. plantarum* as explicitly potent, and proposed that phenolics are utilized as electron acceptors in lactic acid fermentation.

In faba beans, the intense perception of bitterness was partially countered by sweet or umami impressions (Keast & Breslin, 2002; M. J. Kim et al., 2015). For both legumes, a minor salty and slightly metallic perception was mentioned, and fermentation added a very intensive sour impression to both substrates. Generally, the acid perception was rated as too strong for lupines, while it was rated as acceptable or pleasant for faba beans. This is because there are more remaining sugars present in fermented faba bean beverages (mainly maltose), which reflects the analytical measurements (compare Table 1). *Lacticaseibacillus rhamnosus* L1264 was rated as the least sour, which is in line with the measurements as the final pH was the highest, the sum of produced organic acids the lowest, and the sum of remaining sugars the highest,

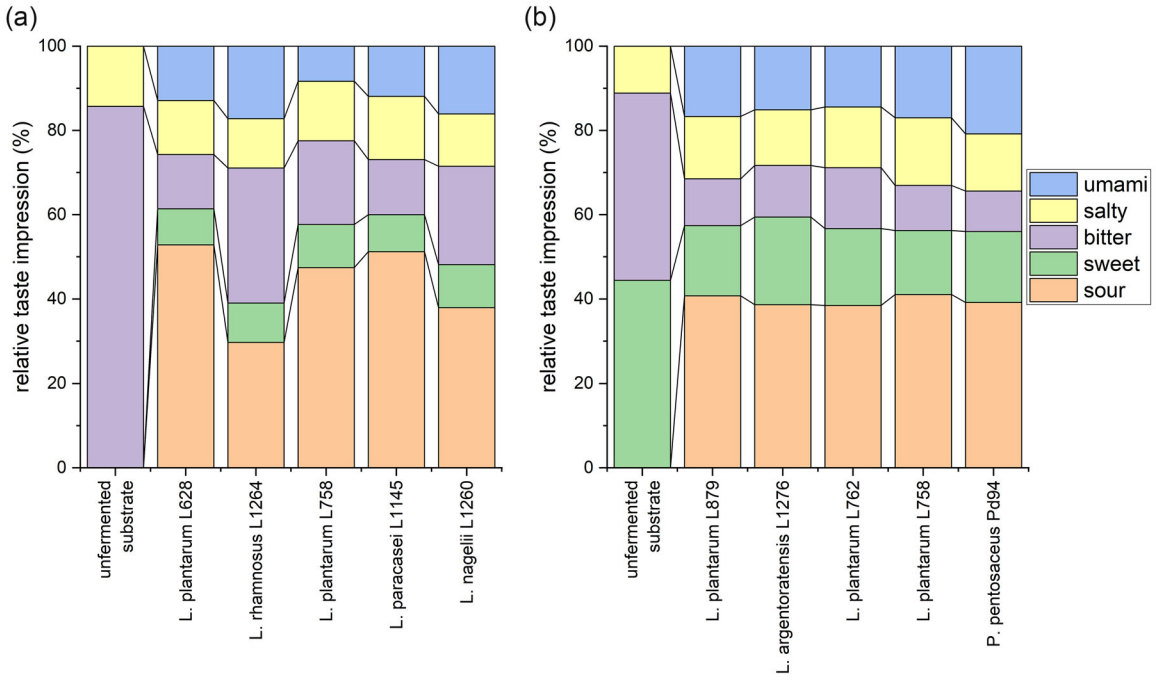

FIGURE 7 Relative taste impression of the unfermented and fermented lupine (a) and faba bean (b) beverages. The unfermented lupine-based substrate showed a very bitter impression, which was highly reduced by fermentation. Moreover, after fermentation, the taste was dominated by sour impressions in both legumes. In faba bean-based substrate, bitter and sweet impressions dominated the taste and changed to strong sour notes and reduced sweet impressions. For both legumes, umami impressions appeared after fermentation. In the sensory session, 18 and 15 persons participated in lupine and faba bean tastings, respectively.

which further counterbalanced sourness. Moreover, *L. plantarum* L758 was rated as less sour than *L. paracasei* L1145, even as it produced more organic acids and reached a lower final pH value. However, the remaining sugars, which can counterbalance sourness, were with 2.8 g/L considerably higher in *L. plantarum* L758 than the 0.3 g/L for *L. paracasei* L1145. This highlights that the pH or the organic acid concentration alone is no indicator for the sour perception of a refreshing beverage but that the remaining sugars have to be always considered as well.

In contrast to faba beans, there is no starch in lupines, which means considerably less sugar in the wort and, consequently, less sugar in the final lupine drinks. However, slightly sweet notes evolved in lupines, the bitterness was highly reduced in both substrates, and minor umami notes were newly introduced. Sourness and bitterness, as well as sweetness and bitterness, can counterbalance each other (Keast & Breslin, 2002). Therefore, the reduced bitterness might be due to such a mitigating taste interaction or as free bitter-tasting amino acids and peptides were metabolized by the LAB.

Regarding the aroma impression, both unfermented substrates were dominated by beany and cooked vegetal-like notes (compare Figure 8). While for lupines, a slight sulfuric, potato-like note was mentioned, earthy impressions were named additionally in faba beans. The aroma impression was strongly improved by fermentation. Fruity and citrus-like aromas, as well as buttermilk-like notes, were often newly introduced. Those fruity impressions might be traced back to aroma compounds like β-damascenone (fruity, cooked apple; Kreissl et al., 2022), γ-nonalactone (coconut-like; Kreissl et al., 2022), and to some extent to esters like ethyl hexanoate (fruity, pineapple-like; Kreissl et al., 2022). Moreover, the buttermilk-like notes are most likely due to diacetyl (sweet, buttery; Kreissl et al., 2022), which is a very common metabolic by-product of LAB. In faba bean-based beverages, higher musty, earthy, and fungus-like notes were mentioned than in lupine-based beverages. This is supported by the higher contribution of 1-octen-3-ol (mushroom-like; Kreissl et al., 2022) in the faba bean samples analyzed in the preselection. Additionally, 1-octen-3-one, the very aroma-active corresponding ketone to 1-octen-3-ol (aroma threshold of 0.016 μg/L in comparison to 45 μg/L of the alcohol; Kreissl et al., 2022) is usually present besides the alcohol. However, it was analytically not identifiable due to the very low amounts and the coinciding retention time on DP-5 columns as used in this study. Interestingly, even as the beany aroma impression was reduced by fermentation in all strains, it was still mentioned as perceivable more or less intense for all strains. The aldehyde hexanal is very often mentioned to be connected to this aroma impression (Tangyu et al., 2023). However, the resulting hexanal concentrations were in all lupine fermentations with 0.06–0.31 μg/L and for some faba bean fermentations clearly beneath its odor threshold of 2.4 μg/L (Kreissl et al., 2022). Therefore, the narrow focus on hexanal regarding the beany aroma impression perceivable in recent years should be broadened again, and further research is required following the first approaches of Vara-Ubol (2004) and Bott and Chambers (2007) to thoroughly understand the complex beany aroma.

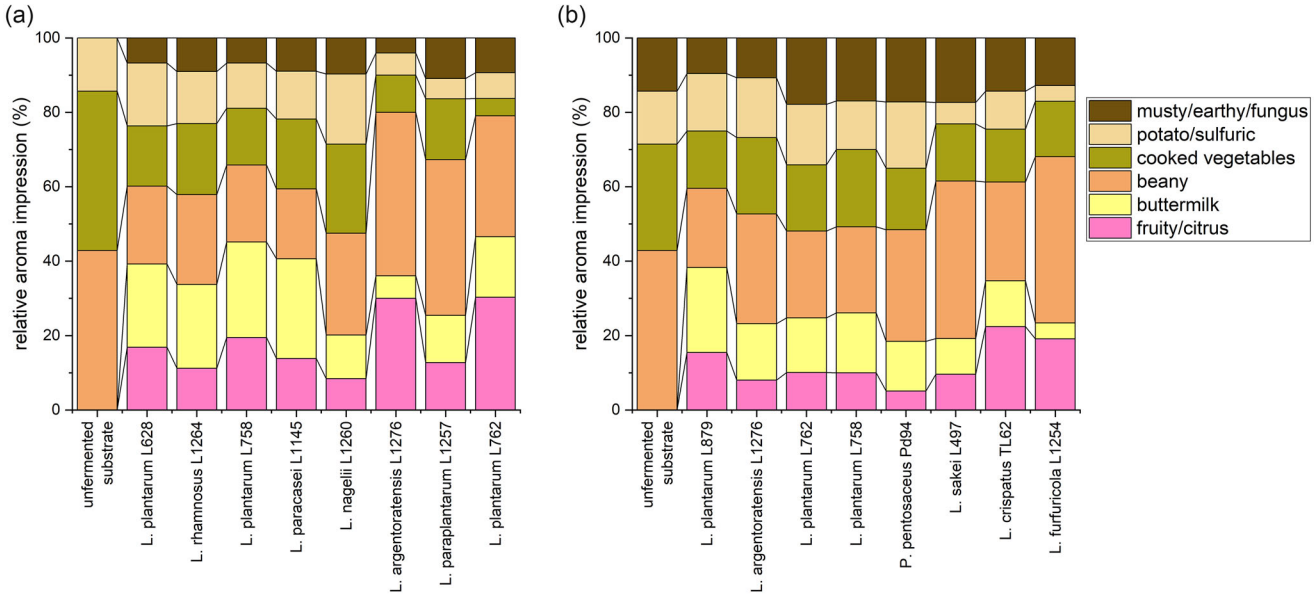

FIGURE 8 Relative aroma impression of the unfermented and fermented lupine (a) and faba bean (b) beverages. Strong beany and cooked vegetable-like aroma impressions characterized both unfermented substrates. In difference to lupines, where only minor sulfuric odors were described, faba beans showed earthy, musty, and fungus-like aroma attributes in addition to sulfuric odors. Fermentation introduced fruity and citrus-like odors as well as buttermilk-like notes in both legumes. Simultaneously, the formerly dominant beany and cooked vegetables-like aromas were strongly reduced. After fermentation, both legumes showed minor sulfuric and earthy odor impressions. The results are from two sensory sessions for each legume, where 14 and 18 persons participated in the lupine tastings and 12 and 15 persons in the faba bean tastings.

The participants in the sensory sessions were also asked to rate their overall preference for fermented lupine and faba bean beverages in a ranking (Figure 9). For lupine-based beverages, strains *L. plantarum* L628, *L. plantarum* L762, and *L. rhamnosus* L1264 were rated as the most preferred ones. All three strains highly reduced the beany and cooked vegetable-like impression while producing strong buttermilk-like notes and modest or strong fruity odors. It should be highlighted that the differences between the preferences of the individual strains were more distinguished in faba beans than in lupines, where the first five strains showed a closely rated preference. The strains *L. paracasei* L1145 and *L. plantarum* L758 showed comparable aroma and taste impressions and were almost as preferable rated as the first three strains and should therefore also be considered as suitable strains for the fermentation of lupine-based substrates. The three least preferred strains (*L. nagelii* 1260, *L. argentoratensis* L1276, and *L. paraplantarum* L1257) were rated with the lowest buttermilk-like impressions, very high beany and cooked vegetable-like odors, and for *L. nagelii* L1260, the highest sulfuric notes. *Lactiplantibacillus plantarum* L879, *L. plantarum* L762, and *L. argentoratensis* L1276 were named as the most preferred for faba bean beverages. Comparing this finding with the aroma impressions, it sticks out that all three strains show low remaining beany impressions and decent buttermilk-like odors. Especially, *L. plantarum* L879 stands out with high buttermilk-like and the lowest beany aroma impressions. The strains *L. crispatus* TL62 and *C. furfuricola* L1254 showed the highest fruity aroma impressions. However, those strains were rated as the least preferred ones. For *C. furfuricola* L1254, this might be due to an almost unchanged beany aroma impression. Also, both strains showed low buttermilk-like odors. The taste impressions showed strong improvements between fermented and unfermented samples but no notable differences between the strains.

The connection between buttermilk-like aroma impressions and higher-rated preference was observed in lupines and faba beans. This indicates that this aroma attribute fits well with the sour taste impression and is consistent with known taste-aroma combinations in fermented dairy products (e.g., kefir, yogurt). Moreover, the buttermilk-like impressions might help to counterbalance negatively perceived odors like sulfuric, cooked vegetables, and beany. Interestingly, the same trend was not observed for fruity aroma impressions. Probably, stronger fruity odors are associated as artificial and/or perished, leading to a decreased preference. For the production of refreshing beverages, the focus should henceforward be put on the formation of a strong buttermilk-like flavor in combination with hints of fruity and citrus. Therefore, future research should focus on process optimization to increase this aroma profile further. This might be accomplished by finding optimized fermentation parameters (e.g., temperature) while monitoring the distinctive key aroma compounds for those aroma attributes (e.g., diacetyl for buttermilk-like or β-damascenone for fruity).

4 | CONCLUSION

For the first time, this study presents a strain screening in germinated lupine- and faba bean-based substrates for the production of refreshingly sour and protein-rich beverages. The most striking result of this study is that, in contrast to other studies, almost all of the used strains

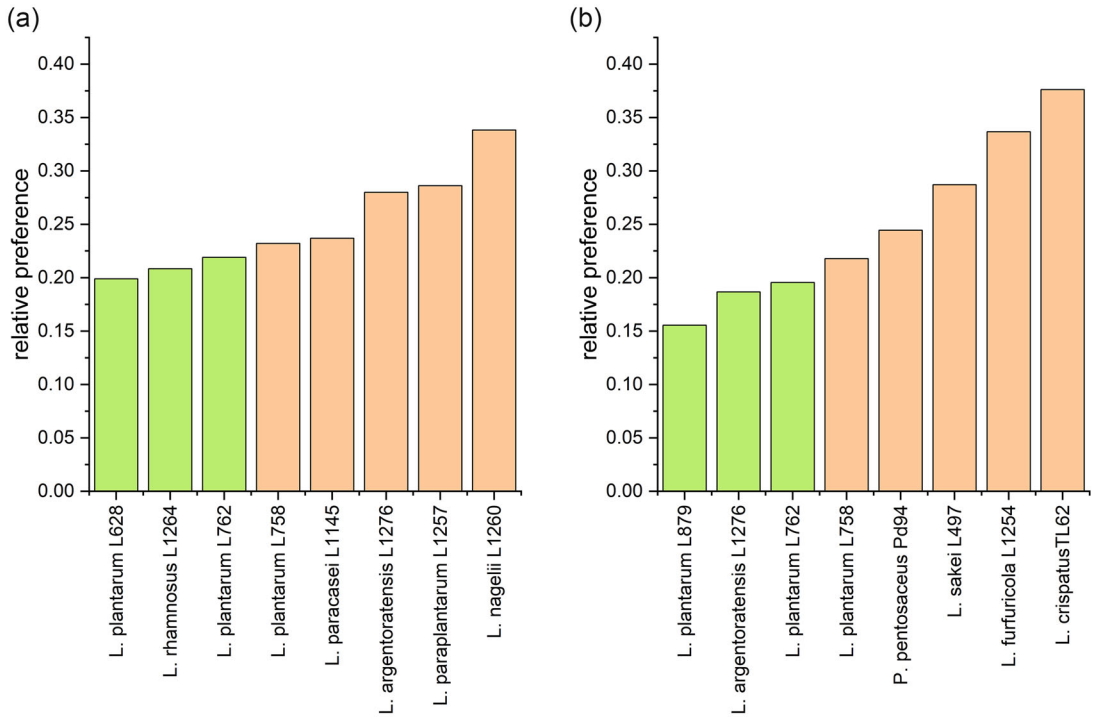

FIGURE 9 Ranking of the overall preference of beverages based on lupine (a) and faba bean (b). Participants were asked to rank the beverages, whereas small numbers indicate a high preference. The results were normalized to allow comparison of two sensory sessions. The three best-rated strains are highlighted in green. The sensory panel consisted of 14 and 18 panelists for the sessions with lupine-based beverages and 12 and 15 for faba bean-based beverages.

grew well on lupine- and faba bean-based substrates. This indicates that germination is a very potent step in substrate preparation to make nutrients easily available and highly broaden the number of usable strains. This is because the choice of strain is no longer restricted to strains that show the ability to break down storage compounds of raw legumes like α-galactosides as carbon source. However, it needs to be mentioned that germination leads to raw material losses due to the germs metabolism and microbial spoilage might occur if seeds are highly contaminated. Nevertheless, it is highly recommended to consider a germination step when using microbial fermentation in the production of legume-based food and beverage applications.

Despite the fact that all strains grew on both substrates, the outcome was considerably different for both substrates in each part of the evaluation. While more acetic acid was produced and the pH was generally lower in faba bean-based samples, the sensory impression of lupine-based samples was recognized as more sour. In contrast, the sweet impressions in faba bean-based samples were often perceived as unpleasant and, in a number of samples, even as impure or spoiled. In the sensory pre-evaluation, it was already clearly perceivable that the majority of faba bean-based samples suffer from unpleasant fungal and cooked vegetable-like odors. In contrast, lupine-based samples showed more pleasant fruity and buttermilk-like aromas. This had a clear impact on the strain selection, as only eight strains were deemed as suitable for faba bean-based substrates, while 22 strains were regarded as promising in lupine-based substrates. Additionally, the fermentation of lupine-based substrates is faster, as the growth kinetics showed. This proves that both strain and raw material have to be considered together in a strain screening as the results gained with one raw material cannot be transferred to a different one, even if the same strain is employed for the fermentation.

Finally, a number of highly suitable strains for the production of appealing beverages were identified for lupine- and faba bean-based substrates by analytical and sensory pre-evaluation and a final sensory analysis with an increased number of trained participants (Table 4). The strains *L. plantarum* L758 and *L. paracasei* L1145 were included for lupine-based substrates as they were almost as preferable as the most preferable three strains.

The fermentations in this study were performed without variations in temperature and inoculum size, as it was required to compare the results of the high number of strains. However, this also shows the limits of this study and further need for research. The fermentation parameters (e.g., temperature, inoculum size) can highly influence the outcome of a fermentation in general and the resulting aroma profile in particular. Therefore, for the most promising strains identified in this study, this impact needs to be investigated by varying the fermentation parameters and comparing the outcome. This might even lead to a further reduction of off-flavors and improved consumer acceptance of the new product. Moreover, the combination of different strains in co-culture fermentations could complement the enzyme capacities of the single strains and might improve performance or flavor production even more. Finally, further research in the origin of the beany aroma impression is required to better understand why this odor evolves,

TABLE 4 Suitable LAB strains for the production of lupine- and faba bean-based beverages.

Lupine-based substrate	
Lactiplantibacillus plantarum L628[a]	Isolated from beer
Lacticaseibacillus rhamnosus L1264[b] (DSM 20021)	Isolated from milk products, fermented vegetables, meat, and fish[c]
Lactiplantibacillus plantarum L762[a]	Isolated from tomato juice
Lactiplantibacillus plantarum L758[a]	Isolated from kombucha
Lactobacillus paracasei subsp. *paracasei* L1145 (DSM 20006)[a]	Isolated from beer
Faba bean-based substrate	
Lactiplantibacillus plantarum L879[a]	Isolated from pickled vegetable, cheese, fermented sorghum porridge and silage[c]
Lactiplantibacillus argentoratensis L1276[b] (DSM 16365)	Isolated from starchy food, fermented food of plant origin, fermented dough[c]
Lactiplantibacillus plantarum L762[a]	Isolated from tomato juice

[a]Obtained from the strain collection of the Chair of Brewing and Beverage Technologies, Freising, Germany.
[b]Obtained from the strain collection of the Chair of Microbiology, Freising, Germany.
[c]According to Zheng et al. (2020).

which interactions are required to trigger the beany impression, and which aroma compounds need to be removed to eliminate the odor efficiently.

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CONFLICT OF INTEREST STATEMENT

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 publication. The sensory evaluations conducted in this work were on a voluntary basis performed by panel members associated with the Chair of Brewing and Beverage Technologies. The aim of the study and possible risks were explained at the beginning of each session. Participants were not forced to participate and could have withdrawn from the sensory evaluations at any time without facing any consequences.

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SUPPORTING INFORMATION

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