LWT - Food Science and Technology 218 (2025) 117516

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Effect of exogenous DPD on fermentation characteristics and flavour formation of *Lactiplantibacillus plantarum* HRB1 *in vitro* and in a dry sausage model: Insights from the quorum sensing system

Jiasheng Lu [a], Yumeng Sui [a], Xin Liu [a], Jiawang Wang [a], Jiatong Li [a], Baohua Kong [a], Qian Chen [a,*], Weiwei Yang [b,**]

[a] *Northeast Agricultural University, Harbin Heilongjiang, 150030, China*
[b] *Department of Food Science, College of Public Health, Shenyang Medical college, Shenyang, Liaoning, 110034, China*

ARTICLE INFO

Keywords:
Lactiplantibacillus plantarum
Quorum sensing
Signal molecule
Volatile compound
Dry sausage

ABSTRACT

The present study aimed to investigate the impact of 4,5-dihydroxy-2,3-pentanedione (DPD; 0, 300, 600, and 900 nmol/L), a crucial precursor in the *LuxS*/AI-2 quorum sensing system, on flavour formation by *Lactiplantibacillus plantarum* HRB1 both *in vitro* and dry-sausage model. Firstly, *L. plantarum* HRB1 was selected from 18 strains of lactic acid bacteria due to its superior AI-2 and protease activity. *In vitro*, DPD significantly increased the activity of the AI-2 and the protease activity of *L. plantarum* HRB1. In the fermented dry-sausage, free amino acid content was significantly higher in the treatment with 600 nmol/L DPD compared to the other treatments. Notably, the dry sausage treated with 600 nmol/L DPD exhibited the highest concentrations of aldehydes (558.50 μg/kg), ketones (192.41 μg/kg), alcohols (1175.56 μg/kg), acids (202.27 μg/kg), esters (1504.48 μg/kg), and terpenes (1319.82 μg/kg). Sensory analysis revealed that the dry sausage treated with 600 nmol/L DPD had the most pronounced fermented and fatty odour. These results suggest that 600 nmol/L DPD enhances the flavour formation of *L. plantarum* HRB1. This research provides a theoretical foundation for utilizing the *LuxS*/AI-2 QS to optimize microbial fermentation processes and improve fermented food quality, especially flavour.

1. Introduction

Dry sausage, a classic fermented meat product, is renowned among consumers for its unique taste and flavour. The fermentation and ripening of traditional dry sausage occur through the action of endogenous enzymes and the microbial community in its natural state (Chen et al., 2017). In this process, the formation of flavour primarily relies on the enzymatic degradation of macromolecules such as lipids, proteins, and carbohydrates by microorganisms within the system (Dong et al., 2024). However, microbial communities in their natural state can result in inconsistent quality. Therefore, starter cultures are recommended to standardize manufacturing, improve product quality, and preserve the unique flavour of fermented product.

It is usual practice to use lactic acid bacteria (LAB) as the main starting culture when fermenting food items. They can enhance flavour profiles through three key metabolic pathways: carbohydrate metabolism, proteolysis catabolism, and lipolysis metabolism (Bintsis, 2018). Moreover, LAB exert inhibitory effects on spoilage microorganisms by metabolizing lactic acid to lower the pH of the system or through bacteriocin production to ensure product safety. Hence, the regulation of LAB metabolism has significant implications for enhancing flavour, extending shelf life, and boosting the nutritional value of fermented meat products.

Certain LAB communicate through specific signal molecules during their growth, enabling the cells to perceive the abundance of their own species in the surrounding environment and regulate gene expression accordingly, thereby facilitating communication within species and genera (Chai et al., 2023; Eickhoff & Bassler, 2018). This phenomenon, where a strain regulates gene expression by sensing changes in the density of similar cells in its surroundings, is called quorum sensing (QS) (Meng et al., 2022). 4,5-dihydroxy-2,3-pentanedione (DPD) plays a pivotal role in the activation of the *LuxS*/AI-2 QS system of LAB, thereby exerting indispensable control over interstrain communication and facilitating fermentation (Sui et al., 2024). Prior research has shown that

* Corresponding author.
** Corresponding author.
E-mail addresses: chenqianego7@126.com (Q. Chen), yangweiwei@symc.edu.cn (W. Yang).

https://doi.org/10.1016/j.lwt.2025.117516
Received 3 December 2024; Received in revised form 20 January 2025; Accepted 10 February 2025
Available online 11 February 2025

AI-2 significantly enhances the probiotic properties of LAB during fermentation by regulating biofilm formation, inhibiting spoilage bacteria growth, and augmenting strain tolerance (Meng et al., 2022). Moreover, it is essential in governing the fermentation process, thereby enhancing the product's sensory attributes (Chai et al., 2023; Meng et al., 2022; Sui et al., 2024). However, the flavour-enhancing effects of LAB based on the modulation of the *LuxS*/AI-2 QS system have been infrequently documented, and the underlying mechanism is still unclear.

This study focuses on 18 LAB strains derived from Harbin dry sausage, which demonstrate superior fermentation performance and flavour-producing potential. The study initially focused on examining the growth, AI-2 activity, and protease activity of the strains during a 24-h incubation period. The goal was to identify LAB strains possessing a strong capability for producing AI-2. Due to the low production and limited stability of AI-2, DPD, the precursor of AI-2, was used in this study. It has the capability to reconfigure molecular structures within the strain to facilitate AI-2 formation (Meng et al., 2022). Firstly, the protease activity and growth of the strains were assessed *in vitro* following the exogenous addition of DPD. Subsequently, the impact of DPD on flavour formation in a dry-sausage model using the superior strain was investigated. The QS system was found to effectively respond to DPD, and the optimal concentration of DPD addition was ascertained. This study establishes a theoretical foundation for investigating the utilization of the *LuxS*/AI-2 QS system as a novel pathway to enhance the fermentation performance of microorganisms and improve the quality of fermented food products, particularly in terms of flavour formation.

2. Materials and methods

2.1. Strains

The LAB strains (*Lactiplantibacillus plantarum* MDJ2, MDJ6, SYS29, HRB1, SH7, and RA6; *Latilactobacillus sakei* MDJ5, DQ2, DQ11, JX2, JX11, SYS4, and 8–2; *Weissella viridescens* MDJ8, DQ9, JX4, JX9, and MDJ10) used in this study were isolated from traditional fermented dry sausages and were preserved in the Meat Science laboratory at Northeast Agricultural University. *Vibrio harveyi* BB170 was acquired from the Shanghai Biotechnology Conservation Center. The broth and agar used in this study were procured from Qingdao Haibo Biotechnology Co. Ltd.

2.2. Selection of LAB with high-yield AI-2

The detection of AI-2 was performed using a bioluminescence assay established by Liu et al. (2022). The initial dilution (ID) was achieved by subjecting this culture to a 5000-fold dilution using 2216E medium. The LAB culture was centrifuged (12000 rpm, 10 min, 4 °C), and the cell-free supernatant (CFS) was obtained by filtering the supernatant through a 0.22-μm sterile syringe filter (Haining de filter new material technology Co., Ltd., Zhejiang, China). The CFS of *V. harveyi* BB170 was obtained through the utilization of an identical methodology. Over a period of 0–6 h, 200 μL of the mixture was collected every 30 min, and its fluorescence intensity was quantified utilizing the chemiluminescence mode of an enzyme labeller (SpectraMax® iD3; Meigu Molecular Instruments Co., Ltd., Shanghai, China).

2.3. Determination of AI-2 production capacity and protease activity of strains

2.3.1. Growth curve and pH

The density of LAB strains selected as described in "Section 2.2" was determined at 600 nm using an enzyme labeller at 4-h intervals from 0 to 24 h. The pH value was assessed using a pH meter (FE28, Hangzhou Wante weighing Instrument Co., Ltd., Zhejiang, China).

Table 1
Primer sequences for assessing the relative gene expression.

Gene		Primer sequence
OppA	forward (5′–3′)	AGTGTTAACGCGCTACGTGA
	reverse (5′–3′)	CATGCTTGACCTTCATCGGC
OppB	forward (5′–3′)	TGGCGTTGTCACTCGCTTAT
	reverse (5′–3′)	TGATCCGCCGCATAGGTAAC
OppF	forward (5′–3′)	GATGTCGGCAGTCCCCATAC
	reverse (5′–3′)	GGAAAACACAACCGGAAGGC
livK	forward (5′–3′)	GTTCCGTTGGCCTGAAAACC
	reverse (5′–3′)	GCTTTCAGCGCATCAACGAT
livH	forward (5′–3′)	GCATGATCAACTTCGCCCAC
	reverse (5′–3′)	CCACCCGTTCGATACTCCAG
luxS	forward (5′–3′)	TCCTACGGAAATTGCCAAAGTT
	reverse (5′–3′)	TGGTCTGAGATGCCTTGTTCA
pfs	forward (5′–3′)	TCTTATTGCGACTGGTGATAGC
	reverse (5′–3′)	CATAGCACGGACAACGATGAA
CysK	forward (5′–3′)	GACTGCGCTTTCGTCTGTTG
	reverse (5′–3′)	AGGCGGTGTTAGGATCAAGC
DSD1	forward (5′–3′)	GGCTGTGTCCAAGATCACGA
	reverse (5′–3′)	ATTGAGGATGCTTCTCGCGT

2.3.2. AI-2 production capacity

The AI-2 activity was evaluated at 4-h intervals over a 24-h period, employing the same methodology described in "Section 2.2".

2.3.3. Protease activity

The protease activity was assessed following the methodology outlined by Han, Baek, Chun, and Jeon (2023) at 4-h intervals from 0 to 24 h. Briefly, LAB CFS (0.5 mL) was combined with the 0.5 mL of casein reagent (Soleibao Technology Co., Ltd., Beijing, China). Subsequently, 2 mL of 0.4 mol/L TCA reagent (Aladdin Biochemical Technology Co., Ltd., Shanghai, China) was added to the solution, followed by centrifugation to obtain 1.0 mL of supernatant. A solution of 5 mL of 0.4 M Na_2CO_3 (Aladdin Biochemical Technology Co., Ltd., Shanghai, China) and 1 mL of Folin-phenol reagent (Aladdin Biochemical Technology Co., Ltd., Shanghai, China) were subsequently added. After incubating at 45 °C for 25 min. The absorbance of the mixture was determined at 680 nm using an enzyme labeller.

2.4. Effect of DPD on AI-2 production capacity and protease activity of *L. plantarum* HRB1 in vitro

The MRS medium was supplemented with DPD, and the concentrations of DPD were adjusted to 0, 300, 600, and 900 nmol/L, respectively. Subsequently, a culture of *L. plantarum* HRB1 with a concentration of 10^7 CFU/mL was injected into the MRS medium. After two precultivations, *L. plantarum* HRB1 was inoculated and incubated (10^7 CFU/mL, 200 rpm, 37 °C) for 24 h. The growth, AI-2 activity, and protease activity of *L. plantarum* HRB1 were assessed at 4-h intervals from 0 to 24 h.

2.5. Determination of gene expression assay of *L. plantarum* HRB1

The gene expression measurements were conducted following by Shi et al. (2024). The MRS medium was supplemented with DPD, and the concentrations of DPD were adjusted to 0 and 600 nmol/L, respectively. Subsequently, a culture of *L. plantarum* HRB1 with a concentration of 10^7 CFU/mL was injected into the MRS medium. After two precultivations, *L. plantarum* HRB1 was inoculated and incubated (10^7 CFU/mL, 200 rpm, 37 °C) for 16 h. Total RNA was isolated from *L. plantarum* HRB1 treated with 0 and 600 nmol/L DPD through E.Z.N.A.® Bacterial RNA kit (Feiyang Biological Engineering Co. Ltd., Guangzhou, China). The 16S rRNA gene was designated as the reference gene, and the primers employed in this study are detailed in Table 1.

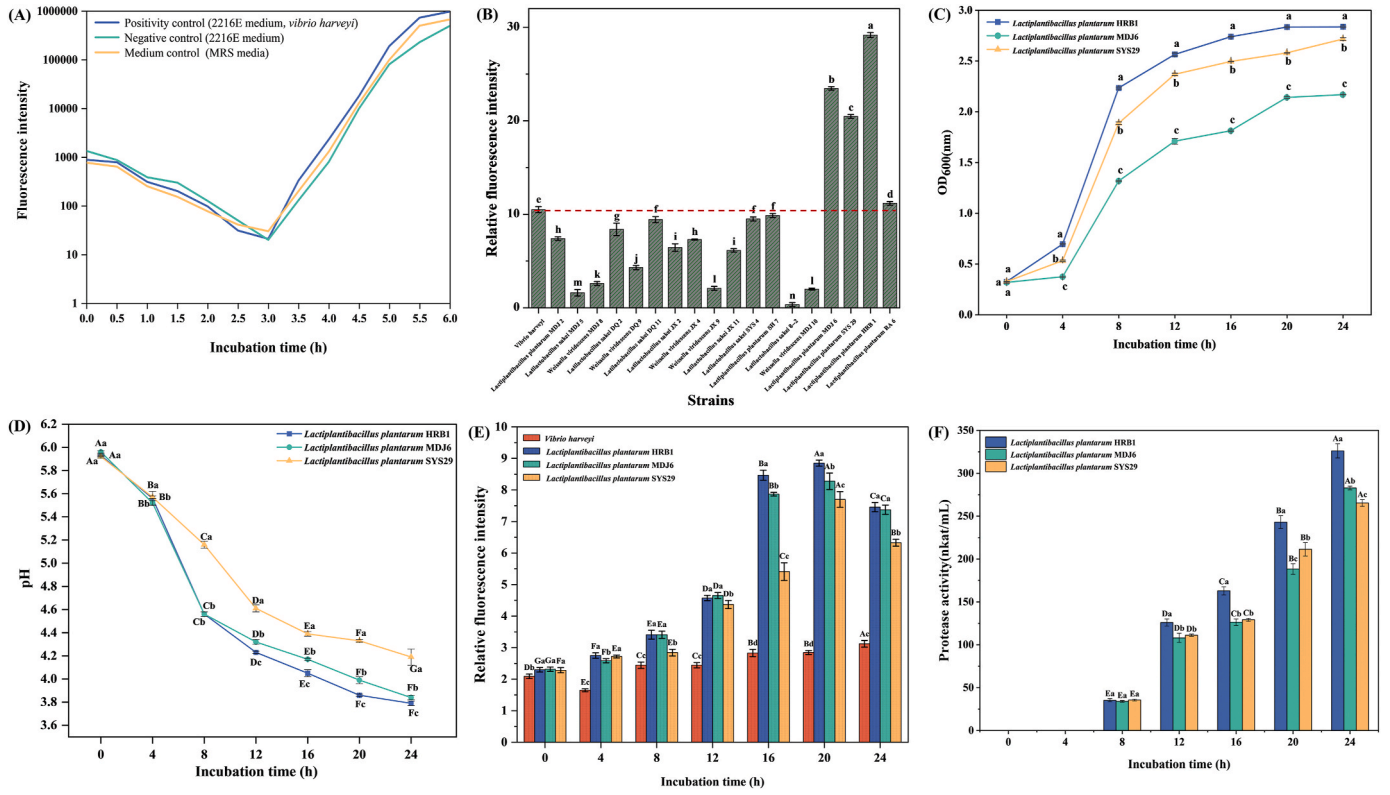

Fig. 1. Screening of lactic acid bacteria based on fluorescence intensity values (A), high yield of AI-2 (B), the growth curve (C), pH (D), AI-2 production ability (E), and protease activity (F) of three strains over a 24-h incubation period. Different lowercase letters (a–o) denote significant differences ($p < 0.05$) among treatments applied for the same duration, while different uppercase letters (A–G) indicate significant differences ($p < 0.05$) among different durations of the same treatment.

2.6. Effect of DPD on the physical properties and flavour development of Harbin dry sausage inoculated with L. plantarum HRB1

2.6.1. Preparation of Harbin dry sausage

The dry sausages were prepared as described by Sui et al. (2024). The meat batters were prepared by 6480 g mincing lean pork and 720 g pork back fat through a 1.5-cm plate, combined with the mixed ingredients of 0.64 g sodium nitrite, 21.6 g monosodium glutamate, 144 g NaCl, 72 g dextrose, 72 g *Daqu* (traditional Chinese wine), 57.6 g mixed spices (including *Foeniculum vulgare* Mill, *Citrus reticulata Blanco*, *Amomum cardamon*, *Syringa oblata* Lindl, *Cinnamomum cassia Presl*, *Amomum villosum*, and *Angelica dahurica*), and *L. plantarum* HRB1 (10^6 CFU per 1 g of meat). Each batch was prepared with DPD at concentrations of 300, 600, and 900 nmol/L. Additionally, a control treatment was included where the dry sausages were prepared without DPD (0 nmol/L). Subsequently, the meat batter was thoroughly mixed in a filling pot (Yiwu Zhanzhi daily necessities Co., Ltd., Zhejiang, China) and then stuffed into porcine casings with a diameter of 30 mm using a sausage stuffer (Yongkang Ayton plastic products Co., Ltd., Zhejiang, Chian). The sausages were dried at 25 ± 2 °C (30%–50% relative humidity) for 1 day and fermented in an incubator (FitoClima 1200 PH, Hao Expansion scientific equipment Co., Ltd., Shanghai, China) at 25 ± 2 °C (65%–70% relative humidity) for 8 days. The dry sausages were sampled on days 0, 3, 6, and 9 for the assessment of their bacterial counts, physical properties, FAAs, and volatile compound analysis, as well as sensory evaluation.

2.6.2. Bacterial counts and physical properties

Plate counts were used to determine the LAB and total viable count of dry sausages according to the method of Sui et al. (2024). LAB counts were determined on MRS agar incubated at 30 °C for 48 h. Total viable counts were determined on Plate Count Agar (MSA) incubated at 37 °C for 36 h. The moisture content was determined according to the method of Sui et al. (2024). The water activity (a_w) was measured using a MB-23 water activity (Qingdao Shengji Instrument System Co., Ltd., Shandong, China), following established protocols. The pH was determined using the methodology established by M. Y. Liu, Luo, Xiao, Chen, Xu, and Li (2024).

2.6.3. Free amino acids

The FAAs present in dry sausage was analysed following the procedure described by Jongsawatsataporn et al. (2021). Dry sausages (10 g) were homogenized with 0.02 mol/L HCl (Laiyang City Kant Chemical Co. Ltd., Laiyang, China) for 30 min and centrifuged at 10000×*g* for 20 min at 4 °C. The supernatant was then filtered through a 0.45-μm aqueous membrane (Sterile Syringe Filter; Chengdu Belanmijin Biotechnology Co., Chengdu, China). Subsequently, the FAA content of the samples was measured using an automated amino acid analyser (LA8080, Hitachi High-Tech Co. Ltd., Tokyo, Japan). The FAA levels in the dry sausages were expressed in milligrams per gram of sausage.

2.6.4. Volatile compounds

The volatile flavor compounds in dry sausages were determined using the method developed by Wen et al. (2019). Minced sausages weighing 3.0 g was placed in a 20 mL headspace vial. Subsequently, the vial was tightly sealed and equilibrated at a temperature of 45 °C for a duration of 25 min in a water bath. The extraction head (50/30 μm DVB/PDMS), which had been conditioned at 250 °C for 60 min in the injection port of the GC-MS instrument, was inserted into the headspace vial at 45 °C for 30 min. Subsequently, the extraction head was promptly transferred to the injection port of the GC-MS instrument for thermal resolution (230 °C for 6 min) to accomplish volatile compound isolation and identification in the samples.

2.6.5. Sensory evaluation

The sensory evaluation was performed as described by Hu et al. (2020a). A total of 24 panellists with expertise in food sensory testing

participated. They underwent rigorous training in sensory analyses and quality standards prior to the assessment. The sensory attributes of all samples were evaluated by the expert panel in a designated room, following the guidelines outlined in ISO 8589–1 (2007). The scores were assigned on a 7-point scale, ranging from 1 (indicating absence of perception) to 7 (representing the highest level of perception).

2.7. Statistical analysis

All indicators were measured in triplicate (replicates) *in vitro*, and three independent batches of sausages (replicates) were prepared. The measurements were performed in triplicate (three observations) for each batch. The study employed a completely randomized design. The data was analysed using a mixed model with Statistix 8.1 software (Analytical Software, St. Paul, MN, USA). Results were expressed as mean ± standard deviation (SD). Tukey's multiple comparisons were used to analyse the significance of differences between treatments ($p < 0.05$). Two-way analysis of variance (ANOVA) was employed to evaluate the interaction between the main factors. *In vitro*, different strains, incubation times, and varying concentrations of exogenous DPD were treated as fixed factors, whereas replicates were treated as random factors. In the dry-sausage model, different concentrations of exogenous DPD, fermentation times, and their interaction were treated as fixed effects, whereas replicates were treated as random effects. Line graphs, bar charts, and box plots were generated using OriginPro®2021b software (Origin-Lab, MA, USA). The chord diagram was produced utilizing a web-based analysis tool (https://circos.ca/).

3. Results and discussion

3.1. Selection of LAB with high-yield AI-2

The bioluminescence in *V. harveyi*, a Gram-negative marine bacterium, is regulated by QS (Chai et al., 2023). The fluorescence intensity values of all samples exhibited a gradual decrease followed by a subsequent increase, as depicted in Fig. 1A. This phenomenon may be due to the initial addition of CFS of *V. harveyi* BB170 to all samples, which produces a small amount of fluorescent material from the AI-2. However, due to the limited effect of *V. harveyi* BB170, the fluorescence diminished over time, leading to a reduction in fluorescence intensity. After an incubation of 3 h, the density of LAB increased to a specific threshold, while the production of the AI-2 continued until it reached a certain level. The fluorescence intensity was minimum at 3 h. The incubation period required for *V. harveyi* BB170 to reach the luminescence-inducing threshold, as inferred from the negative control consisting solely of indicator bacteria diluted with 2216E medium, was determined to be 3 h (Sui et al., 2024). Therefore, this time point (3 h) was adopted as a reference to calculate the relative fluorescence intensity. After an incubation of 3 h, the bacterial density reached a specific threshold, triggering the production of AI-2 and subsequently inducing an increase in fluorescence intensity. (Qian et al., 2022a). The relative fluorescence intensities (RFI) of 18 LAB strains and *V. harveyi* BB170 are depicted in Fig. 1B. The RFI of *L. plantarum* MDJ6, SYS29, HRB1, and *Lac. sakei* RA6 were observed to be higher compared to that of *V. harveyi* BB170 in this study (23.47, 20.47, 29.17, 11.17, and 10.5, respectively; $p < 0.05$). Consequently, strains with a high AI-2 activity, namely *L. plantarum* MDJ6, SYS29, and HRB1, were selected for further experimentation.

3.2. Analysis of protease activity and AI-2 producing capacity of strains

3.2.1. Growth and pH analysis

As shown in Table S1, both the different strains and incubation times significantly influenced the growth of the strains and pH levels ($p < 0.05$). The growth of *L. plantarum* MDJ6, SYS29, and HRB1 displayed a sigmoidal pattern, as depicted in Fig. 1C, consistent with the characteristic S-shaped curve. The time intervals of 0–4, 4–12, 12–20, and 20–24 h were designated as the lag, exponential, stationary, and decline phases, respectively, based on the temporal curve analysis (Vereecken & Van Impe, 2002). The growth performance of all three strains appeared to be favourable within a 24-h period. *L. plantarum* MDJ6 and HRB1 exhibited higher growth rates compared to *L. plantarum* SYS29, as indicated by OD_{600} measurements. Fig. 1D illustrates a decreasing trend in pH values over the 24-h period. The rapid decrease in pH effectively ensures the safety of fermented meat products by inhibiting the proliferation of spoilage bacteria (Sui et al., 2024). At 20 h, the pH levels of *L. plantarum* MDJ6 and HRB1 declined to 3.99 and 3.86, respectively. *L. plantarum* HRB1, with a pH of 3.79, exhibited the highest acidification capacity among the three strains after 24 h (3.84 and 4.19 for *L. plantarum* MDJ6 and SYS29, respectively; $p < 0.05$). This pH level is comparable to that reported by Sui et al. (2024) for *L. plantarum* SH7, which exhibited a pH of approximately 3.74 after 24 h of incubation. In terms of interaction between the strains and incubation times, statistically significant differences were observed in the OD_{600} and pH ($p < 0.05$).

3.2.2. AI-2 production capacity analysis

The production of AI-2 by strains exhibited an initial increase within 20 h, as depicted in Fig. 1E, followed by a subsequent decrease between 20 and 24 h. The observed trends correspond to the exponential and decline phases of the strain. This can be ascribed to the intracellular transport of AI-2, which subsequently regulates various physiological functions (Mok & K., 2014). These findings further support the close correlation between the QS system and strain density (Mok & K., 2014). This result aligns with previous findings (Gori et al., 2011; Sui et al., 2009). In conjunction with the 24-h growth profiles of the three strains of LAB, the observed increase in cell density facilitated the secretion of the signal molecule AI-2. The peak AI-2 activity for most LAB strains was observed during the transition from the exponential phase to the stationary phase. The RFI showed significant variations among the strains at 20 h in this study (8.85, 8.28, and 7.70 for *L. plantarum* HRB1, MDJ6, and SYS29 respectively; $p < 0.05$). Moreover, *L. plantarum* HRB1 exhibited a higher relative fluorescence intensity than *L. plantarum* MDJ6 and SYS29 from 16 to 24 h ($p < 0.05$). In terms of interaction between the strains and incubation times, statistically significant differences were observed in the AI-2 activity ($p < 0.05$; Table S1).

3.2.3. Protease activity analysis

As depicted in Fig. 1F, the protease activity of the strains remained at zero from 0 to 8 h, then gradually increased from 8 to 24 h, with the maximum protease activity observed at 24 h. Sun et al. (2020) also noted a similar trend of increasing protease activity from 0 to 24 h. During the production of microbial proteases, variations in the pH of the medium and strain density can exert influence on transmembrane transport mechanisms governing diverse growth factors, potentially leading to unfavourable conditions (Singh et al., 2012). The metabolic efficiency is enhanced when the microorganism is exposed to an optimal pH or during exponential growth phases, which can directly or indirectly impact the activity of microbial proteases (Singh et al., 2012). It is noteworthy that the protease activity of all three strains was 0 before 8 h. This phenomenon may be attributed to the fact that, in the early stages of culture, microorganisms adapt to their environment by modulating metabolic pathways and enzyme activities, making it challenging to detect microbial protease activity within the first 8 h (Gameiro & Struhl, 2018; Kuatsjah et al., 2024). The protease activity displayed significant variations among the strains at 24 h in this study (326.07, 282.89, and 265.39 nkat/mL for *L. plantarum* HRB1, MDJ6, and SYS29 respectively; $p < 0.05$). The L. *plantarum* HRB1 strain was selected for further investigation based on the aforementioned indicators.

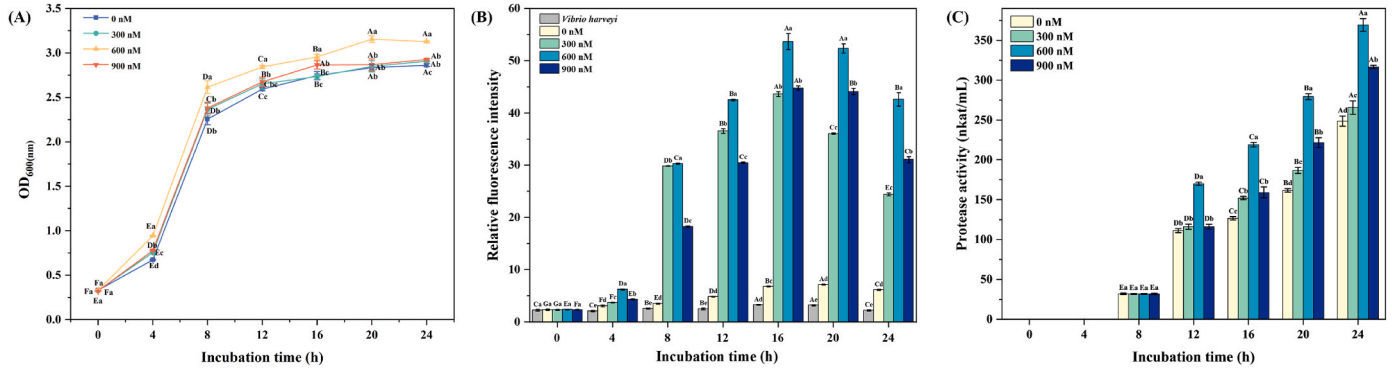

Fig. 2. Effects of different concentrations of exogenous AI-2 precursor on the growth (A), AI-2 production ability (B), and protease activity (C) of *Lactiplantibacillus plantarum* HRB1. Different lowercase letters (a–e) indicate significant differences ($p < 0.05$) among treatments applied for the same duration, while different uppercase letters (A–G) indicate significant differences ($p < 0.05$) among different durations of the same treatment.

3.3. Effects of DPD on the AI-2 activity and protease activity of L. plantarum HRB1 in vitro

3.3.1. Growth curve

Fig. 2A shows the influence of DPD on the growth of *L. plantarum* HRB1 over a 24-h period. The OD_{600} values were higher for DPD concentrations of 600 nmol/L and 900 nmol/L ($p < 0.05$). The OD_{600} values did not show any significant difference between the treatment with 600 nmol/L and treatment with 900 nmol/L ($p > 0.05$). It is noteworthy that the growth of the strain was unaffected by low concentrations of DPD, whereas concentrations of 600 nmol/L and 900 nmol/L exhibited a growth-promoting effect. This is because the 600 nmol/L DPD reaching the sensory recognition threshold of AI-2 for *L. plantarum* HRB1 (Sui et al., 2024). Moreover, once the level of AI-2 surpassed the strain's recognized threshold value, any subsequent impact of the excessive signal molecule on the strain became indiscernible (Zhao et al, 2018).

3.3.2. AI-2 producing capacity

The impact of DPD on the AI-2 producing capacity of the strain for 24 h of incubation is depicted in Fig. 2B. AI-2 activity exhibited a significant increase in the 300 nmol/L, 600 nmol/L, and 900 nmol/L treatments compared to the 0 nmol/L treatment ($p < 0.05$). On this basis, considering the crucial role of the *luxS* gene in AI-2 biosynthesis (Sui et al., 2024), we investigated its expression levels using qPCR. The results demonstrated that the relative expression level of the *luxS* gene was significantly elevated by approximately 5-fold following the addition of exogenous DPD (Fig. 6A). These results demonstrate the presence of *LuxS*/AI-2 quorum sensing in *L. plantarum* HRB1 and confirm that the addition of DPD enhances *luxS* gene expression, thereby promoting AI-2 activity. This finding offers compelling evidence that substantiates the proposed connection between exogenous DPD addition, enhanced *luxS* gene expression, and the consequent increase in AI-2 activity. These elements collectively form a critical component of the regulatory

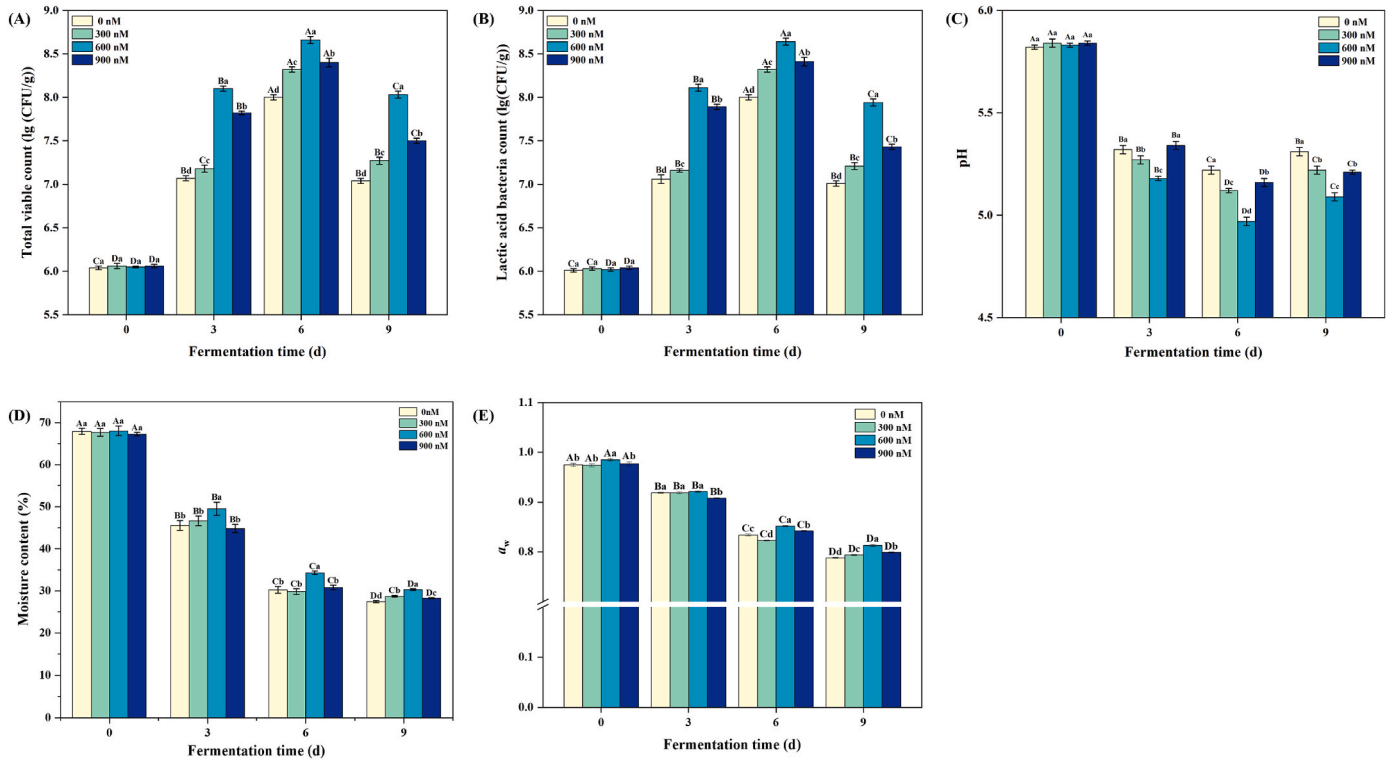

Fig. 3. Effects of different concentrations of exogenous AI-2 precursor on microbial (A and B) and physical (C–E) properties of dry sausage inoculated with *Lactiplantibacillus plantarum* HRB1. Different uppercase letters (a–d) indicate significant differences ($p < 0.05$) among treatments applied for the same duration, while different lowercase letters (A–D) indicate significant differences ($p < 0.05$) among different durations of the same treatment.

mechanism under investigation. Additionally, AI-2 activity exhibited a concentration-dependent relationship within the range of 0–600 nmol/L DPD. However, the 900 nmol/L treatment resulted in a lower AI-2 activity compared to the 600 nmol/L treatment. This could be due to the inhibitory effects of AI-2 on bacterial physiological properties, which occur when its concentration surpasses the recognition threshold (Mok & K., 2014; Zhao et al, 2018).

3.3.3. Protease activity

The moderate protein hydrolysis carried out by LAB, along with the subsequent release of specific enzymes upon cellular disruption, plays a fundamental role in shaping the sensory characteristics and overall quality of fermented foods (Ma et al., 2022). As illustrated in Fig. 2C, the protease activity was significantly higher at 600 nmol/L than in the other treatments ($p < 0.05$). Furthermore, the protease activity of DPD-added treatments exhibited a significantly higher level compared to the treatment with 0 nmol/L DPD ($p < 0.05$). It is hypothesized that DPD could potentially increase the expression of genes involved in protease biosynthesis in *L. plantarum* HRB1, thereby enhancing the efficiency of the protease transport system and subsequently increasing strain protease activity (Singh et al., 2010). The protease activity in the 600 nmol/L treatment (369.41 nkat/mL) was significantly higher as compared to the 0 nmol/L treatment (248.72 nkat/mL), showing a substantial increase of 48.53% within 24 h ($p < 0.05$).

3.4. Effects of the exogenous DPD on the physical properties and flavour development of Harbin dry sausage inoculated with L. plantarum HRB1

3.4.1. Bacterial counts and physical analysis

The impact of DPD on dry sausages inoculated with *L. plantarum* HRB1 was investigated by assessing microbial counts, pH, moisture content, and a_w over a 9-day fermentation period. The initial LAB and total viable counts of the sausages were 6.02 and 6.05 log CFU/g, respectively (Fig. 3A and B). All treatments reached peak levels of LAB and total viable counts on day 6 (7.01–9.94 log CFU/g and 7.04–8.03 log CFU/g, respectively; $p < 0.05$). The LAB counts in the 600 nmol/L treatment (7.94 log CFU/g) were significantly higher compared to other treatments (7.01, 7.21, and 7.43 log CFU/g for 0 nmol/L, 300 nmol/L, and 900 nmol/L treatments, respectively; $p < 0.05$). The continuous synthesis of AI-2 induced by exogenous DPD, which activates the QS system, might explain the continuous expansion of *L. plantarum* (Li et al., 2019; Wen et al., 2021).

The pH values of all dry sausages during treatments were around 5.83, with no significant variation across the treatments (Fig. 3C). The pH values on day 6 exhibited a significant reduction in all treatments, reaching minimum levels of 5.31, 5.22, 5.09, and 5.21 for dry sausages with DPD concentrations of 0 nmol/L, 300 nmol/L, 600 nmol/L, and 900 nmol/L, respectively ($p < 0.05$). It was observed that the pH of dry sausages treated with DPD was lower, possibly due to the exogenous DPD-induced carbohydrate metabolism in *L. plantarum* HRB1 (Sui et al., 2024). However, the pH values displayed a significant increase between day 6 and day 9 ($p < 0.05$), likely due to the synthesis of nitrogenous substances primarily derived from protein (Cruxen et al., 2018).

Furthermore, the moisture content and a_w exhibited a significant decrease throughout the fermentation process of all dry sausages, as depicted in Fig. 3D and E ($p < 0.05$). A previous study (Hu, Li, Zhu, Kong, & Chen, 2021) also reported a decreasing trend in moisture content and a_w of dry sausage as fermentation progressed. This reduction may be attributed to lower relative humidity conditions as well as the decrease in pH, which leads to muscle protein denaturation, muscle bundle contraction, and water loss from both intra- and inter-fibre protein networks (Visessanguan et al., 2006). The highest moisture content and a_w were observed in 600 nmol/L treatment ($p < 0.05$), potentially attributed to the regulatory role of LAB in water release (Wen et al., 2023).

The findings described above demonstrate that 600 nmol/L DPD can induce alterations in the physical properties of dry sausages and facilitate the fermentation process.

Table 2
Effect of different concentrations of exogenous AI-2 precursor on the levels (mg/g dry matter) of free amino acids (FAAs) in dry sausages inoculated with *Lactiplantibacillus plantarum* HRB1 at the end of fermentation.

	0 nM	300 nM	600 nM	900 nM
FAA				
Asp	n.d.	0.46 ± 0.01^{a}	0.08 ± 0.01^{b}	n.d.
Glu	n.d.	n.d.	1.13 ± 0.01^{a}	n.d.
Ser	0.16 ± 0.01^{c}	0.81 ± 0.01^{b}	0.97 ± 0.01^{a}	0.14 ± 0.01^{d}
Gly	0.43 ± 0.02^{b}	0.31 ± 0.02^{c}	0.64 ± 0.01^{a}	0.17 ± 0.01^{d}
His	1.80 ± 0.03^{c}	3.91 ± 0.03^{a}	3.76 ± 0.02^{b}	1.23 ± 0.02^{d}
Thr	3.24 ± 0.02^{b}	0.78 ± 0.01^{d}	6.67 ± 0.02^{a}	1.47 ± 0.02^{c}
Ala	2.20 ± 0.02^{c}	2.05 ± 0.02^{d}	3.97 ± 0.01^{a}	3.44 ± 0.02^{b}
Arg	2.17 ± 0.02^{c}	4.12 ± 0.03^{a}	3.45 ± 0.02^{b}	0.76 ± 0.02^{d}
Pro	1.15 ± 0.02^{c}	1.88 ± 0.01^{b}	3.37 ± 0.05^{a}	0.63 ± 0.03^{d}
Tyr	0.08 ± 0.01^{d}	0.27 ± 0.01^{c}	0.36 ± 0.01^{b}	0.45 ± 0.03^{a}
Val	0.48 ± 0.01^{b}	0.62 ± 0.02^{a}	0.31 ± 0.01^{c}	0.18 ± 0.01^{d}
Met	0.98 ± 0.02^{b}	0.29 ± 0.02^{c}	1.03 ± 0.02^{a}	1.04 ± 0.02^{a}
Cys	0.32 ± 0.01^{b}	5.30 ± 0.04^{a}	0.32 ± 0.01^{b}	0.23 ± 0.02^{c}
Ile	0.28 ± 0.01^{c}	n.d.	1.28 ± 0.02^{a}	0.32 ± 0.01^{b}
leu	0.69 ± 0.01^{c}	1.07 ± 0.02^{b}	1.82 ± 0.01^{a}	0.70 ± 0.02^{c}
Phe	0.17 ± 0.01^{c}	0.12 ± 0.01^{d}	1.15 ± 0.01^{a}	0.34 ± 0.02^{b}
Lys	2.27 ± 0.02^{c}	2.23 ± 0.02^{c}	2.68 ± 0.02^{b}	3.04 ± 0.05^{a}
Σ Sweet taste FAAs	7.18 ± 0.08^{b}	5.83 ± 0.07^{c}	15.61 ± 0.09^{a}	5.86 ± 0.08^{c}
Σ Bitter taste FAAs	2.60 ± 0.06^{b}	2.10 ± 0.06^{c}	5.58 ± 0.06^{a}	2.58 ± 0.07^{b}
Σ Acid taste FAAs	1.80 ± 0.03^{c}	4.37 ± 0.04^{b}	4.97 ± 0.03^{a}	1.23 ± 0.02^{d}
Σ Aged taste FAAs	2.35 ± 0.02^{d}	2.96 ± 0.04^{c}	3.11 ± 0.03^{b}	3.49 ± 0.08^{a}
Σ Total taste FAAs	16.42 ± 0.22^{c}	24.22 ± 0.25^{b}	32.97 ± 0.24^{a}	14.16 ± 0.28^{d}

Different lowercase letters (a–d) indicate significant differences ($p < 0.05$) among treatments.
n.d., not detected; Ser, Serine; Gly, Glycine; Thr, Threonine; Ala, Alanine; Lys, Lysine; Pro, Proline; Asp, Aspartic acid; Glu, Glutamic acid; Arg, Arginine; His, Histidine; Tyr, Tyrosine; Val, Valine; Phe, Phenylalanine; Ile, Isoleucine; Leu, Leucine; Met, Methionine; Cys, Cysteine; Σ Sweet taste FAAs, sum of the amino acids responsible for sweet taste (Ala, Gly, Thr, Ser, and Pro); Σ Bitter taste FAAs, sum of the amino acids responsible for bitter taste (Leu, Val, Ile, Met, and Phe); Σ Acid taste FAAs, sum of the amino acids responsible for acid taste (Glu, Asp and His); Σ Aged taste FAAs, sum of the amino acids responsible for aged taste (Asp, Tyr, and Lys).

3.4.2. Free amino acid analysis

During fermentation, microbial enzymes with protein hydrolysis activity ultimately degrade proteins into FAAs (Wang et al., 2022). Some amino acids possess distinct flavours, while others serve as precursors for taste and odour molecules (Toldrá, 2006; Hu et al., 2020b). The FAA's content for day 9 is presented in Table 2. The 600 nmol/L treatment exhibited the highest content of FAAs (32.97 mg/g dry matter), compared to the 0 nmol/L, 300 nmol/L, and 600 nmol/L treatments (16.42, 24.22, and 14.16 mg/g dry matter, respectively; $p < 0.05$). This may result from the initial activation of *LuxS*/AI-2 QS by exogenous DPD, subsequently leading to the upregulation of protease-related genes and consequent enhancement in protease activity within the strain (Sui et al., 2024). The FAAs were categorized into four groups based on their taste characteristics (Table 2): sweet taste FAAs, bitter taste FAAs, acid taste FAAs, and aged taste FAAs (Hu et al., 2021). Table 2 demonstrates a notable increase in the levels of sweet taste FAAs, bitter taste FAAs, and acid taste FAAs in the 600 nmol/L treatment (15.61, 5.58, and 4.97 mg/g dry matter, respectively; $p < 0.05$), which can be attributed to exogenous DPD stimulation, promoting the production of microbial proteases to enhance the sweet, bitter, and sour taste FAAs in the final products.

These results demonstrated that treatment with 600 nmol/L DPD resulted in increased levels of FAAs, potentially promoting flavour formation. Furthermore, the use of DPD to enhance the flavour of dry

Table 3
Effect of different concentrations of exogenous AI-2 precursor on the levels (μg/kg) of volatile compounds in dry sausages inoculated with *Lactiplantibacillus plantarum* HRB1 at the end of fermentation.

Number	CAS	Volatile compound	Odour description(s)	LRI	0 nM	300 nM	600 nM	900 nM
Aldehydes (6)								
V1	66-25-1	Hexanal	Apple, fat, fresh, green, oil	1081	87.65 ± 0.75^{c}	94.21 ± 1.76^{b}	112.67 ± 1.87^{a}	n.d.
V2	18829-55-5	Trans-2-heptenal	Almond, fat, fruit	1323	15.11 ± 0.58^{b}	13.81 ± 0.30^{c}	18.77 ± 0.83^{a}	n.d.
V3	124-19-6	1-Nonanal	Fat, citrus, green	1326	221.07 ± 1.93^{b}	121.20 ± 2.18^{c}	256.38 ± 1.86^{a}	114.19 ± 0.57^{d}
V4	2548-87-0	(E)-2-Octenal	Green, nut, fat	1436	n.d.	n.d.	31.76 ± 0.03^{a}	n.d.
V5	104-55-2	Cinnamaldehyde	Spice, sweet, aldehydic	2035	25.23 ± 0.37^{c}	45.23 ± 0.85^{b}	69.44 ± 0.89^{a}	68.97 ± 0.95^{a}
V6	14371-10-9	Trans-Cinnamaldehyde	Cinnamon, paint	2015	15.13 ± 0.14^{c}	35.33 ± 0.31^{b}	69.48 ± 0.14^{a}	69.99 ± 0.32^{a}
		Total			364.19 ± 3.77^{b}	309.78 ± 5.40^{c}	558.50 ± 5.62^{a}	253.15 ± 1.84^{d}
Ketones (9)								
V7	110-93-0	6-Methyl-5-hepten-2-one	Pepper, mushroom, rubber	1325	2.41 ± 0.26^{c}	3.25 ± 0.23^{b}	3.46 ± 0.05^{b}	4.19 ± 0.14^{a}
V8	693-54-9	2-Decanone	Fat, fruit	1476	n.d.	n.d.	8.66 ± 0.17^{a}	n.d.
V9	112-12-9	2-Undecanone	Fresh, green, orange, rose	1512	19.62 ± 0.21^{d}	22.22 ± 0.83^{c}	29.46 ± 0.57^{a}	24.67 ± 0.97^{b}
V10	98-86-2	Acetophenone	Almonds, flower, meat	1616	21.78 ± 0.57^{c}	25.42 ± 0.32^{b}	27.80 ± 0.32^{a}	26.14 ± 0.60^{b}
V11	89-81-6	Piperitone	Mint, fresh	1232	7.54 ± 0.10^{b}	5.54 ± 0.40^{c}	7.43 ± 0.29^{b}	8.63 ± 0.17^{a}
V12	111-13-7	2-Octanone	Fat, fragrant	1302	n.d.	n.d.	15.83 ± 0.37^{a}	15.82 ± 0.35^{a}
V13	513-86-0	Acetoin	Butter, cream	1275	27.10 ± 0.22^{c}	37.14 ± 0.45^{b}	67.26 ± 1.01^{a}	67.25 ± 1.01^{a}
V14	821-55-6	2-Nonanone	Fragrant, fruit, green, hot milk	1325	n.d.	n.d.	32.49 ± 1.01^{b}	38.87 ± 0.68^{a}
V15	585-25-1	2,3-Octanedione	Green	1310	n.d.	n.d.	n.d.	2.23 ± 0.12^{a}
		Total			78.46 ± 1.36^{d}	93.59 ± 2.23^{c}	192.41 ± 3.79^{a}	187.83 ± 4.04^{b}
Alcohols (23)								
V16	64-17-5	Ethanol	Wine	939	131.92 ± 2.31^{b}	133.26 ± 1.17^{ab}	134.58 ± 0.83^{ab}	135.86 ± 1.02^{a}
V17	78-83-1	2-Methyl-1-propanol	Wine, solvent	1036	8.73 ± 0.16^{a}	n.d.	n.d.	n.d.
V18	71-36-3	1-Butanol	Fruit	1165	33.21 ± 1.54^{b}	27.73 ± 1.30^{c}	42.82 ± 0.62^{a}	17.37 ± 0.23^{d}
V19	111-27-3	1-Hexanol	Banana, flower, grass	1312	135.82 ± 0.40^{b}	57.31 ± 0.70^{c}	171.69 ± 1.68^{a}	36.30 ± 0.58^{d}
V20	15537-55-0	Trans-4-thujanol	Wood, balsamic	1479	2.41 ± 0.17^{ab}	2.48 ± 0.19^{a}	2.14 ± 0.04^{b}	2.34 ± 0.10^{ab}
V21	104-76-7	2-Ethylhexanol	Green, rose	1459	20.84 ± 0.41^{a}	13.63 ± 0.55^{c}	16.80 ± 0.25^{b}	12.45 ± 0.38^{d}
V22	628-99-9	2-Nonanol	Cucumber	1534	2.73 ± 0.10^{a}	2.75 ± 0.05^{a}	2.53 ± 0.06^{b}	2.50 ± 0.02^{b}
V23	78-70-6	Linalool	Coriander, floral, lavender, lemon, rose	1506	5.71 ± 0.13^{d}	6.37 ± 0.16^{c}	9.60 ± 0.17^{a}	7.79 ± 0.12^{b}
V24	111-87-5	1-Octanol	Bitter almond, fat, floral	1544	38.71 ± 1.13^{b}	23.66 ± 0.57^{c}	52.39 ± 1.16^{a}	n.d.
V25	513-85-9	2,3-Butanediol	Fruit, onion	1466	n.d.	103.83 ± 0.73^{b}	197.52 ± 1.10^{a}	196.33 ± 0.76^{a}
V26	143-08-8	1-Nonanol	Fat, floral, green, oil	1664	25.91 ± 0.38^{b}	22.21 ± 0.31^{c}	64.21 ± 1.76^{a}	18.17 ± 0.54^{d}
V27	98-55-5	Alpha-terpineol	Anise, fresh, mint, oil	1159	2.22 ± 0.10^{b}	1.41 ± 0.26^{c}	3.75 ± 0.09^{a}	2.22 ± 0.08^{b}
V28	1653-30-1	2-Undecanol	Fruit	1706	15.56 ± 0.50^{a}	n.d.	n.d.	n.d.
V29	1197-01-9	2-(4-Methylphenyl) propan-2-ol	Citrus	1156	22.46 ± 1.28^{d}	28.22 ± 0.25^{b}	31.27 ± 0.98^{a}	25.35 ± 1.28^{c}
V30	100-51-6	Benzyl alcohol	Sweet, flower	1312	21.66 ± 1.26^{c}	21.68 ± 0.75^{c}	29.83 ± 0.32^{a}	25.50 ± 0.44^{b}
V31	60-12-8	Phenethyl alcohol	Honey, spice, rose, lilac	1625	36.21 ± 0.67^{a}	26.17 ± 0.58^{b}	36.36 ± 0.55^{a}	25.92 ± 1.42^{b}
V32	112-53-8	1-Dodecanol	Fat, wax	1926	4.12 ± 0.08^{b}	8.93 ± 0.06^{a}	3.34 ± 0.04^{c}	2.20 ± 0.08^{d}
V33	122-97-4	3-Phenyl-1-propanol	Arise, Cinnamon, Fruit	1515	48.58 ± 0.12^{b}	n.d.	48.61 ± 0.50^{b}	59.36 ± 0.79^{a}
V34	104-54-1	Cinnamyl alcohol	Floral, honey, sweet, hyacinth	2218	n.d.	n.d.	179.36 ± 1.22^{a}	n.d.
V35	543-49-7	2-Heptanol	Citrus, fried, oil	1306	n.d.	12.78 ± 0.05^{b}	16.48 ± 0.05^{a}	n.d.
V36	150-86-7	Phytol	Flower	2611	n.d.	8.89 ± 0.30^{a}	n.d.	n.d.
V37	123-96-6	2-Octanol	Mushroom, fat	1415	n.d.	n.d.	14.67 ± 0.67^{a}	n.d.
V38	3391-86-4	1-Octen-3-ol	Cucumber, fat, floral, mushroom	1409	n.d.	n.d.	117.51 ± 1.10^{a}	99.53 ± 1.26^{b}
		Total			556.87 ± 10.75^{c}	501.37 ± 7.98^{d}	1175.56 ± 13.17^{a}	669.24 ± 9.09^{b}
Acids (9)								
V39	64-19-7	Acetic acid	Acid, fruit, sour, vinegar	1325	3.56 ± 0.10^{c}	3.16 ± 0.06^{d}	5.18 ± 0.04^{b}	6.12 ± 0.11^{a}
V40	79-31-2	Isobutyric acid	Butter, cheese	1532	31.29 ± 0.49^{c}	37.45 ± 0.55^{b}	39.38 ± 0.41^{a}	24.43 ± 1.16^{d}
V41	107-92-6	Butyric acid	Rancid, cheese, sweat	1644	2.17 ± 0.07^{d}	2.35 ± 0.08^{c}	2.56 ± 0.11^{b}	2.86 ± 0.10^{a}
V42	142-62-1	Hexanoic acid	Sweat	1715	112.34 ± 0.90^{c}	115.72 ± 0.80^{b}	122.41 ± 1.06^{a}	115.45 ± 0.56^{b}
V43	124-07-2	Octanoic acid	Sweat, cheese	1915	12.74 ± 0.38^{c}	14.24 ± 0.11^{b}	16.83 ± 0.16^{a}	14.49 ± 0.33^{b}
V44	112-05-0	Nonanoic acid	Green, fat	1912	6.63 ± 0.11^{a}	4.26 ± 0.10^{b}	n.d.	n.d.
V45	334-48-5	Decanoic acid	Rancid, fat	2013	3.35 ± 0.10^{a}	2.16 ± 0.14^{b}	n.d.	n.d.
V46	503-74-2	Isovaleric acid	Sweat, acid, rancid	1659	n.d.	n.d.	2.53 ± 0.07^{a}	n.d.

(*continued on next page*)

Table 3 (*continued*)

Number	CAS	Volatile compound	Odour description(s)	LRI	0 nM	300 nM	600 nM	900 nM
V47	143-07-7	Lauric acid	Metal	1856	n.d.	n.d.	13.38 ± 0.93^{a}	n.d.
		Total			172.08 ± 2.16^{c}	179.34 ± 1.83^{b}	202.27 ± 2.76^{a}	163.35 ± 2.26^{c}
Esters (16)								
V48	141-78-6	Ethyl acetate	Pineapple	865	35.86 ± 0.40^{c}	45.71 ± 0.53^{b}	50.66 ± 0.48^{a}	45.25 ± 0.42^{b}
V49	105-54-4	Ethyl butyrate	Apple	1026	18.96 ± 0.41^{d}	25.00 ± 0.39^{c}	28.82 ± 0.33^{a}	26.33 ± 0.51^{b}
V50	108-64-5	Ethyl isovalerate	Fruit	1075	46.05 ± 0.70^{a}	17.92 ± 0.55^{b}	n.d.	n.d.
V51	539-82-2	Ethyl valerate	Yeast, fruit	1058	19.43 ± 1.22^{c}	38.29 ± 0.54^{b}	42.67 ± 0.40^{a}	n.d.
V52	106-70-7	Methyl hexanoate	Fruit, fresh, sweet	1165	303.06 ± 4.86^{c}	329.12 ± 1.17^{b}	560.66 ± 3.15^{a}	355.23 ± 1.51^{d}
V53	106-30-9	Ethyl heptanoate	Fruit	1126	14.95 ± 0.61^{d}	21.72 ± 0.25^{b}	33.50 ± 0.43^{a}	18.64 ± 0.28^{c}
V54	97-64-3	Ethyl lactate	Fruit	1322	295.80 ± 3.06^{d}	377.42 ± 1.33^{b}	408.43 ± 1.94^{a}	349.50 ± 0.79^{c}
V55	111-11-5	Caprylic acid methyl ester	Orange	1356	32.81 ± 0.36^{a}	25.39 ± 0.65^{b}	n.d.	n.d.
V56	106-32-1	Ethyl caprylate	Fruit, fat	1422	8.55 ± 0.10^{d}	16.76 ± 0.36^{b}	23.37 ± 0.96^{a}	10.99 ± 0.41^{c}
V57	80-26-2	Terpinyl acetate	Wax	1656	68.72 ± 0.58^{c}	97.51 ± 1.84^{a}	65.81 ± 0.90^{d}	71.42 ± 1.03^{b}
V58	105-87-3	Geranyl acetate	Rose	1755	22.48 ± 0.64^{c}	35.63 ± 0.72^{a}	29.16 ± 0.46^{b}	30.16 ± 0.73^{b}
V59	2021-28-5	Ethyl-3-phenylpropionate	Flower	1855	54.49 ± 1.20^{d}	108.28 ± 1.00^{a}	102.19 ± 1.38^{b}	81.83 ± 0.98^{c}
V60	7452-79-1	Ethyl 2-methylbutyrate	Apple	1023	n.d.	n.d.	7.34 ± 0.10^{a}	n.d.
V61	110-38-3	Ethyl caprate	Grape	1629	n.d.	50.10 ± 0.82^{b}	69.94 ± 2.10^{a}	51.32 ± 1.25^{b}
V62	115-95-7	Linalyl acetate	Sweet, fruit	1523	n.d.	n.d.	45.73 ± 1.70^{a}	45.65 ± 0.47^{b}
V63	123-66-0	Ethyl hexanoate	Apple peel, fruit	1235	n.d.	n.d.	12.31 ± 0.83^{b}	12.95 ± 0.78^{a}
		Total			940.17 ± 14.69^{d}	1207.18 ± 11.15^{b}	1504.48 ± 15.49^{a}	1099.26 ± 9.16^{c}
Terpenes (15)								
V64	79-92-5	Camphene	Camphor	1052	52.10 ± 0.62^{d}	54.91 ± 0.51^{c}	71.48 ± 0.11^{a}	65.78 ± 0.42^{b}
V65	3387-41-5	Sabinene	Pepper, turpentine, wood	989	9.55 ± 0.27^{b}	8.65 ± 0.10^{c}	12.30 ± 0.16^{a}	n.d.
V66	99-86-5	α-Terpinene	Lemon	1156	7.50 ± 0.18^{b}	15.03 ± 0.39^{a}	n.d.	n.d.
V67	586-62-9	Terpinolene	Pine, plastic	1266	51.93 ± 0.63^{b}	n.d.	94.84 ± 0.56^{a}	n.d.
V68	99-85-4	Gamma-terpinene	Turpentine	1239	225.92 ± 1.60^{c}	234.03 ± 1.77^{b}	238.56 ± 1.29^{a}	224.23 ± 1.00^{c}
V69	127-91-3	β-Pinene	Pine, resin, turpentine	1078	n.d.	n.d.	92.50 ± 1.32^{a}	n.d.
V70	3856-25-5	α-Copaene	Wood, spice	1023	215.44 ± 0.39^{d}	231.31 ± 0.77^{c}	254.71 ± 1.59^{b}	265.18 ± 0.96^{a}
V71	87-44-5	β-Caryophyllene	Wood, spice	1533	112.33 ± 0.90^{a}	n.d.	86.85 ± 0.52^{b}	40.74 ± 0.79^{c}
V72	6753-98-6	α-Caryophyllene	Wood	1359	14.75 ± 0.40^{ab}	15.41 ± 0.24^{a}	12.26 ± 0.93^{c}	13.70 ± 0.52^{b}
V73	495-61-4	β-Bisabolene	Balsamic	1615	160.63 ± 0.46^{b}	185.65 ± 1.12^{a}	133.84 ± 0.67^{d}	148.59 ± 1.05^{c}
V74	10208-80-7	α-Muurolene	Wood	1314	86.89 ± 0.63^{d}	101.61 ± 0.70^{c}	122.41 ± 1.15^{a}	119.34 ± 0.75^{b}
V75	644-30-4	α-Curcumene	Herb	1755	26.50 ± 0.17^{c}	36.23 ± 1.43^{a}	26.79 ± 1.03^{c}	30.89 ± 0.68^{b}
V76	21391-99-1	α-calacorene	Wood	1031	37.74 ± 1.20^{c}	62.40 ± 1.06^{a}	53.32 ± 1.25^{b}	61.03 ± 0.57^{a}
V77	123-35-3	Myrcene	Balsamic, must, spice	1156	11.15 ± 0.85^{c}	17.56 ± 0.50^{a}	14.66 ± 0.32^{b}	n.d.
V78	20307-83-9	β-Sesquiphellandrene	Wood	1539	n.d.	211.54 ± 0.87^{a}	n.d.	n.d.
		Total			1045.88 ± 9.21^{d}	1248.27 ± 10.17^{b}	1319.82 ± 11.45^{a}	1143.69 ± 8.67^{c}

Different lowercase letters (a–d) indicate significant differences ($p < 0.05$) among treatments.
LRI, linear retention index; n.d., not detected.

sausage was further verified through GC/MS analysis.

3.4.3. Volatile compound analysis

A total of 78 volatile compounds were identified in all dry sausages, as indicated in Table 3 and Fig. 4, which were further categorized into six distinct classes: 6 aldehydes, 9 ketones, 23 alcohols, 9 acids, 16 esters, and 15 terpenes.

Six aldehydes were observed in dry sausages, predominantly generated via lipid oxidation and amino acid metabolism (Li et al., 2023). Among them, the low threshold short chain fatty acid derivatives (C_5−C_9) exhibit a robust aroma profile and are considered favourable flavour components in fermented meat products (Wang, Gao, Wang, Xi, & Li, 2021). Hexanal imparts apple, fresh, and oil odours, while 1-nonanal imparts fat, citrus, and leaf odours (Chen et al., 2021). The highest hexanal concentration was observed in the 600 nmol/L treatment (112.67 μg/kg), followed by the 300 nmol/L and 0 nmol/L treatments (94.21 and 87.65 μg/kg, respectively; $p < 0.05$). This could be because *L. plantarum* HRB1 can effectively utilize DPD to form AI-2, thereby upregulating genes involved in lipid metabolism and ultimately leading to increased hexanal production. The levels of 1-nonana were significantly higher in the 600 nmol/L treatment (256.38 μg/kg) compared to the other treatments (221.07, 121.20, and 114.19 μg/kg for the 0 nmol/L, 300 nmol/L, and 900 nmol/L treatments, respectively; $p < 0.05$). These results indicate that DPD may upregulate gene expression related to alcohol dehydrogenase and lipid oxidation pathways, leading to increased aldehyde production (Chen et al., 2021). In addition, the presence of (E)-2-octenal was exclusively detected in the 600 nmol/L treatment (31.76 μg/kg), contributing to the characteristic green, nutty, and fatty aromas (Wang, Gao, Wang, Xi, & Li, 2021).

Nine ketones were identified in dry sausages, predominantly

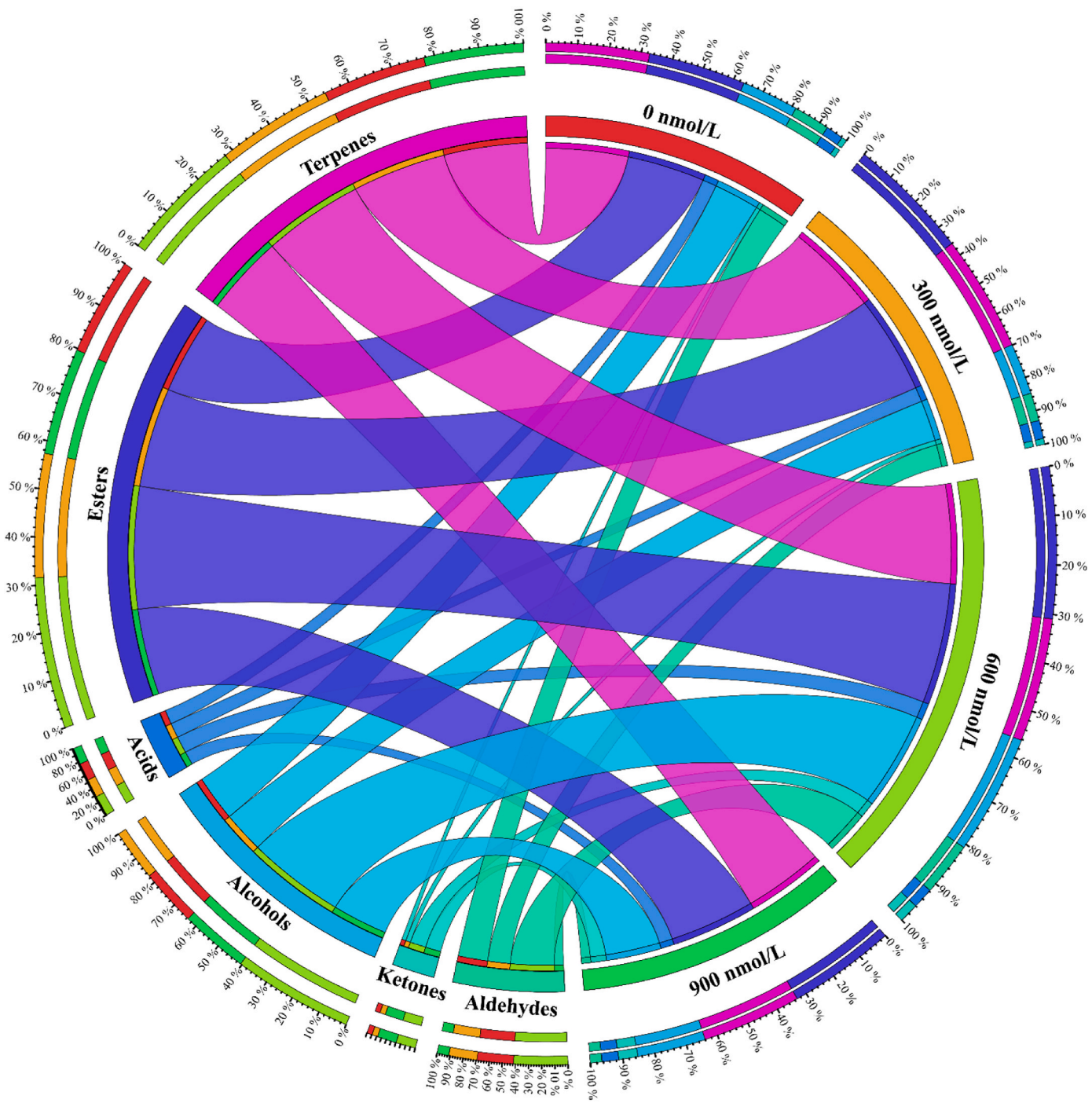

Fig. 4. A chord diagram of volatile compounds present in dry sausage inoculated with *Lactiplantibacillus plantarum* HRB1. The diagram includes external and internal chords. The external chord is the outermost measurement circle and indicates the content of each object, while the internal chord illustrates the relationship between the samples and classes of volatile compounds. Different colours (nodes) represent different samples or classes of volatile compounds, and connecting lines between nodes indicate the relationship between them: the strength of the relationship is indicated by the width of the lines and the width of the contact between the lines and the circle.

resulting from lipid oxidation and the degradation of branched-chain amino acids (Wang et al., 2023). The ketone content in all dry sausages is relatively low; however, due to their low odour threshold, they can significantly contribute to the flavour profile (Wang et al., 2023). The 600 nmol/L and 900 nmol/L treatments led to significantly higher acetylene levels at the end of fermentation compared to the 300 nmol/L treatment ($p < 0.05$). The supplementation of 600 nmol/L DPD was found to reach the threshold required for acetoin production by *L. plantarum* HRB1. Several studies have consistently demonstrated that acetoin is a distinctive flavour compound present in fermented meat products, characterized by its buttery, creamy, and fresh odour (Wang et al., 2023). The generation of acetoin can arise not only from the action of LAB and pyruvate metabolism, but also through 2,3-butanediol (Hu et al., 2021). Further, the contents of 2-undecanone and acetophenone were significantly higher in the 600 nmol/L treatment compared to the other treatments ($p < 0.05$). These findings provide evidence that 600 nmol/L DPD effectively enhances the synthesis of flavour compounds derived from ketones in dry sausages.

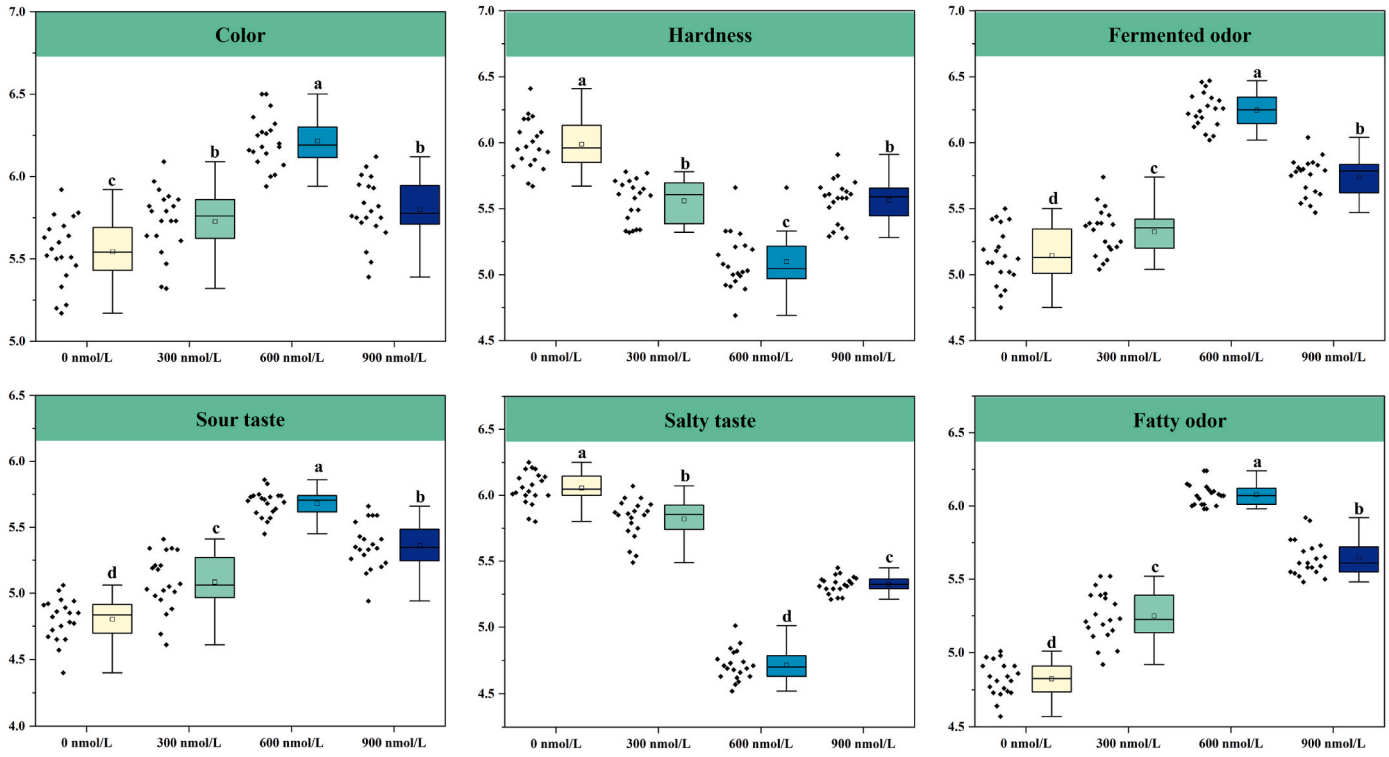

Fig. 5. Effects of different concentrations of exogenous AI-2 precursor on the sensory evaluation of dry sausage inoculated with *Lactiplantibacillus plantarum* HRB1. Different lowercase letters (a–d) indicate significant differences ($p < 0.05$) among treatments.

A total of 23 alcohols were identified in dry sausages. Their biosynthetic metabolic pathways in fermented meat products primarily involve carbohydrate metabolism (Assaf et al., 1997). Ethanol was the most prevalent of the 23 alcohols, primarily derived from wine in the raw material ($p < 0.05$). Due to its substantial concentration and low detection threshold (1 μg/kg), 1-octen-3-ol, known for its mushroom-like aroma (Shen, Wu, Wang, Li, & Yang, 2021; Zhao et al., 2021), was also detected. The presence of 1-octen-3-ol in the 600 nmol/L and 900 nmol/L treatments may be attributed to the upregulation of AI-2 activity following DPD addition, surpassing the QS system threshold and subsequently triggering gene expression.

A total of 9 acids were detected in dry sausage. Due to their higher threshold, acids have a limited direct contribution to the flavour profile of dry sausages. However, they play a crucial role as essential precursors in ester formation and exert a significant influence on fermented flavour (Wang et al., 2023). The total acid content in the 600 nmol/L treatment (202.27 μg/kg) was markedly elevated compared to other treatments (172.08, 179.34, and 163.35 μg/kg for the 0 nmol/L, 300 nmol/L, and 900 nmol/L treatments, respectively; $p < 0.05$).

Esters are mainly formed through the enzymatic reaction of alcohols and acids (Li et al., 2023). Esters were found in relatively high proportions in all dry sausages (Fig. 4), contributing fruity and wine-like odours. The dry sausages contained a total of 11 esters, and the concentration of esters in the 600 nmol/L treatment (1504.48 μg/kg) was significantly higher compared to the other treatments. (972.27, 1227.25, and 1047.09 μg/kg for the 0, 300, and 900 nmol/L treatments respectively; $p < 0.05$). The metabolic impact of the exogenously added DPD can be ascribed to its enhancement of carbohydrate conversion into acids involved in ester synthesis (Hu, Li, Zhu, Kong, & Chen, 2021). The highest ethyl butyrate content was observed in the 600 nmol/L treatment (28.82 μg/kg; $p < 0.05$). The trend for methyl caproate is consistent with the observations for ethyl butyrate above. The findings of this study demonstrate that DPD enhances the esterase activity of *L. plantarum* HRB1. This finding provides evidence supporting the hypothesis that DPD enhances AI-2 synthesis and accumulation, thereby facilitating microbial-mediated esterification processes. In addition, it is possible that the addition of DPD promoted the expression of the esterase gene in *L. plantarum* HRB1, thereby enhancing esterase activity and promoting ester production.

In addition to the findings mentioned above, the terpenes α-terpinene, gamma-terpinene, α-copaene, β-pinene, and myrcene contribute a pleasant fragrance and sweet odour to dry sausages primarily through their presence in mixed spices during sausage preparation (Montanari et al., 2016).

3.4.4. *Sensory evaluation*

As shown in Fig. 5, DPD treatment at a concentration of 600 nmol/L resulted in the highest colour score, followed by the treatments with 900 nmol/L, 300 nmol/L, and 0 nmol/L DPD. The hardness score was lowest in the 600 nmol/L treatment, likely due to its relatively higher moisture content. The salty taste score was lowest in the treatment with 600 nmol/L DPD; however, the 600 nmol/L DPD treatment exhibited significantly higher scores for sour taste compared to the other treatments ($p < 0.05$). The higher scores for fermented and fatty odour in the 600 nmol/L treatment can be attributed to elevated levels of aldehydes, alcohols, and esters (Table 3). Consequently, the 600 nmol/L DPD imparts more positive taste and odour profiles, as well as an exceptional sensory quality to the dry sausage.

3.5. *qRT-PCR validation*

The results confirmed the upregulation of genes associated with branched-chain amino acid transport systems (*livK* and *livH*), oligopeptide transport system (*OppA*, *OppB*, and *OppF*), quorum sensing (*luxS and pfs*), and pyruvate metabolism (*CysK*) upon 600 nmol/L DPD treatment (Fig. 6A and B). The expression levels of *OppA*, *OppB*, and *OppF* genes in the oligopeptide transport system of *L. plantarum* HRB1 were significantly upregulated by 3.13-fold, 3.01-fold, and 2.57-fold, respectively, upon DPD addition (Fig. 6A). The growth of *L. plantarum* HRB1 can be promoted by amino acids generated through oligopeptide metabolism,

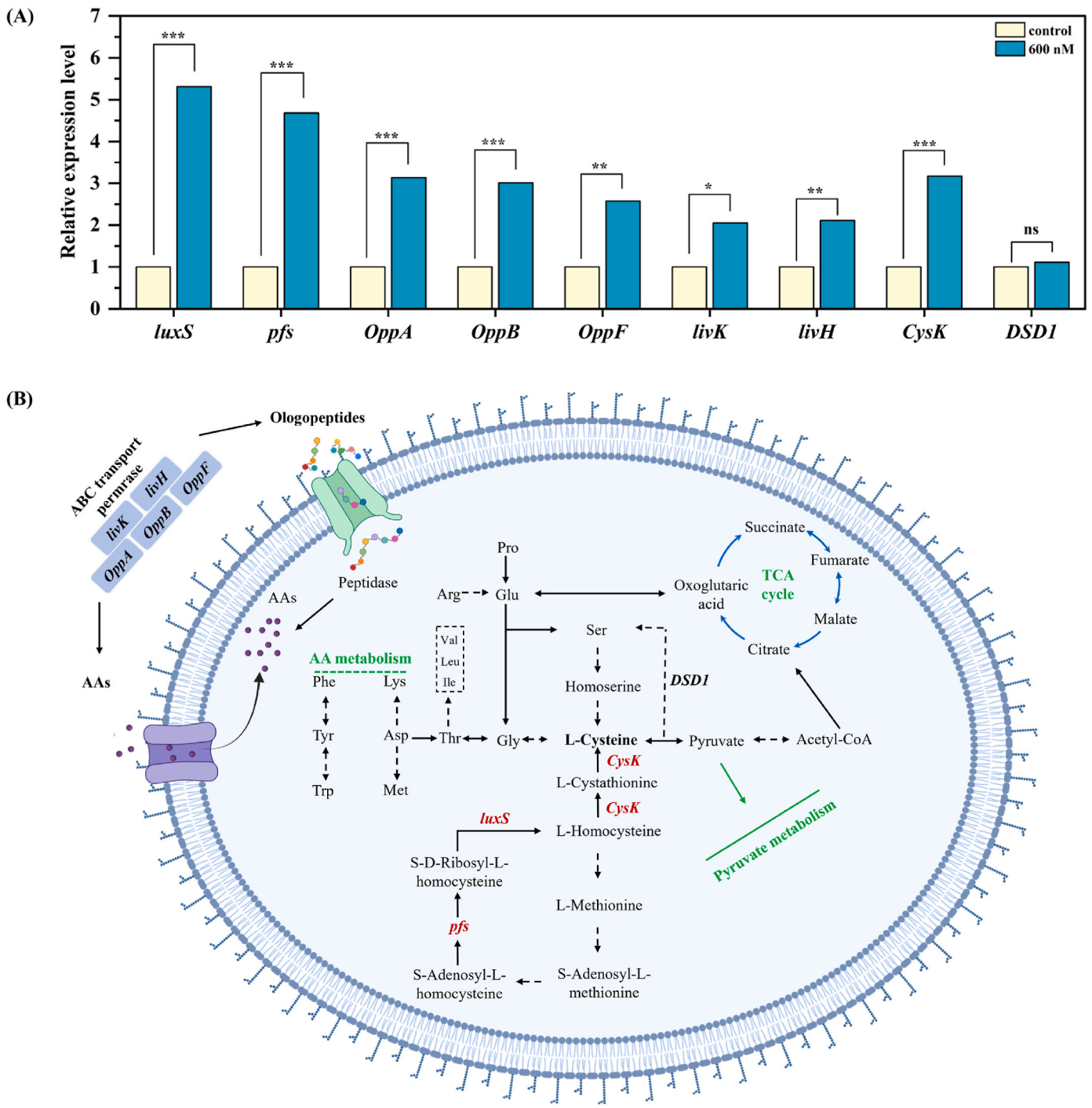

Fig. 6. Metabolic pathway of *Lactiplantibacillus plantarum* HRB1. The construction of pathways was based on the information provided by the KEGG database.

as demonstrated in related studies (Kunji et al., 1996). Notably, the addition of DPD significantly enhanced the growth ability of *L. plantarum* HRB1, as observed in our experimental results (Fig. 2 A) ($p < 0.05$). The addition of DPD was shown to induce rapid growth of *L. plantarum* HRB1 by up-regulating the expression of oligopeptide transporter system genes (*OppA*, *OppB*, and *OppF*), while increasing strain density also enhanced the QS system (Sui et al., 2024).

The expression levels of *pfs* and *luxS* genes in the QS system of *L. plantarum* HRB1 were significantly upregulated by 4.68-fold and 5.31-fold, respectively, upon addition of DPD. The *pfs* and *luxS* genes were found to directly participate in the metabolic pathways of amino acids, particularly facilitating the biosynthesis of L-cysteine and L-serine (Fig. 6B). The addition of DPD enhances the amino acid metabolism of the strain, thereby augmenting L-cysteine and L-serine production via synergistic interactions with the ABC transporter. Notably, L-cysteine and L-serine played a direct role in the production of pyruvate. The accumulation of pyruvate will provide ample energy for the physiological activities of the strain (Qian et al., 2022b). It has been demonstrated that exogenous DPD primarily participates in the cysteine metabolic pathway and oligopeptide metabolic pathway, while simultaneously providing energy and precursors for other metabolic pathways during the overall metabolic process (Qian et al., 2022a). Therefore, we hypothesize that the augmentation of the *LuxS*/AI-2 QS can confer ample energy to LAB for supporting diverse physiological activities, including

protein metabolism, pyruvate metabolism, lipid metabolism, and other related processes. Consequently, the metabolic byproducts can serve as ample precursors for the synthesis of volatile flavour compounds, thereby contributing significantly to flavour formation.

4. Conclusions

In this study, *L. plantarum* HRB1, *L. plantarum* MDJ6, and *L. plantarum* SYS29 exhibited the highest AI-2 activity among the 18 LAB strains tested, with *L. plantarum* HRB1 exhibiting superior ability in both AI-2 and protease activity. The supplementation of exogenous DPD significantly enhanced the AI-2 activity and protease activity of *L. plantarum* HRB1. The addition of 600 nmol/L DPD significantly increased the levels of acids, alcohols, aldehydes, and esters in dry sausages. Overall, the 600 nmol/L DPD treatment was found to significantly increase the levels of FAAs and volatile flavour compounds in dry sausage inoculated with *L. plantarum* HRB1. In the metabolic pathway, DPD significantly enhanced the up-regulation of *luxS* and *pfs* genes in the *LuxS*/AI-2 QS system, as well as *OppA*, *OppB*, *OppF*, *livK*, and *livH* genes in the ABC transporter system of *L. plantarum* HRB1, thereby promoting flavor formation. These results lay the groundwork for a deeper understanding of the flavour formation mechanism regulated by LAB through the *LuxS*/AI-2 QS system. In addition, the high cost associated with DPD in this study limits its applicability in large-scale scenarios. However, our research has elucidated the mechanism of *LuxS*/AI-2 QS. In a follow-up study, we can explore the development of engineered bacteria through genetic engineering technology, which holds promise for achieving large-scale production and broad application of high-yielding AI-2 signal molecule starter cultures.

CRediT authorship contribution statement

Jiasheng Lu: Writing – review & editing, Methodology, Investigation. **Yumeng Sui:** Investigation, Data curation. **Xin Liu:** Methodology, Investigation. **Jiawang Wang:** Methodology. **Jiatong Li:** Investigation. **Baohua Kong:** Project administration, Funding acquisition. **Qian Chen:** Writing – review & editing, Funding acquisition, Conceptualization. **Weiwei Yang:** Supervision.

Ethical statement

No coercion to participate, full disclosure of study requirements and risks, written or verbal consent of participants, no release of participant data without their knowledge, ability to withdraw from the study at any time.

Declaration of competing interest

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

Acknowledgments

This study was funded by the National Natural Science Foundation of China (32172232 and U22A20547).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.117516.

Data availability

Data will be made available on request.

References

Assaf, S., Hadar, Y., & Dosoretz, C. G. (1997). 1-Octen-3-ol and 13-hydroperoxylinoleate are products of distinct pathways in the oxidative breakdown of linoleic acid by Pleurotus pulmonarius. *Enzyme and Microbial Technology, 21*(7), 484–490. https://doi.org/10.1016/S0141-0229(97)00019-7

Bintsis, T. (2018). Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. *Aims Microbiology, 4*(4). https://doi.org/10.3934/microbiol.2018.4.665

Chai, Y. M., Ma, Q. W., Nong, X., Mu, X. Y., & Huang, A. X. (2023). Dissecting LuxS/AI-2 quorum sensing system-mediated phenyllactic acid production mechanisms of *Lactiplantibacillus plantarum* L3. *Food Research International, 166*, Article 112582. https://doi.org/10.1016/j.foodres.2023.112582

Chen, Q., Hu, Y. Y., Wen, R. X., Wang, Y., & Kong, B. H. (2021). Characterisation of the flavour profile of dry fermented sausages with different NaCl substitutes using HS-SPME-GC-MS combined with electronic nose and electronic tongue. *Meat Science, 172*(1), Article 108338. https://doi.org/10.1016/j.meatsci.2020.108338

Chen, Q., Kong, B. H., Han, Q., Xia, X. F., & Xu, L. (2017). The role of bacterial fermentation in lipolysis and lipid oxidation in Harbin dry sausages and its flavour development. *LWT - Food Science and Technology, 77*, 389–396. https://doi.org/10.1016/j.lwt.2016.11.075

Cruxen, C. E. D. S., Braun, C. L. K., Fagundes, M. B., Gularte, M. A., Wagner, R., Wladimir, P. D. S., et al. (2018). Development of fermented sausage produced with mutton and native starter cultures. *LWT - Food Science and Technology, 95*, 23–31. https://doi.org/10.1016/j.lwt.2018.04.060

Dong, K., Wang, Q., Li, X., Li, X., An, F., Luo, Z., et al. (2024). An effective means to improve the flavor quality of traditional fermented sour meat: The salt reduction strategy. *LWT - Food Science and Technology, 209*, Article 116726. https://doi.org/10.1016/j.lwt.2024.116726

Eickhoff, M. J., & Bassler, B. L. (2018). SnapShot: Bacterial quorum sensing. *Cell, 174*(5), 1328. https://doi.org/10.1016/j.cell.2018.08.003

Gameiro, P. A., & Struhl, K. (2018). Nutrient Deprivation elicits a transcriptional and translational inflammatory response coupled to decreased protein synthesis. *Cell Reports, 24*(6), 1415–1424. https://doi.org/10.1016/j.celrep.2018.07.021

Gori, K., Moslehi-Jenabian, S., Purrotti, M., & Jespersen, L. (2011). Autoinducer-2 activity produced by bacteria found in smear of surface ripened cheeses. *International Dairy Journal, 21*(1), 48–53. https://doi.org/10.1016/j.idairyj.2010.06.009

Hu, Y. Y., Li, Y. J., Zhu, J. M., Kong, B. H., Liu, Q., & Chen, Q. (2021). Improving the taste profile of reduced-salt dry sausage by inoculating different lactic acid bacteria. *Food Research International, 145*, Article 110391. https://doi.org/10.1016/j.foodres.2021.110391

Hu, Y. Y., Zhang, L., Wen, R. X., Chen, Q., & Kong, B. H. (2020a). Role of lactic aicid bacteria in flavor development in traditional Chinese fermented foods: A review. *Critical Reviews in Food Science and Nutrition, 62*(10), 2741–2755. https://doi.org/10.1080/10408398.2020.1858269

Hu, Y. Y., Zhang, L., Zhang, H., Wang, Y., Chen, Q., & Kong, B. H. (2020b). Physicochemical properties and flavour profile of fermented dry sausages with a reduction of sodium chloride. *LWT - Food Science and Technology, 124*, Article 109061. https://doi.org/10.1016/j.lwt.2020.109061

Jongsawatsataporn, N., Suzuki, Y., & Tanaka, R. (2021). Evaluation of functional chemical components and radical scavenging activity in 11 fermented fish products from Thailand. *Journal of Aquatic Food Product Technology, 30*(6), 746–762. https://doi.org/10.1080/10498850.2021.1937422

Kuatsjah, E., Schwartz, A., Zahn, M., Tornesakis, K., Kellermyer, Z. A., Ingraham, M. A., et al. (2024). Biochemical and structural characterization of enzymes in the 4-hydroxybenzoate catabolic pathway of lignin-degrading white-rot fungi. *Cell Reports, 43*(12), Article 115002. https://doi.org/10.1016/j.celrep.2024.115002

Kunji, E. R. S., Mierau, I., Hagting, A., Poolman, B., & Konings, W. N. (1996). The proteolytic systems of lactic acid bacteria. *Antonie Van Leeuwenhoek, 70*(2), 187–221. https://doi.org/10.1007/bf00399500

Li, Y., Li, W. J., Li, C. S., Li, L. H., Yang, D. Q., Wang, Y. Q., et al. (2023). Novel insight into flavor and quality formation in naturally fermented low-salt fish sauce based on microbial metabolism. *Food Research International, 166*, Article 112586. https://doi.org/10.1016/j.foodres.2023.112586

Li, J. P., Yang, X. Y., Shi, G. C., Chang, J., Liu, Z. Y., & Zeng, M. Y. (2019). Cooperation of lactic acid bacteria regulated by the AI-2/LuxS system involve in the biopreservation of refrigerated shrimp. *Food Research International, 120*, 679–687. https://doi.org/10.1016/j.foodres.2018.11.025

Liu, J., Liu, K. Q., Zhao, Z., Wang, Z., Wang, F. C., Xin, Y. X., et al. (2022). The LuxS/AI-2 quorum-sensing system regulates the algicidal activity of *Shewanella xiamenensis* lzh-2. *Frontiers in Microbiology, 12*. https://doi.org/10.3389/fmicb.2021.814929

Liu, M. Y., Luo, H. T., Xiao, Q., Chen, C. G., Xu, B. C., & Li, P. J. (2024). Effect of *Latilactobacillus sakei* and *Staphylococcus xylosus* on the textural characteristics of dry fermented sausages. *Food Bioscience, 59*, Article 103972. https://doi.org/10.1016/j.fbio.2024.103972

Ma, H., Wang, L., Yu, H. J., Wang, W. W., Wu, G. F., Qin, G. Y., et al. (2022). Protease-producing lactic acid bacteria with antibacterial properties and their potential use in soybean meal fermentation. *Chemical and Biological Technologies in Agriculture, 9*(1). https://doi.org/10.1186/s40538-022-00303-5

Meng, F. Q., Zhao, M. W., & Lu, Z. X. (2022). The LuxS/AI-2 system regulates the probiotic activities of lactic acid bacteria. *Trends in Food Science & Technology, 127*, 272–279. https://doi.org/10.1016/j.tifs.2022.05.014

Montanari, C., Bargossi, E., Gardini, A., Lanciotti, R., Magnani, R., Gardini, F., et al. (2016). Correlation between volatile profiles of Italian fermented sausages and their size and starter culture. *Food Chemistry, 192*, 736–744. https://doi.org/10.1016/j.foodchem.2015.07.062

Qian, Y. L., Li, Y., Tang, Z. X., Wang, R. R., Zeng, M. Y., & Liu, Z. Y. (2022a). The role of AI-2/LuxS system in biopreservation of fresh refrigerated shrimp: Enhancement in competitiveness of *Lactiplantibacillus plantarum* for nutrients. *Food Research International, 161*, Article 111838. https://doi.org/10.1016/j.foodres.2022.111838

Qian, Y. L., Li, Y., Xu, T. T., Zhao, H. J., Zeng, M. Y., & Liu, Z. Y. (2022b). Dissecting of the AI-2/LuxS mediated growth characteristics and bacteriostatic ability of *Lactiplantibacillus plantarum* SS-128 by integration of transcriptomics and metabolomics. *Foods, 11*(5), 638. https://doi.org/10.3390/foods11050638

Shen, Y. Y., Wu, Y. Y., Wang, Y. Q., Li, L. H., Li, C. S., Zhao, Y. Q., et al. (2021). Contribution of autochthonous microbiota succession to flavor formation during Chinese fermented Mandarin fish (*Siniperca chuatsi*). *Food Chemistry, 348*(2), Article 129107. https://doi.org/10.1016/j.foodchem.2021.129107

Singh, S. K., Singh, S. K., Tripathi, V. R., & Garg, S. K. (2012). Purification, characterization and secondary structure elucidation of a detergent stable, halotolerant, thermoalkaline protease from *Bacillus cereus* SIU1. *Process Biochemistry, 47*(10), 1479–1487. https://doi.org/10.1016/j.procbio.2012.05.021

Singh, S. K., Tripathi, V. R., Jain, R. K., Vikram, S., & Garg, S. K. (2010). An antibiotic, heavy metal resistant and halotolerant *Bacillus cereus* SIU1 and its thermoalkaline protease. *Microbial Cell Factories, 9*(1), 1–7. https://doi.org/10.1186/1475-2859-9-59

Sui, Y. M., Lu, J. S., Zhu, Y. A., Kong, B. H., Wang, H., Qin, L., et al. (2024). Effects of exogenous autoinducer-2 precursor on the ability of *Lactiplantibacillus plantarum* SH7 to degrade biogenic amines: In vitro and in a dry-sausage model. *Food Bioscience, 60*, Article 104263. https://doi.org/10.1016/j.fbio.2024.104263

Sun, F. D., Hu, Y. Y., Yin, X. Y., Kong, B. H., & Qin, L. (2020). Production, purification and biochemical characterization of the microbial protease produced by *Lactobacillus fermentum* R6 isolated from Harbin dry sausages. *Process Biochemistry, 89*, 37–45. https://doi.org/10.1016/j.procbio.2019.10.029

Toldrá, F. (2006). The role of muscle enzymes in dry-cured meat products with different drying conditions. *Trends in Food Science & Technology, 17*(4), 164–168. https://doi.org/10.1016/j.tifs.2005.08.007

Vereecken, K. M., & Van Impe, J. F. (2002). Analysis and practical implementation of a model for combined growth and metabolite production of lactic acid bacteria. *International Journal of Food Microbiology, 73*(2), 239–250. https://doi.org/10.1016/S0168-160501)00641-9

Visessanguan, W., Benjakul, S., Smitinont, T., Kittikun, C., Thepkasikul, P., & Panya, A. (2006). Changes in microbiological, biochemical and physico-chemical properties of Nham inoculated with different inoculum levels of *Lactobacillus curvatus*. *LWT - Food Science and Technology, 39*(7), 814–826. https://doi.org/10.1016/j.lwt.2005.05.006

Wang, F., Gao, Y. Q., Wang, H. B., Xi, B., He, X. N., Yang, X. L., et al. (2021). Analysis of volatile compounds and flavor fingerprint in Jingyuan lamb of different ages using gas chromatography–ion mobility spectrometry (GC–IMS). *Meat Science, 175*(1), Article 108449. https://doi.org/10.1016/j.meatsci.2021.108449

Wang, H. P., Sui, Y. M., Liu, J. Q., Kong, B. H., Li, H. Y., Qin, L. G., et al. (2023). Analysis and comparison of the quality and flavour of traditional and conventional dry sausages collected from northeast China. *Food Chemistry X, 20*, Article 100979. https://doi.org/10.1016/j.fochx.2023.100979

Wang, H., Xu, J. H., Liu, Q., Xia, X. F., Sun, F. D., & Kong, B. H. (2022). Effect of the protease from *Staphylococcus carnosus* on the proteolysis, quality characteristics, and flavor development of Harbin dry sausage. *Meat Science, 189*, Article 108827. https://doi.org/10.1016/j.meatsci.2022.108827

Wen, R. X., Dong, Z. M., Lv, Y. C., Liu, H. T., Bayana, B., Kong, B. H., et al. (2023). Comparative evaluation of the flavour-promoting role of autochthonous yeast strains on dry sausages. *LWT - Food Science and Technology, 184*, Article 115032. https://doi.org/10.1016/j.lwt.2023.115032

Wen, R. X., Hu, Y. Y., Zhang, L., Wang, Y., Chen, Q., & Kong, B. H. (2019). Effect of NaCl substitutes on lipid and protein oxidation and flavor development of Harbin dry sausage. *Meat Science, 156*, 33–43. https://doi.org/10.1016/j.meatsci.2019.05.011

Wen, R. X., Li, X. A., Han, G., Chen, Q., & Kong, B. (2021). Fungal community succession and volatile compound dynamics in Harbin dry sausage during fermentation. *Food Microbiology, 99*(2), Article 103764. https://doi.org/10.1016/j.fm.2021.103764

Zhao, J., Quan, C. S., Jin, L. M., & Chen, M. (2018). Production, detection and application perspectives of quorum sensing autoinducer-2 in bacteria. *Journal of Biotechnology, 268*, 53–60. https://doi.org/10.1016/j.jbiotec.2018.01.009

Zhao, Y., Wang, Y. Q., Li, C. S., Li, L. H., Yang, X. Q., Wu, Y. Y., et al. (2021). Novel insight into physicochemical and flavor formation in naturally fermented tilapia sausage based on microbial metabolic network. *Food Research International, 141*, Article 110122. https://doi.org/10.1016/j.foodres.2021.110122