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Assessing the effects of homofermentative *Lactiplantibacillus plantarum* K25 and heterofermentative *Limosilactobacillus fermentum* 13-1 on the flavor and functional characteristics of fermented milk analyzed by metabolomics approach

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ORIGINAL ARTICLE

Abstract

Lactic acid bacteria (LAB) play a significant role in milk fermentation; however, the impact of different LAB fermentation types on the metabolic characteristics of fermented milk remains poorly understood. In this study, homofermentative *Lactoplantibacillus plantarum* K25 and heterofermentative *Limosilactobacillus fermentum* 13-1 were utilized in yogurt fermentation to produce fermented milk samples D-Lp and D-Lf, respectively, which were compared to the control yogurt sample (D) in terms of flavor and functional properties. Analysis revealed that strain K25 enhanced the production of milky aroma compounds such as 2,3-butanedione (8.82±1.13 ng/g) and hexanoic acid (28.94±1.39 ng/g), while strain 13-1 led to a decrease in 2,3-butanedione (0 ng/g) and acetoin (0 ng/g) levels along with an increase in the content of both acetic acid (23.58±1.99 ng/g) and ethanol (9.92±0.93 ng/g). Both strains demonstrated potential in reducing the bitterness of fermented milk by decreasing the levels of bitter amino acids and dipeptides in samples. Untargeted metabolomics analysis indicated that strain 13-1 induced more significant metabolic changes in fermented milk compared to strain K25. Both strains influenced various amino acid metabolisms, with strain K25 promoting lysine degradation and strain 13-1 enhancing tyrosine metabolism. Furthermore, an increase in different types of bioactive compounds was observed in different fermented milk samples. This study enhances our understanding of LAB strain metabolism in fermented milk.

Keywords: fermented milk; flavor; functional characteristics; *Lactoplantibacillus plantarum* K25; *Limosilactobacillus fermentum* 13-1; metabolomics analysis

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Introduction

Yogurt is a widely consumed dairy product globally; it is favored by consumers for its healthy nutritional value and smooth texture as well as characteristic flavors (Gu *et al.*, 2020; Innocente *et al.*, 2016; Tang *et al.*, 2024). In recent years, the addition of probiotics or other functional lactic acid bacteria (LAB) to yogurt fermentation, along with the standard *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Nemati *et al.*, 2023), has become a popular practice to enhance the yogurt's flavor, texture, appearance, and probiotic properties (Aziz *et al*., 2024a; Aziz *et al*., 2024b). Commonly added LAB strains include homofermentative *Lactobacillus acidophilus*, *Lacticaseibacillus casei*, *Lactoplantibacillus plantarum,* etc., and heterofermentative *Bifidobacterium animalis*, *Bifidobacterium longum*, etc. (Adugna and Andualem, 2023). LAB strains are responsible for producing various metabolites, such as volatile compounds, peptides, and organic acids, during milk fermentation. These metabolites have diverse effects on the technological, nutritional, and sensory properties of the product (Zhou *et al.*, 2024a; Zhou *et al.*, 2024b; Naveed *et al.*, 2024; Naveed *et al.*, 2023; .Isık *et al.*, 2023; Aziz *et al.*, 2021, Aziz *et al.*, 2020).

Different types of LAB strains, such as homofermentative and heterofermentative, possess different carbohydrate metabolism and fermentation products, exhibiting different effects on the flavor and functional properties of yogurt (Gänzle, 2015). Homofermentative LAB metabolizes hexoses, typically glucose, primarily via the Embden–Meyerhof–Parnas (EMP) pathway, resulting in lactic acid as the sole product. On the other hand, heterofermentative LAB utilizes the phosphoketolase (PK) pathway to metabolize both hexoses and pentoses, resulting in a variety of metabolites (Zotta *et al.*, 2018). Some homofermentative LAB strains are also capable of heterofermentation, and are referred to as facultative heterofermentative strains. *Bifidobacterium* undergoes a unique form of heterofermentation, producing lactic acid and acetic acid as end products. It was reported that homofermentative *L. plantarum* can improve the quality of silage (Wang *et al.*, 2020b; Xu *et al.*, 2019), while some heterofermentative LAB, such as *Leuconostoc citreum* GR1, performed well in kimchi fermentation (Moon *et al.*, 2018). However, in the production of fermented milk, the traditional starters remain the obligately homofermentative *Lactobacillus delbrueckii* and *Streptococcus thermophilus*. The impact on flavor of fermented milk when supplementing these starters with specific facultatively heterofermentative and obligately heterofermentative strains for auxiliary fermentation is difficult to ascertain. This complexity arises because the intermediary metabolites generated through different fermentation pathways serve as precursors for crucial flavor compounds. For example, precursor of the highly significant 2,3-butanedione in yogurt originates from pyruvate within the EMP pathway. In recent years, assumptive attempts have been made to introduce a variety of strains with distinct fermentation types into yogurt fermentation research. The addition of homofermentative *Lactobacillus casei* and *Lactobacillus acidophilus* significantly impacted the levels of acetaldehyde and ketones in yogurt, respectively (Tian *et al.*, 2017). The addition of heterofermentative *Lactobacillus fermentum* HY01 in yak milk increased its acetic acid content (Zhang *et al.*, 2022), and heterofermentative *Bifidobacterium* CCFM5871 provided a rich taste and aroma to yogurt (Tian *et al.*, 2022), although the obligate heterofermentative *Lactobacillus brevis* was not suitable for independent yogurt fermentation (Rönkä *et al.*, 2003). However, the limited research data available are insufficient to elucidate the general patterns of the influence of fermentation types on yogurt flavor. Further studies are required to delve deeper into the mechanisms underlying the synthesis and metabolism of flavor compounds.

Metabolomics is a convenient tool used for high-throughput screening and analyzing metabolites in complex substrates by investigating dynamic changes in their contents and biological phenotypes (Han *et al.*, 2024a). Metabolomics-based approaches are extensively used in scientific research on different fermented foods, such as red wine (Wang *et al.*, 2023a), coffee (Chan *et al.*, 2021), fermented pickle (Tomita *et al.*, 2018), and sausages (Zhao *et al.*, 2022b). Zhao *et al*. (2023) conducted a metabolomics study on the important differential metabolites in fermented milk with *Lactobacillus plantarum* NMGL2 during the storage period. Xia *et al*. (2023) revealed important metabolic pathways associated with the flavor of fermented milk produced by *Lactococcus lactis* subsp. *lactis* by a metabolomics approach. However, limited information is available on the metabolomics studies of functional metabolites and mechanism of the health promotion of probiotic fermentation in yogurt (Zha *et al.*, 2021).

In order to better understand the effects of different fermentation types of strains on the flavor and metabolic pathway of fermented milk, a homofermentative *Lp. plantarum* K25 and a heterofermentative *L. fermentum* 13-1 were employed for co-fermentation with a commercial yogurt starter culture in our study. The effects of two bacterial strains on the changes in volatile flavor compounds in fermented milk were investigated using gas chromatography–mass spectrometry (GC-MS) technique. Metabolomics analysis was performed using ultra-performance liquid chromatography coupled with time-of-flight mass spectrometry (UPLC-Q-TOF-MS) to describe differences in metabolite profiles and relevant metabolic pathways by multivariate statistical methods.

This study aimed to offer theoretical guidance for the potential application of different types of LAB strains in milk fermentation.

Materials and Methods

Materials and reagents

Bovine milk (protein 3.0%, fat 3.4%, w/v) was obtained from Beijing Sanyuan Food Co. Ltd (Beijing, China). Yogurt starter YO-MIX 300 LYO containing *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* was purchased from Danisco (Denmark). LAB strains *Lp. plantarum* K25 and *L. fermentum* 13-1 were previously isolated from Kefir grains collected from Tibet and preserved in our laboratory (Aziz et al., 2022; Jiang *et al*., 2021). 2-Methyl-3-heptanone and normal alkanes (C10–C25) were acquired from Sigma-Aldrich® (Germany). Helium gas with a purity of 99.99% was supplied by Air Products and Chemicals Inc. (USA). HPLC-grade methanol, acetonitrile, isopropanol, and formic acid were sourced from Merck (Germany). L-2-chlorophenylalanine was purchased from Shanghai Macklin Biochemical Technology Co. Ltd (China).

Preparation of fermented milk

The milk was heated at 65°C for 30 min, followed by cooling to about 40°C and inoculation with the respective microbial strains. The inoculated milk samples were then placed in an incubator (Shanghai Yiheng, China) and incubated at 42°C till pH 4.5 was attained. After fermentation, the fermented milk samples were cooled and stored at 4°C for further analysis. Three experimental groups of samples were utilized in the experiment: control samples (D) inoculated only with yogurt starter (10^7 CFU/mL), group D-Lp samples inoculated with yogurt starter (10^7 CFU/mL) and *Lp. plantarum* K25 (10^7 CFU/mL), and group D-Lf samples inoculated with yogurt starter (10^7 CFU/mL) and *L. fermentum* 13-1 (10^7 CFU/mL). The experiment was performed in triplicate.

Determination of pH value

Dairy fermentation monitor (AMS Alliance, France) was used to monitor changes in pH values during fermentation.

Detection of volatile organic compounds (VOCs)

The methodology used by Han *et al.* (2024c) with slight modifications was followed. VOCs were extracted from a 10-g sample of fermented milk using solid-phase microextraction (SPME) and analyzed by GC-MS (7890A-7000; Agilent Technologies Inc., Santa Clara, CA, USA). The fermented milk was placed in a 30-mL extraction bottle and incubated with 1 µL of 2-methyl-3-heptanone (0.816 µg/µL) at 40°C for 30 min. A 2-cm long divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (50/30 µm; Supelco, Bellefonte, PA, USA) was inserted in the headspace bottle for volatile extraction for 30 min. The sampler was then inserted into a gas chromatography (GC) injector at 250°C for 5 min for desorption. Gas chromatography was carried out with a DB-Wax high-polarity polyethylene glycol chromatographic column (30 m × 0.25 mm, 0.25 µm) in the injector port in splitless mode using helium carrier gas with a flow rate of 1.2 mL/min. The temperature rise program was performed at 40°C for 3 min, then raised to 200°C (5°C/min), to a final temperature of 230°C (10°C/min, maintained for 3 min). Mass spectrometry (MS) was performed using an electronic ionization source with an electronic energy of 70 eV. The transfer line temperature was set at 280°C, the ion source temperature at 230°C, and the quadrupole temperature at 150°C. The mass scanning range was set from 40 m/z to 250 m/z. Identification of VOCs was performed using mass spectral databases (NIST 14 spectral library [https://chemdata.nist.gov]) and retention index (RI) comparison (actual and reference RIs), as described by Han *et al.* (2024b), enabling qualitative analysis. Quantitative analysis of the VOCs identified by GC-MS was performed using an internal standard semi-quantitative method. The concentrations of VOCs were calculated based on the ratio of the compound peak areas to the known concentration of internal standard. The actual RI was obtained by the calculated formula and the retention times of the series of n-alkanes (Qiu *et al.*, 2024).

Untargeted metabolomics analysis

The fermented milk sample (100 µL) was subjected to extraction by mixing with 400 µL of extraction solvent (acetonitrile–methanol = 1:1, containing an internal standard of L-2-chlorophenylalanine at a concentration of 0.02 mg/mL). After 30 s of vortex, the mixture was subjected to ultrasound extraction for 30 min at 5°C with a frequency of 40 kHz. The treated samples were precipitated at -20°C for 30 min, followed by centrifugation for 15 min at 4°C and 13,000×*g*. The supernatant was dried by nitrogen, and further dissolved in 120 µL of a mixed solution of acetonitrile and water (1:1). The resulting solution was treated with the low-temperature ultrasound extraction at 40 kHz (5°C, 5 min). Finally, the solution was centrifuged for 5 min (4°C, 13,000×*g*). The supernatant was collected after filtration through a sterile membrane with a pore size of 0.22 µm and used for further analysis by using UPLC-Q-TOF-MS (Triple TOF5600, AB SCIEX, USA).

Chromatographic conditions: The separation was achieved on a BEH C18 column (100 × 2.1 mm, 1.8 μm) purchased from Waters Corporation (Milford, CT, USA). The mobile phase consisted of solvent A and solvent B. Solvent A was an aqueous solution containing 0.1% formic acid. Solvent B was a 1:1 mixture of acetonitrile and isopropanol, containing 0.1% formic acid. The flow rate was controlled at 0.40 mL/min and the column temperature was kept at 40°C.

Mass spectrometric conditions: The sample was acquired in positive and negative ionization modes within a mass scanning range of 50–1,000 m/z. The spray voltage was set at 5,000 V (positive) and 4,000 V (negative). The declustering potential was 80 V, and the spray gas pressure, auxiliary heating gas pressure, and curtain gas pressure were set at 50, 50, and 30 psi, respectively. The ion source heating temperature was 500°C, and collision energy was set at 20–60 V.

Data processing

Flavor data were analyzed using Excel 2019, and the Origin Pro 2022 software. Simca 14.1.0 was used to perform partial least squares discriminant analysis (PLS-DA) to visualize discrimination between samples. To indicate contribution of each variable to classification, the variable importance in projection (VIP) value of each variable was calculated.

Raw liquid chromatography–mass spectrometry (LC-MS) data were processed using the metabolomics software Progenesis QI (Waters Corporation). The MS values were matched against metabolite databases, Human Metabolome Database (HMDB) and METLIN metabolomics database, to obtain metabolite information. The preprocessed data were uploaded to the Majorbio Cloud platform (https://cloud.majorbio.com) for further data analysis, including principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), Student's *t*-test, fold change analysis, and VIP analysis. The annotation of metabolic pathways and the classification of metabolites were carried out using the *Kyoto Encyclopedia of Genes and Genomes* (KEGG) database and HMDB database, respectively.

Results and Discussion

Monitoring of pH changes

Figure 1 illustrates pH changes during milk fermentation with yogurt starter only (control D), together with *Lp. plantarum* K25 (D-Lp) and *L. fermentum* (D-Lf). The pH curves of the three groups exhibited a similar decreasing

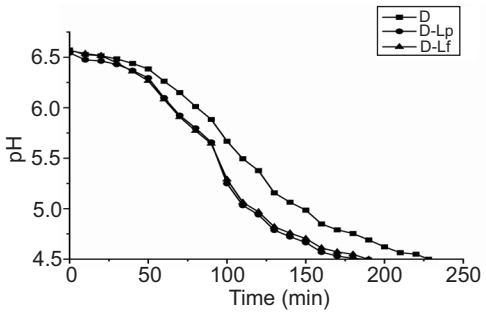


Figure 1. Changes in pH values during milk fermentation with yogurt starter (D), together with *Lp. plantarum* K25 (D-Lp) and *L. fermentum* 13-1 (D-Lf).

trend, but differences were observed in the time taken to reach the endpoint of fermentation (pH 4.5). Addition of *Lp. plantarum* K25 and *L. fermentum* 13-1 was found to promote milk fermentation with 180 min and 190 min, respectively, compared to the control with 228 min to reach pH 4.5. Tian *et al.* (2017) reported that the addition of probiotics accelerated decrease in pH during milk fermentation. Similar findings were obtained by Li *et al.* (2021), who observed that milk samples fermented with *thermophilic Streptococci* took longer time to reach the fermentation endpoint compared to the co-fermented samples with other LAB strains.

Identification of VOCs in fermented milk

In all, 20 VOCs were detected by SPME-GC-MS analysis in the three groups of fermented milk samples (D, D-Lp, and D-Lf), as presented in Table 1 and Figure 2. These compounds included two aldehydes, four ketones, six acids, four alcohols, and four other compounds. To investigate variation in flavor characteristics between the experimental groups, PLS-DA analysis was used to describe key VOCs. As shown in Figure 3A, the parallel processing of the same samples was clustered well with PC1 and PC2 being 41.3% and 24.9%, respectively. The cumulative variance contribution proportion of 66.2% was sufficient to reflect main variation of the original variable information. Based on the criteria of VIP > 1 and $p < 0.05$, 12 compounds were identified as key VOCs, which were hexanal, nonanal, 2,3-butanedione, acetoin, acetic acid, hexanoic acid, ethanol, 2-heptanol, 2,3-butanediol, dimethyl sulfone, and phenol (Figure 3C). These aroma compounds contributed different flavor characteristics to fermented milk with addition of homofermentative *Lp. plantarum* K25 and heterofermentative *L. fermentum* 13-1. In Table 1, data were expressed as mean ± standard deviation (SD) from replicate analyses (n = 3) of three replicate samples.

Aldehydes, such as hexanal and nonanal, are important components of odor, although their contents in

Table 1. Content of aroma volatile organic compounds in fermented milk samples.

Compounds	CAS	Content (ng/g)			Actual RI	Reference RI	Method of identification
		D	D-Lp	D-Lf			
Hexanal	66-25-1	0.18±0.04^{a}	–	–	1,079	1,084	MS-RI
Nonanal	124-19–6	0.87±0.01^{a}	–	–	1,359	1,381	MS-RI
2,3-Butanedione	431-03-8	4.98±0.26^{b}	8.82±1.13^{a}	–	956	973	MS-RI
2-Heptanone	110-43-0	2.47±0.53^{a}	2.06±0.13^{a,b}	1.56±0.25^{b}	1,194	1,180	MS-RI
Acetoin	513-86-0	–	6.88±1.11^{a}	–	1,282	1,287	MS-RI
2-Nonanone	821-55-6	1.34±0.46^{a}	1.32±0.30^{a}	0.45±0.04^{b}	1,394	1,387	MS-RI
Acetic acid	64-19-7	9.82±1.08^{b}	11.75±0.74^{b}	23.58±1.99^{a}	1,440	1,441	MS-RI
Butyric acid	107-92-6	5.73±0.55^{a}	4.88±0.42^{a}	5.35±0.35^{a}	1,615	1,628	MS-RI
Hexanoic acid	142-62-1	19.17±1.11^{c}	28.94±1.39^{a}	22.74±1.38^{b}	1,918	1,846	MS-RI
Octanoic acid	124-07-2	14.30±1.78^{a}	14.69±1.68^{a}	16.78±1.51^{a}	2,034	2,060	MS-RI
Decanoic acid	334-48-5	4.62±1.07^{a}	5.41±0.11^{a}	5.55±0.35^{a}	2,174	2,290	MS-RI
Benzoic acid	65-85-0	4.49±0.73^{a}	4.17±0.47^{a}	4.69±0.60^{a}	2,408	2,412	MS-RI
Ethanol	64-17-5	1.57±0.42^{b}	–	9.92±0.93^{a}	959	934	MS-RI
2-Heptanol	543-49-7	–	–	0.69±0.13^{a}	1,318	1,316	MS-RI
2-Ethyl-1-hexanol	104-76-7	1.01±0.20^{a}	1.32±0.55^{a}	1.51±0.16^{a}	1,485	1,474	MS-RI
2,3-Butanediol	513-85-9	–	–	3.54±0.24^{a}	1,522	1,539	MS-RI
γ-Butyrolactone	96-48-0	0.61±0.13^{a}	0.72±0.09^{a}	0.68±0.03^{a}	1,596	1,613	MS-RI
2-(2-Ethoxyethoxy)-ethanol	111-90-0	4.37±0.35^{a}	4.36±0.26^{a}	4.19±1.32^{a}	1,604	1,622	MS-RI
Dimethyl sulfone	67-71-0	0.46±0.10^{a}	0.24±0.05^{b}	0.41±0.04^{a,b}	1,930	1,912	MS-RI
Phenol	108-95-2	–	0.37±0.02^{a}	0.24±0.05^{b}	2,016	2,008	MS-RI

Different lowercase letters in each row indicate significant differences between samples ($p < 0.05$).
Symbol "-" means not found. D: control sample fermented with yogurt starter only; D-Lp: sample fermented with yogurt starter and *Lp. plantarum* K25; D-Lf: sample fermented with yogurt starter and *L. fermentum* 13-1; MS-RI: mass spectrometry–retention index.

fermented dairy products are usually low. The primary reasons for the low levels of aldehydes may be attributed to two aspects. On the one hand, aldehydes themselves are susceptible to reduction into alcohols or oxidation into acids (Chi *et al.*, 2024); on the other hand, the inherent antioxidant background of yogurt (Shori *et al.*, 2022) hinders the formation of aldehydes derived from the oxidative decomposition of fatty acids. Table 1 and Figure 3B show that hexanal and nonanal were only detected in the control sample.

Hexanal, typically derived from the auto-oxidation of linoleic acid (Zhao *et al.*, 2022a), exhibits an aroma reminiscent of fresh grass (Wang *et al.*, 2020a). Nonanal, primarily originating from unsaturated fatty acids through the formation of peroxide intermediates, serves to impart floral and citrus aroma characteristics to fermented milk (Zhang *et al.*, 2024). The low levels or absence of these two aldehydes may be attributed to the yogurt environment itself. Relevant research indicates that the pH value is crucial for the successful neutralization of lipid oxidation in oil-in-water (O/W) emulsions containing antioxidants (Cantele *et al.*, 2024). In acidic conditions, compared to neutral environments, the negative charge on oil droplets is weaker, making them less likely to attract cations, such as Fe^{3+}, that initiate chain reactions, thereby inhibiting lipid oxidation. Consequently, antioxidants can more effectively retard the formation of hexanal and nonanal. In addition, the antioxidant activity of these compounds is also dependent on the prevailing pH during the reaction, often exhibiting enhanced performance in acidic environments (Bayram *et al.*, 2023). Fermented milk can be approximated as an O/W emulsion, and its low pH environment delays fatty acid oxidation, thus inhibiting the formation of hexanal and nonanal.

Ketones, such as 2,3-butanedione and acetoin, are significant volatile compounds in yogurt, although the composition of ketones varied greatly among different fermented milk samples. The relative abundance of 2,3-butanedione and acetoin is strongly correlated with citrate metabolism, and other ketones can be formed through fatty acid oxidation, thermal degradation, amino acid degradation, and microbial metabolism (Gómez-Torres *et al.*, 2016). 2,3-Butanedione gives the yogurt a buttery flavor (Innocente *et al.*, 2016), but acetoin helps to provide a mild creamy aroma (Liu *et al.*, 2022b). A study focusing on beef burgers (Botta *et al.*, 2022) indicated

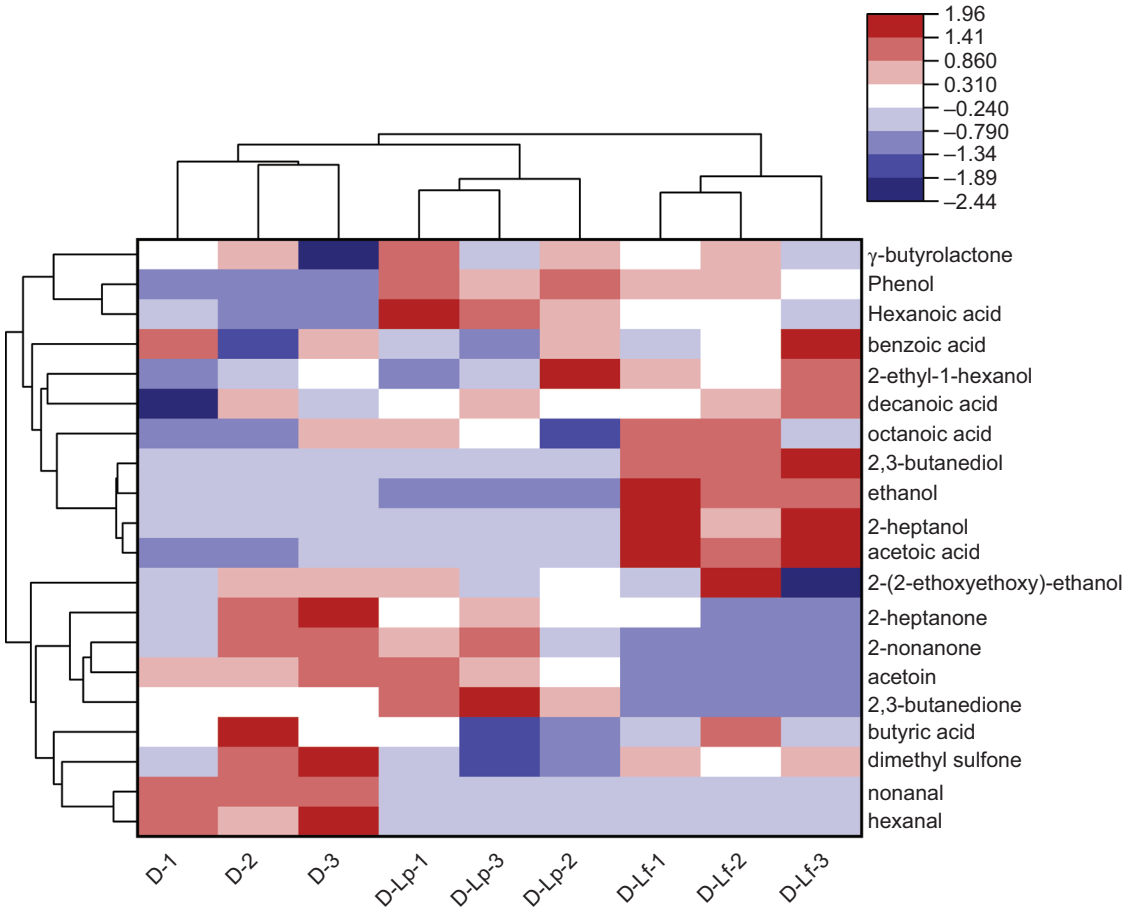


Figure 2. Heatmap of the aroma compounds in the fermented milk samples D, D-Lp and D-Lf. D: control sample fermented with only the yogurt starter; D-Lp: sample fermented with yogurt starter and *Lp. plantarum* K25; D-Lf: sample fermented with yogurt starter and *L. fermentum* 13-1.

that the accumulation of acetoin was primarily a consequence of homofermentative metabolism conducted by LAB, specifically *Carnobacterium divergens*. This finding underscored the intimate association between homofermentation and the formation of these buttery flavor compounds. Figure 3B shows that among the distributed dots representing each volatile compound, these two ketones are found to be near the points representing D and D-Lp. This suggested that the addition of homofermentative *Lp. plantarum* K25 promoted formation of ketones, specially 2,3-butanedione with significantly higher content ($p < 0.05$) in D-Lp than that in D. Moreover, neither of these two ketones was detected in the D-Lf samples, suggesting that the addition of heterofermentative *L. fermentum* 13-1 had a negative effect on the formation of these specific yogurt flavor ketones. Previously, Mukisa *et al.* (2017) also did not detect 2,3-butanedione and acetoin in the fermented milk produced by *L. fermentum* MNC34. Zhang *et al.* (2022) reported similar results using *Lactobacillus fermentum* HY01.

Acid is an essential component of fermented milk flavor, which mainly focuses on the formation of characteristic sour and fermentation odor (Tang *et al.*, 2024). The content of acetic acid in the D-Lf sample was significantly higher ($p < 0.05$) than that in the D and D-Lp samples, probably because of the heterofermentative carbohydrate metabolism of *L. fermentum*. Ferrocino *et al.* (2018) reported that heterofermentative LAB strains could produce acetic acid through pyruvate metabolism, and *L. sakei* with high acetate kinase activity promoted the formation of acetic acid. However, too high concentration of acetic acid results in a vinegar flavor, which negatively affects the overall milk flavor. Furthermore, the content of hexanoic acid was highest in D-Lp sample, followed by D-Lf and D samples. Hexanoic acid appears in the fermentation and storage of dairy products, providing fatty, sweaty, and cheesy odor (Wang *et al.*, 2023b).

Alcohols, which are mainly produced from oxidation of fats and reduction of aldehydes and ketones, contribute

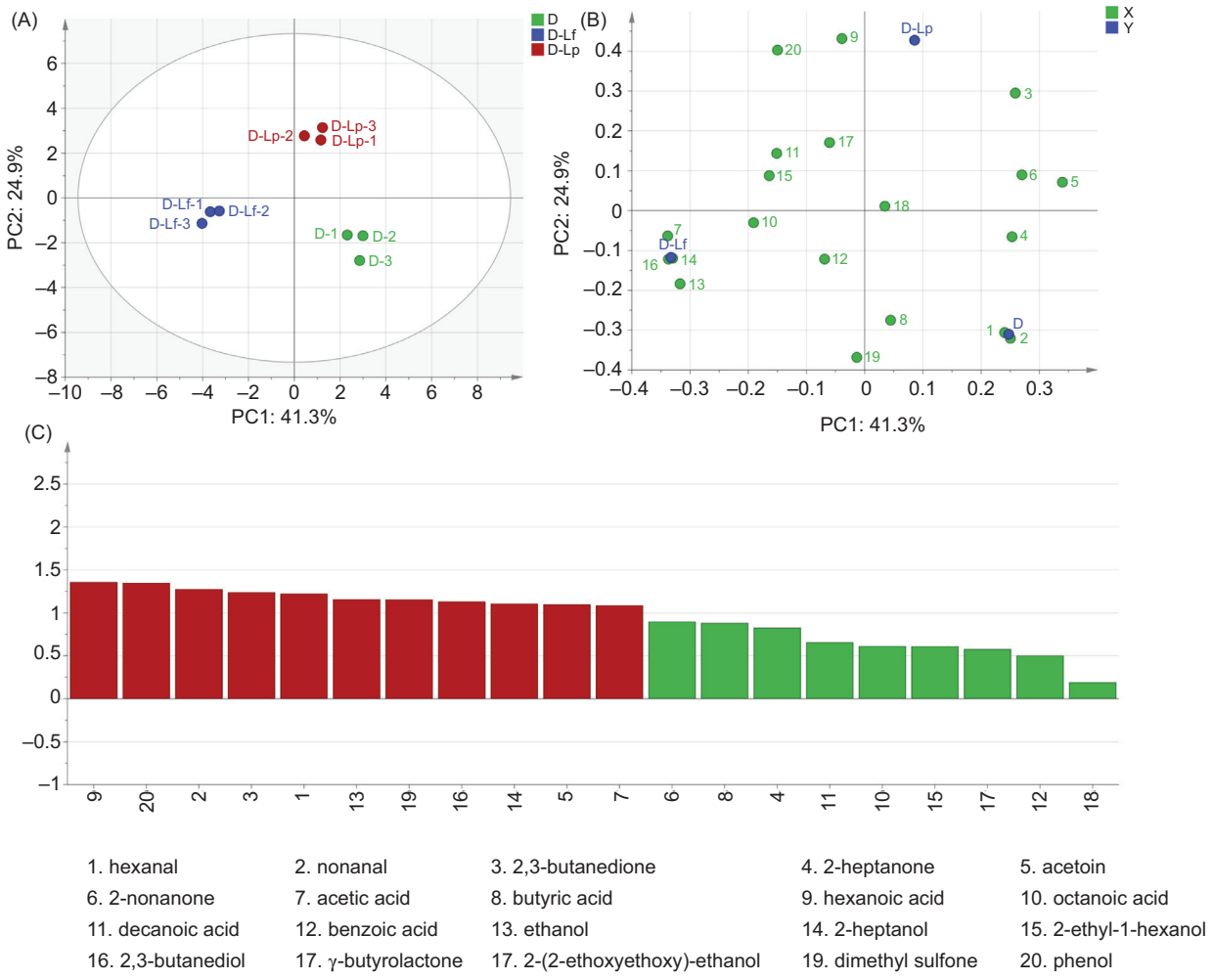


Figure 3. Analysis of the volatile compounds in the fermented milk samples by PLS score plot (A), PLS loading of the scatter plot of volatile compounds (B), and calculation of the variable importance in projection (VIP) values from the PLS (C). (D) control sample fermented with only the yogurt starter; D-Lp: sample fermented with yogurt starter and *Lp. plantarum* K25; D-Lf: sample fermented with yogurt starter and *L. fermentum* 13-1.

special flavors to fermented milk. Alcohols weaken the sour taste, although they have little effect on flavor because of high threshold and low concentration (Zhao *et al.*, 2018). Compared to both D and D-Lp samples, the D-Lf sample contained significantly more alcohols ($p < 0.05$), especially ethanol with high content because of production from heterofermentative fermentation (Table 1 and Figure 2). In the study conducted by Botta *et al.* (2022), a positive correlation between ethanol and heterofermentative *Leuconostoc gelidum* was observed. Ethanol provides typical alcohol odor. 2-Heptanol may be related to lipolysis, offering green and floral aroma (Wang *et al.*, 2023b). 2,3-Butanediol, derived from the reduction of acetoin, also contributes to creamy odor (Liu *et al.*, 2022c). It is worth noting that although 2,3-butanediol was found in D-Lf sample, no acetoin or 2,3-butanedione was observed. The only speculation is that *Lactobacillus fermentum* has the ability to convert all acetoin into 2,3-butanediol.

In the present study, dimethyl sulfone and phenol were also identified as key volatile compounds in yogurt samples. Dimethyl sulfone, a type of sulfur-containing compound, was first noticed by Liu *et al.* (2022c). Heat treatment of milk prior to fermentation, microbial degradation of sulfur-containing amino acids, and catabolism of sulphydryl groups could produce sulfur-containing compounds (Pereda *et al.*, 2008). Phenol was rarely identified as a key flavor compound and was occasionally discovered in dairy products, typically at low levels (Liu *et al.*, 2022d; Wang *et al.*, 2024).

Metabolomics analysis of the fermented milk

Metabolite clustering and multivariate analysis in D, D-Lp, and D-Lf samples

Metabolomics analysis of fermented milk samples demonstrated a total of 601 metabolites discovered in the three groups of samples, with 283 in cationic mode and 318 in anionic mode. The clustering and relative abundance of all metabolites are shown in Figure 4. Based on compound classification in HMDB, lipids and lipid-like molecules (322 species) and organic acids and derivatives (117 species) were found to be the predominant metabolites in yogurt samples. Clustering results showed that D and D-Lp clustered together, meaning that these two groups were more comparable in terms of the composition and content of metabolites, but D-Lf was different from these two groups.

Principal component analysis was performed on D-Lp, D-Lf, and D samples, as shown in Figure 5A. The cumulative variance contribution rates in positive and negative modes were found to reach 72.0% and 66.4%, respectively, explaining changes in original variable information. OPLS-DA is a supervised recognition method that

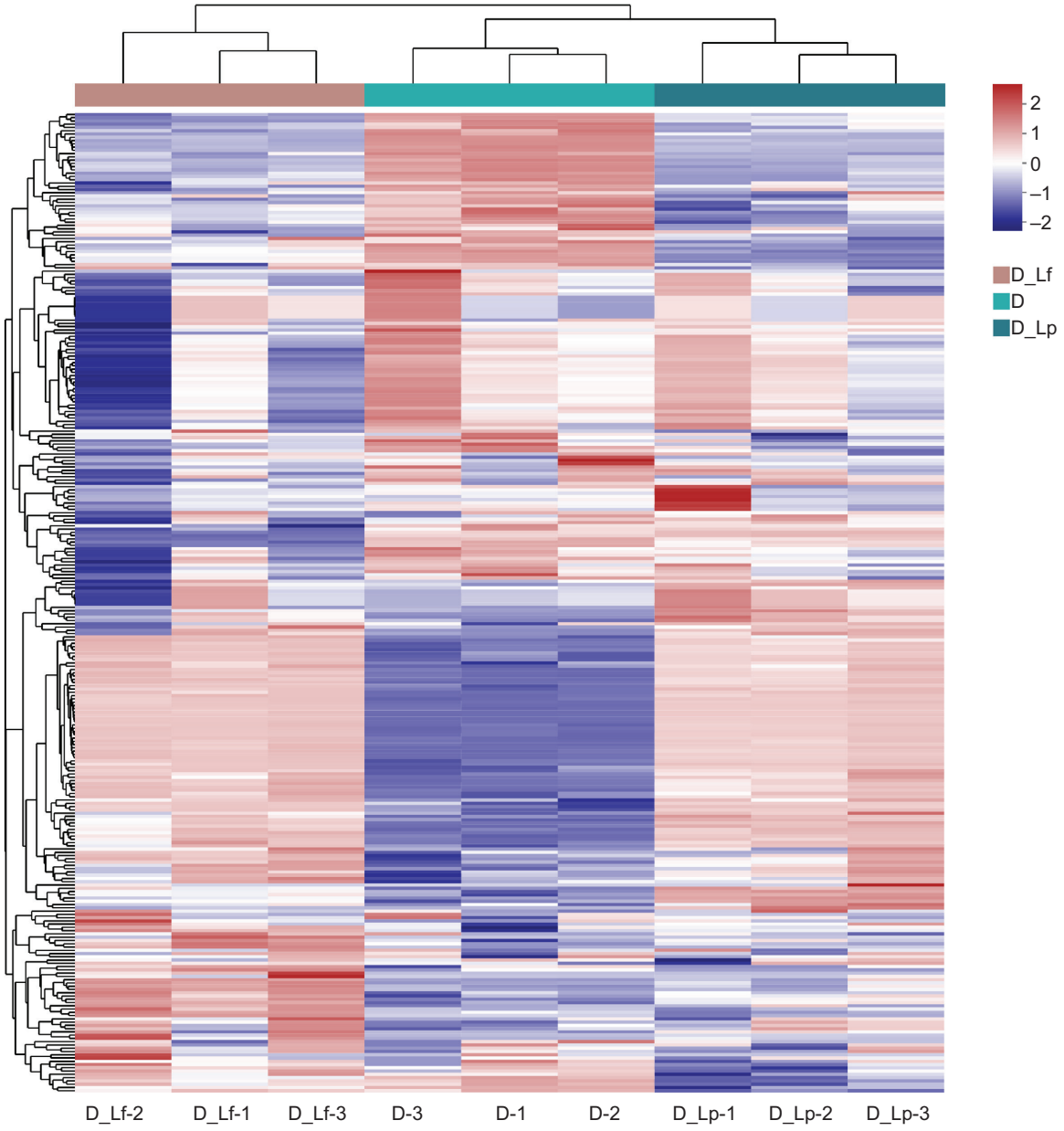


Figure 4. Metabolite clustering heat map of the fermented milk samples (D, D-Lp and D-Lf), displaying compounds with abundance ranking in the top 300. D: control sample fermented with only the yogurt starter; D-Lp: sample fermented with yogurt starter and *Lp. plantarum* K25; D-Lf: sample fermented with yogurt starter and *L. fermentum* 13-1.

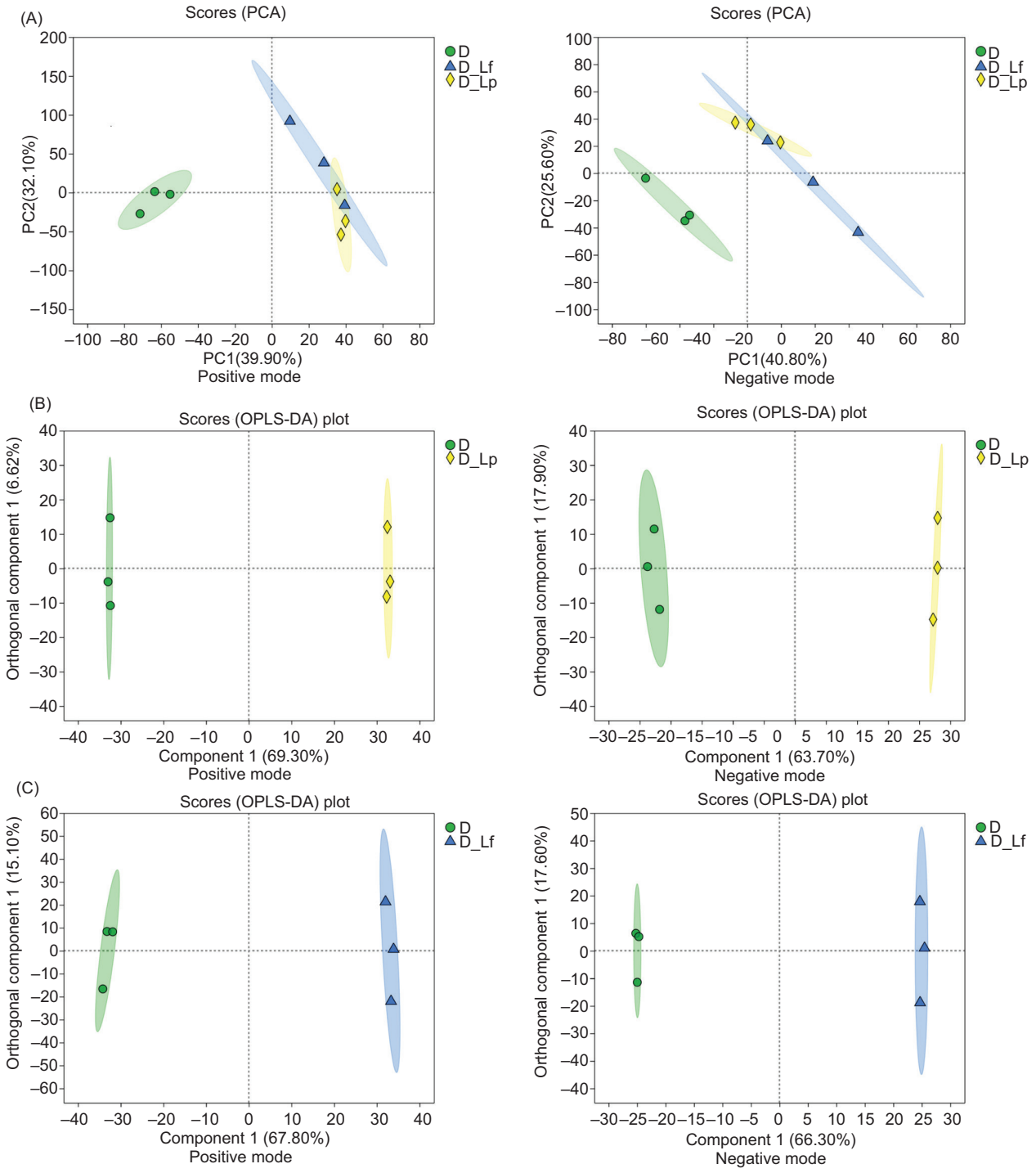


Figure 5. (A) PCA scores plot of D-Lp, D-Lf, and D samples. (B) OPLS-DA scores plot of D-Lp vs D model. (C) OPLS-DA scores plot of D-Lf vs D model. D: control sample fermented with only the yogurt starter; D-Lp: sample fermented with yogurt starter and *Lp. plantarum* K25; D-Lf: sample fermented with yogurt starter and *L. fermentum* 13-1.

adopts orthogonalization to remove data irrelevant to categorizing information, and is considered a highly effective approach for the classification and establishment of discriminative model of samples (Du *et al.*, 2021). In Figures 5B and 5C, the score plots of the D-Lp versus D model and the D-Lf versus D model clearly demonstrate that D-Lp is distinctly segregated into two classes from D, and D-Lf is separated into two classes from D, because of the considerable distances between different sample groups. In addition, the model was tested with

200 permutations to prevent over-fitting. The R^2X, R^2Y, and Q^2 values for the D-Lp versus D model in positive and negative ion modes were 0.693, 0.998, and 0.986, and 0.637, 0.998, and 0.978, respectively. The R^2X, R^2Y, and Q^2 values for the D-Lf versus D model in positive and negative ion modes were 0.678, 0.99, and 0.973, and 0.663, 0.969, and 0.944, respectively. R^2X and R^2Y reflect the ability to interpret information from X and Y matrices, and Q^2 refers to the predictive power of the model. The closer these three values are to 1, the higher the credibility. Thus, both models exhibited good explanatory and predictive power.

Analysis of metabolites that affect the taste of fermented milk

The flavor of fermented milk comprises two components: aroma and taste. Taste compounds are generally composed of hydrophilic small molecules or ions, including amino acids, peptides, organic acids, and sugars. Among them, amino acids and their derivatives, and peptides with taste activity are commonly found in various fermented foods, exerting a significant influence on food flavor (Zhao *et al.*, 2016). Using a non-targeted metabolomics approach, a total of 53 amino acids, peptides, and the related amino acid derivatives were detected from the three groups of samples (Figure 6B). By establishing the PLS-DA model (Figures 6A and 6C), with the criteria of VIP >1 and $p < 0.05$, 10 key taste compounds were identified, including 3 amino acids: L-isoleucine (L-Ile), L-phenylalanine (L-Phe), and L-tryptophan (L-Try); 5 dipeptides: arginyl-proline (Arg-Pro), glutaminyl-proline (Gln-Pro), glycyl-phenylalanine (Gly-Phe), leucyl-glycine (Leu-Gly) and γ-glutamylarginine (γ-Glu-Arg); and 2 amino acid derivatives: 2-hepteneoylglycine and N-acetyl-L-glutamic acid. These taste compounds were primarily associated with bitterness and kokumi sensation as discussed below.

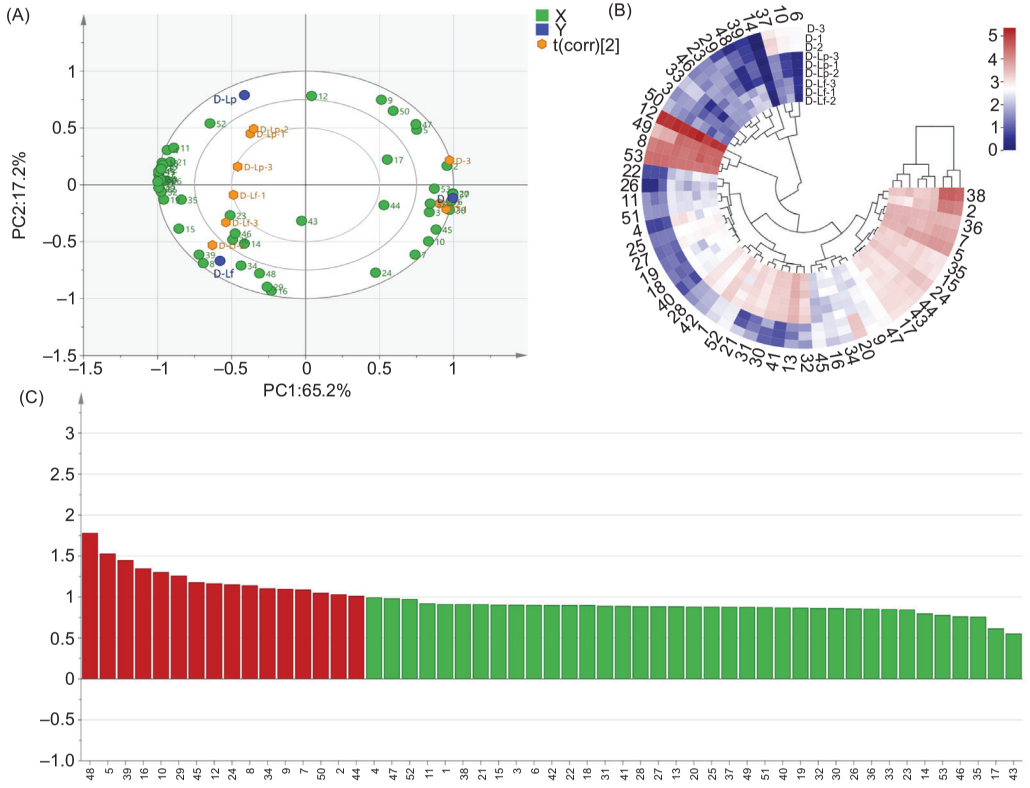
In fermented foods, taste compounds are generally derived from hydrolysis of proteins (Zhao *et al.*, 2016). Free amino acids, such as L-Ile, L-Phe, and L-Try, as identified in this study, were reported to be bitter amino acids in some fermented foods, such as cheese and soy sauce (Zhao *et al.*, 2016). In the samples added with *Lp. plantarum* K25 and *L. fermentum* 13-1, the richness of these three bitter amino acids was significantly decreased, suggesting the effect of reducing the bitterness of fermented milk by these two LAB strains. Furthermore, in the D-Lp sample, the content of bitter dipeptides, Gln-Pro and Leu-Gly, decreased significantly, while in the D-Lf sample, the bitter Arg-Pro and γ-Glu-Arg increased significantly ($p < 0.05$). The bitterness of these dipeptides was associated with their hydrophobic properties (Fu *et al.*, 2018). Kim and Li-Chan (2006) found that in di- or tripeptides, large hydrophobic amino acids at the C-terminus contributed significantly to bitterness, and amino acids with highly hydrophobic side chains included Try, Ile, Tyr, Phe, Pro, Leu, and Val. When arginine was adjacent to proline, the bitterness of peptide was more stronger (Liu *et al.*, 2022a). Bitter peptides had a much greater impact than bitter amino acids on the bitter taste of food. However, γ-glutamyl peptides were known as kokumi peptides that mainly contributed to the kokumi taste (Diez-Simon *et al.*, 2020).

Analysis of functional metabolites in fermented milk and the related metabolic pathways

Using $p < 0.05$ and VIP > 1 as screening criteria, the two groups of fermented milk samples (D-Lp and D-Lf) were compared to the control sample (D) in a paired manner. Both D-Lp and D-Lf samples were found with 122 differential metabolites when each of them was compared with D sample (refer to the Supplementary Material for detailed information), and the cluster heatmaps of differential metabolites are shown in Figures 7A and 7B. The Venn diagram analysis showed 98 common differential metabolites for the two groups, with 24 specific ones that were different from each other (Figure 7C).

Based on the 122 differential metabolites in D-Lp compared to D, the pathway enrichment analysis of KEGG (Figure 8A) revealed that the addition of *Lp. plantarum* K25 led to changes in several metabolic pathways in fermented milk, particularly amino acid metabolism, including phenylalanine metabolism, valine, leucine, and isoleucine biosynthesis, cyanoamino acid metabolism, arginine and proline metabolism, and D-glutamine and D-glutamate metabolism. Sun *et al.* (2023) also found enrichment in arginine and proline metabolism, and alanine, aspartate, and glutamate metabolism in the co-fermented milk with *Bifidobacterium adolescentis* B8589 and *Lacticaseibacillus paracasei* PC-01. *Lp. plantarum* L3 was found to have a significant impact on phenylalanine metabolism in yogurt as analyzed by metabolomics method (Wang *et al.*, 2023c). Amino acids and derivatives were shown to be closely related to yogurt flavor, for example, the volatile ketones produced from the degradation of amino acids (Zhang *et al.*, 2023), and sulfides, such as dimethyl sulfone, produced from the decomposition of sulfur-containing amino acids (Liu *et al.*, 2022d). Further analysis using the KEGG database revealed some differential metabolites to be explicitly involved in metabolic processes, including (R)-(+)-2-pyrrolidone-5-carboxylic acid, 2-hydroxycinnamic acid, linamarin, phenylacetic acid, L-proline, 2-phenylacetamide, pyroglutamic acid, 2-isopropylmalic acid, β-D-3-ribofuranosyluric acid, L-4-hydroxyglutamate semi-aldehyde, acetylcholine, and D-1-piperideine-2-carboxylic acid. Their relative horizontal changes are reflected in a volcano plot (Figure 9A).

Figure 8B illustrates the metabolic pathway changes in D-Lf based on differential metabolites compared to D. Changes in amino acid metabolism in D-Lf were similar



1: (R)-(+)-2-Pyrrolidone-5-carboxylic acid
2: L-Isoleucine
3: L-Lysine
4: Pyroglutamic acid
5: L-Phenylalanine
6: L-Proline
7: L-Tryptophan
8: Arginyl-Proline
9: Cysteinyl-Histidine
10: Glutaminyl-Proline
11: Glutamylalanine
12: Glutamylcysteine
13: Glutamylisoleucine
14: Glutamylmethionine
15: Glutamylproline
16: Glycyl-Phenylalanine
17: Glycylproline
18: Hydroxyprolyl-Alanine
19: Hydroxyprolyl-Proline
20: Hydroxyprolyl-Hydroxyproline
21: Isoleucyl-Hydroxyproline
22: Isoleucyl-Arginine
23 : Isoleucyl-Methionine
24: Leucyl-Glycine
25: Lysyl-Proline
26: Methionyl-Hydroxyproline
27: Methionyl-Proline
28: Pyroglutamylvaline
29: Phenylalanyl-Arginine
30: Phenylalanyl-γ-glutamate
31: L-phenylalanyl-L-hydroxyproline
32: Prolyl-Alanine
33: Prolylhydroxyproline
34: Tryptophyl-Valine
35: Tryptophyl-Aspartate
36: Tyrosyl-Arginine
37: Tyrosyl-Proline
38: Tyrosyl-Valine
39: γ-Glutamylarginine
40: γ-Glutamylphenylalanine
41: γ-Glutamyltryptophan
42: Acetyl-DL-Leucine
43: Acetyl-DL-Valine
44: Cinnamoylglycine
45: 2-Hepteneoylglycine
46: 4-Hydroxyphenylacetylglutamic acid
47: Indolylacryloylglycine
48: N-Acetyl-L-glutamic acid
49: N-(l-Deoxy-1-fructosyl)phenylalanine
50: N-(l-Deoxy-1-fructosyl)methionine
51: N-(l-Deoxy-1-fructosyl)histidine
52: N-(l-Deoxy-1-fructosyl)isoleucine
53: Phenylacetylglycine

Figure 6. PLS biplot based on taste compounds (A), circle heatmap of taste compounds (B), VIP calculation based on the PLS model of taste compounds in the fermented milk samples (C). (D) control sample fermented with only the yogurt starter; D-Lp: sample fermented with yogurt starter and *Lp. plantarum* K25; D-Lf: sample fermented with yogurt starter and *L. fermentum* 13-1.

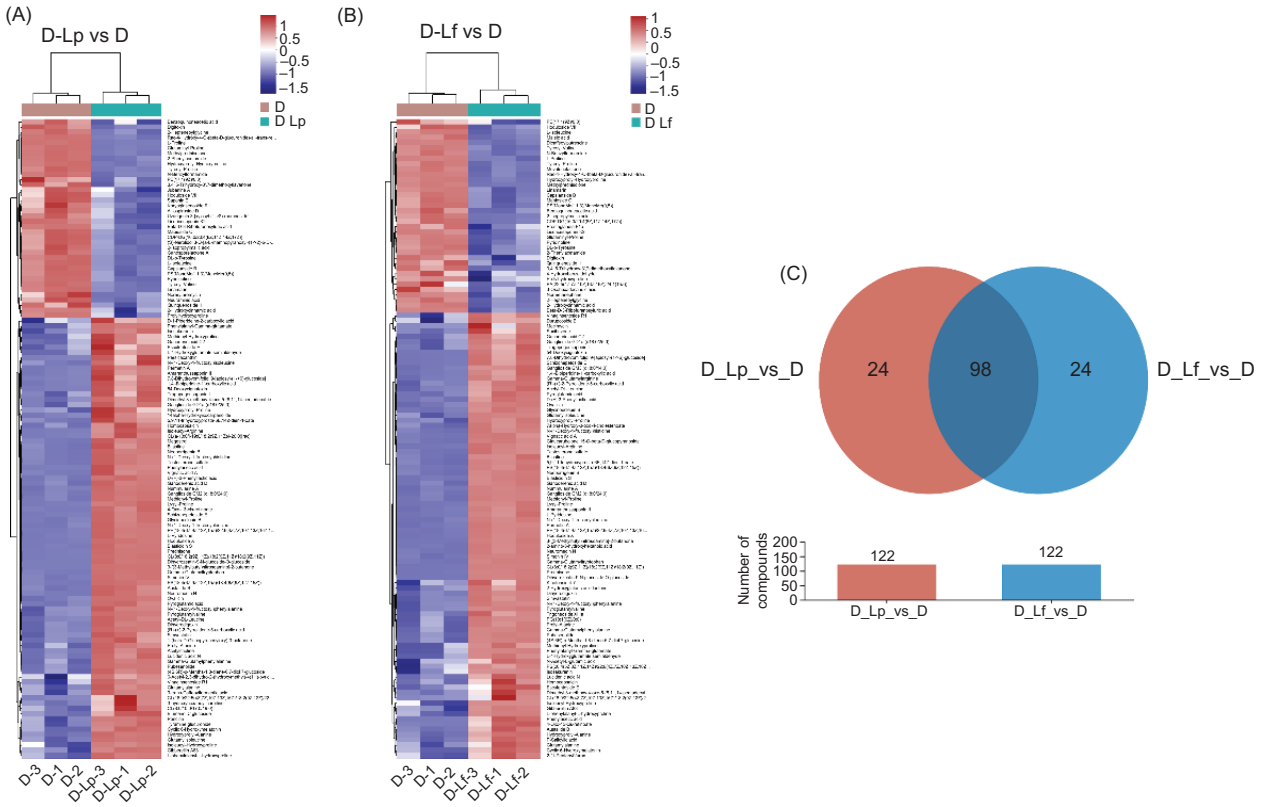


Figure 7. Cluster heatmaps of the differential metabolites in D-Lp (A, B), and Venn diagram of the differential metabolites in D-Lp vs D model and D-Lf vs D model (C). (D) control sample fermented with only the yogurt starter; D-Lp: sample fermented with yogurt starter and *Lp. plantarum* K25; D-Lf: sample fermented with yogurt starter and *L. fermentum* 13-1.

to those in D-Lp, but with different degrees of impact, and these pathways included phenylalanine metabolism, valine, leucine and isoleucine biosynthesis, cyanoamino acid metabolism, and arginine and proline metabolism. Furthermore, the increased promotion of tyrosine metabolism in D-Lf was attributed to the significantly decreased levels of normetanephrine and maleic acid ($p < 0.05$) (Figures 8B and 9B). In contrast to D-Lp, the degradation of toluene and bisphenol was significantly promoted in D-Lf, as indicated by the discovery of 4-hydroxybenzaldehyde and P-salicylic acid. It was reported that 4-hydroxybenzaldehyde was present as a natural benzaldehyde in *Gastrodia elata* (Loh *et al.*, 2022), and it possessed wound-healing properties and potential therapeutic effects in treating headaches (Chen *et al.*, 2021). P-Salicylic acid, also known as 4-hydroxybenzoic acid, has antioxidant, antibacterial, and cardioprotective activities (Joshi *et al.*, 2022). In the toluene degradation pathway, P-salicylic acid is directly converted from 4-hydroxybenzaldehyde by catalyzing with 4-hydroxybenzaldehyde dehydrogenase. N-acetyl-L-glutamate, derived from L-glutamate, was observed with a twice higher level in D-Lf than that in D (Figure 9B), and it exerted its primary biological activity by modulating the allosteric regulation of carbamoyl phosphate synthetase I (Harper *et al.*, 2009).

As shown in Figure 10, a notable rise in phenylacetic acid and significantly decreased levels of 2-phenylacetamide and 2-hydroxycinnamic acid in D-Lp indicated the promoted phenylalanine metabolism ($p < 0.05$). Phenylacetic acid acted as an inhibitor of tyrosinase, a copper-containing enzyme catalyzing browning reaction in fruits and vegetables, and it also had antimicrobial activity (Zhu *et al.*, 2011).

The substantially decreased level of L-proline accompanied by the increased content of L-4-hydroxyglutamate semi-aldehyde ($p < 0.05$) reflected stimulation of proline metabolism. The level of pyroglutamic acid (pGlu) significantly increased ($p < 0.05$) due to the intramolecular dehydration and cyclization of L-glutamate in glutathione metabolism, and pGlu had antitumor, mitotic promotion, antidiabetic, and lipid-lowering effects (Aiello *et al.*, 2022).

Moreover, (R)-(+)-2-pyrrolidone-5-carboxylic acid, linamarin, 2-isopropylmalic acid, and β-D-3-ribofuranosyluric acid were involved in several

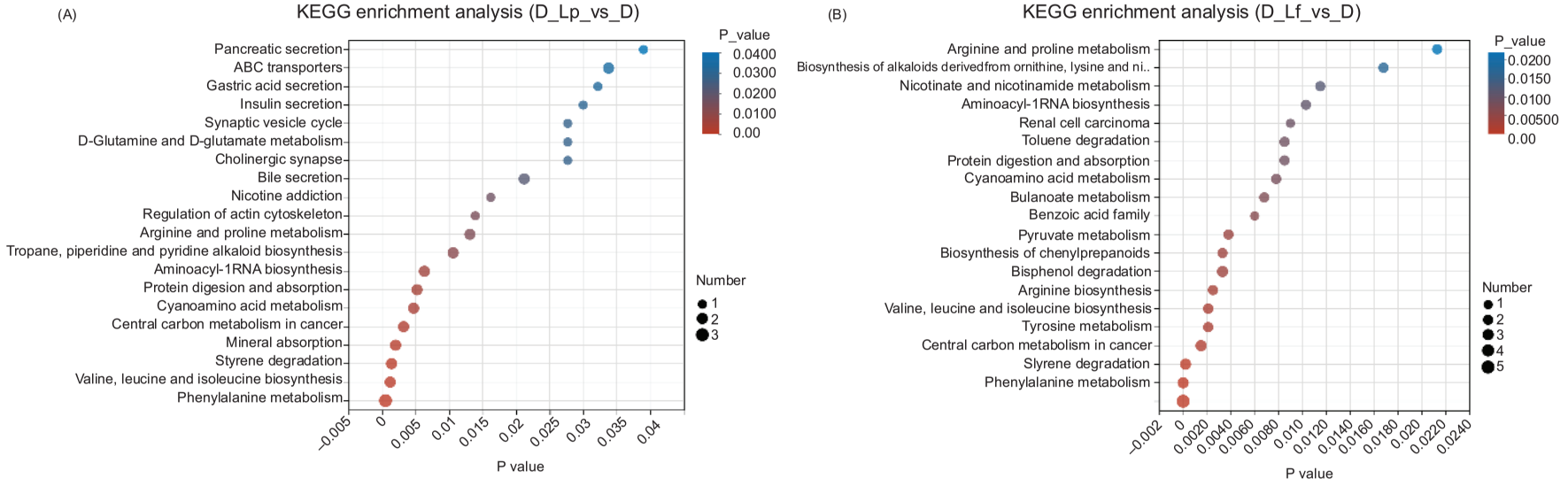


Figure 8. Bubble chart of significantly enriched metabolic pathways in D-Lp (A) and D-Lf (B). The horizontal axis represents the enrichment significance p-value. The smaller the p-value, the more significant it is in statistics. Generally, a p-value less than 0.05 indicates a significant enrichment term for this function; The vertical axis represents the KEGG pathway. The size of the bubbles in the figure represents the amount of compound enriched in the metabolic concentration in this pathway. (D) control sample fermented with only the yogurt starter; D-Lp: sample fermented with yogurt starter and *Lp. plantarum* K25; D-Lf: sample fermented with yogurt starter and *L. fermentum* 13-1.

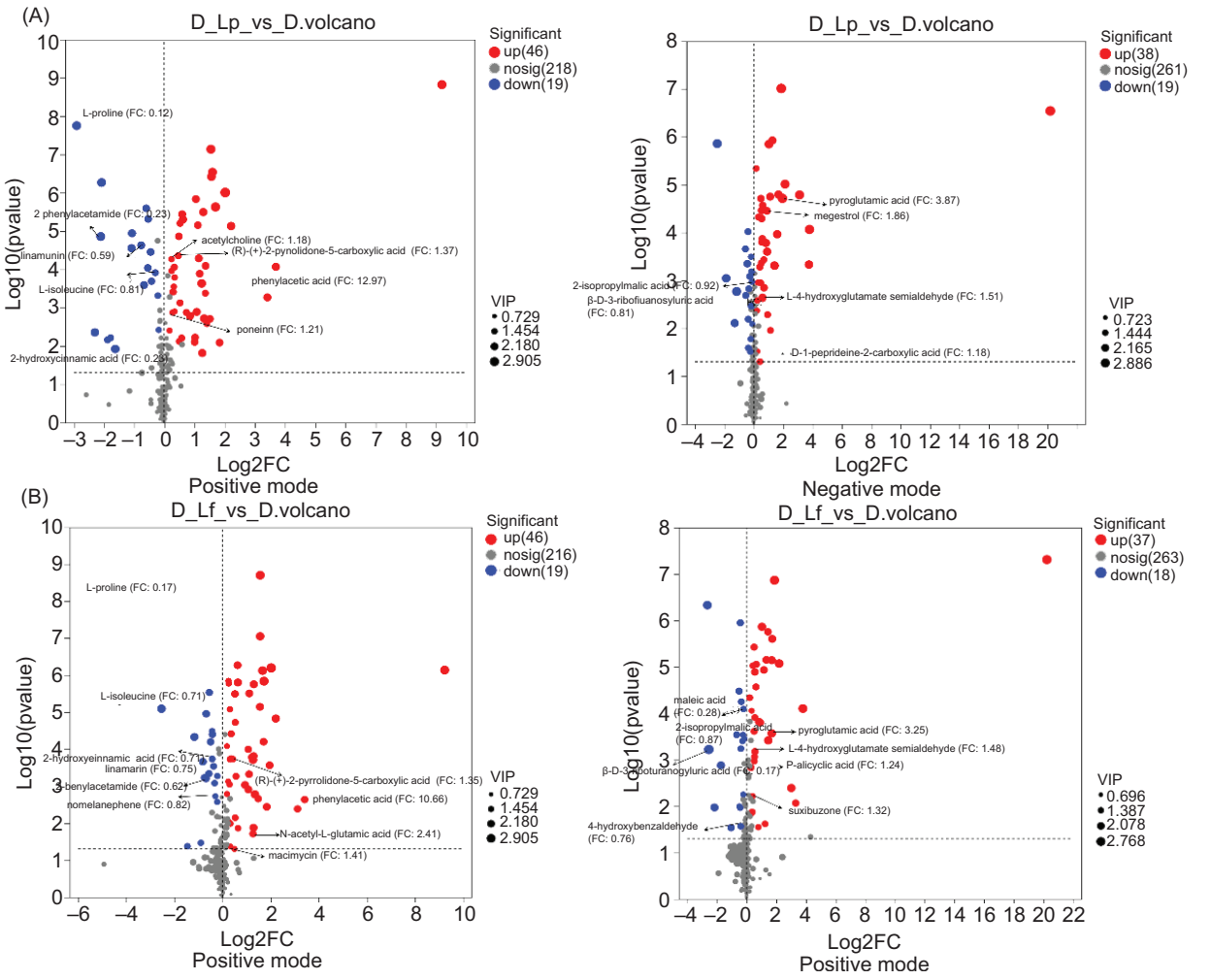


Figure 9. Volcano plot of metabolic products in D-Lp vs D model (A) and D-Lf vs D (B). FC stands for fold change. Red represents a significant increase, while blue represents a significant decrease. The larger the sample point, the larger the VIP. (D) control sample fermented with only the yogurt starter; D-Lp: sample fermented with yogurt starter and *Lp. plantarum* K25; D-Lf: sample fermented with yogurt starter and *L. fermentum* 13-1.

metabolism pathways, including D-amino acid metabolism, cyanogenic amino acid metabolism, and metabolism for the synthesis of valine, leucine, isoleucine, and purine. As a precursor for the biosynthesis of L-leucine and L-valine, 2-isopropylmalic acid had mild antioxidant activity and weak anti-pathogenic activity against some food-borne pathogens (Ricciutelli *et al.*, 2020). Linamarin had neurotoxic effects, and it could induce motor disorders in mice (Rivadeneyra-Domínguez *et al.*, 2013).

The significantly increased expression of D-1-piperideine-2-carboxylic acid ($p < 0.05$) indicated promoted lysine degradation by addition of *Lp. plantarum* K25. In addition, among the 24 specific differential metabolites in D-Lp, acetylcholine (Ach) was significantly up-regulated (Figure 8). ACh is a neurotransmitter widely present in the peripheral and central nervous systems, and it plays an important role in cognitive functions, encompassing thought processing, learning, and memory (Chen *et al.*, 2023). As an oral flavonoid, poncirin is a flavonoid glycoside derivative with multiple pharma and other therapeutic effects (Yousof Ali *et al.*, 2020), such as gastric protection (Lee *et al.*, 2009), promotion of osteoblast differentiation (Yoon *et al.*, 2011), and anti-inflammatory effects (Kang and Kim, 2016).

Conclusions

Addition of different fermentation types of LAB strains, such as the homofermentative *Lp. plantarum* K25 and heterofermentative *L. fermentum* 13-1, was shown to promote yogurt fermentation. *Lp. plantarum* K25 could increase the content of 2,3-butanedione and hexanoic acid whereas *L. fermentum* 13-1 caused a loss of 2,3-butanedione and acetoin, and greatly increased the

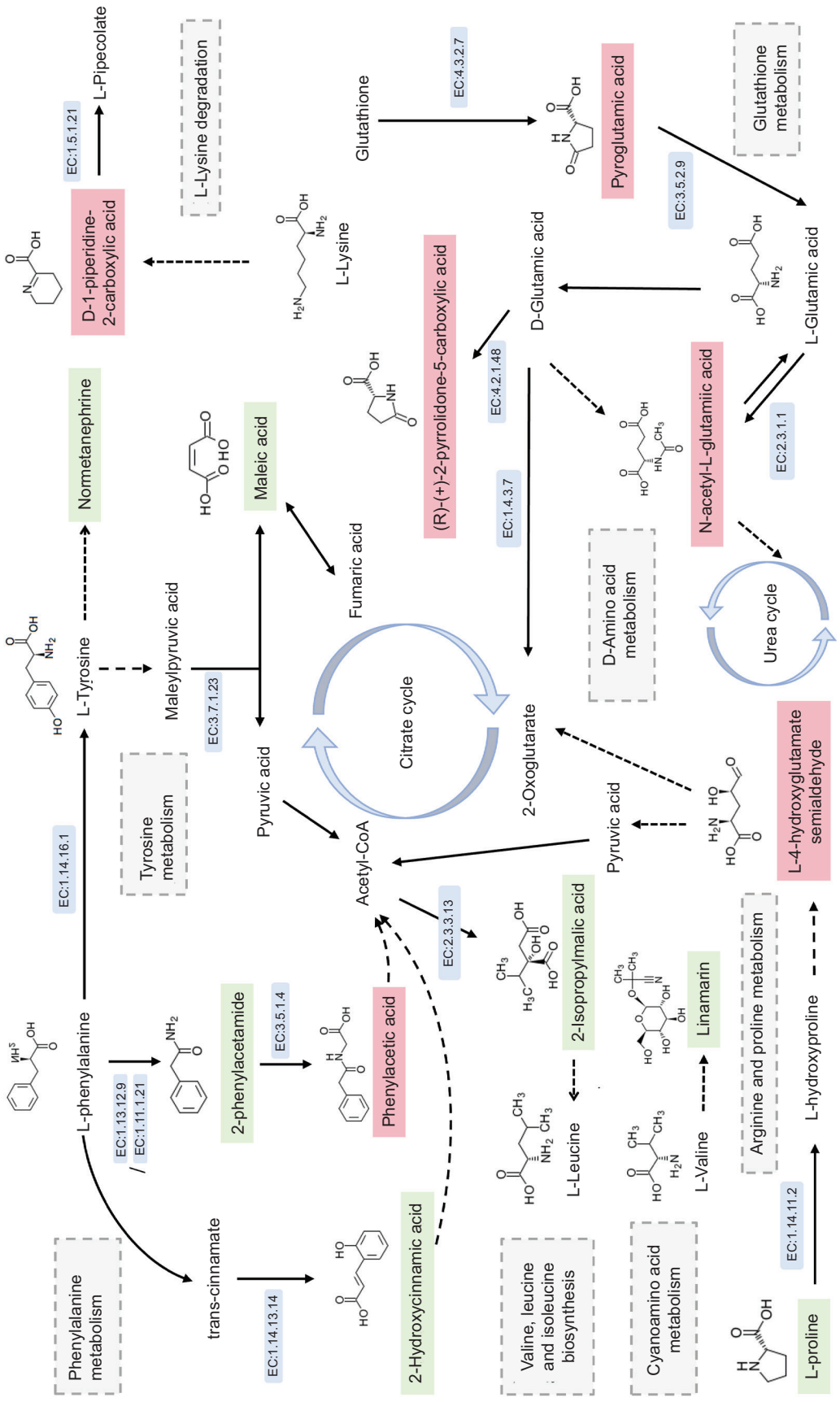


Figure 10. The metabolic pathway network of amino acids based on KEGG analysis. The colored metabolites indicate the identified metabolites, with red indicating increased abundance and green indicating decreased abundance. Dashed arrows represent multi-step conversion, while solid arrows represent single-step reactions, with adjacent enzymes in blue catalyzing this step.

content of acetic acid and ethanol in fermented milk. Both *Lp. plantarum* K25 and *L. fermentum* 13-1 could significantly reduce the content of bitter amino acids, such as L-Ile, L-Phe, and L-Try, bitter dipeptides, such as Gln-Pro and Leu-Gly in D-Lp sample, and bitter Arg-Pro in D-Lf sample, suggesting the potential of these two strains to reduce bitterness of fermented milk.

Further analysis of metabolomics revealed that *L. fermentum* 13-1 caused more metabolic changes in fermented milk than *Lp. plantarum* K25. Both strains had similar effects on several amino acid metabolism, including phenylalanine, valine, leucine, isoleucine, arginine, and proline, although *Lp. plantarum* K25 promoted lysine degradation, and *L. fermentum* 13-1 enhanced tyrosine metabolism. Moreover, a significant increase in bioactive compounds ($p < 0.05$) was found in fermented milk, for example, Ach associated with cognitive function and medicinal compound poncirin in D-Lp, suggesting the potential of these two strains to improve the functional characteristics of fermented milk. Future studies must focus on the possible synergistic effects of different flavor compounds and consumer perception-based sensory tests to evaluate changes in sensory characteristics. Further, future research should also concentrate on *in vivo* animal models to investigate the functional aspects of fermented milk by LAB strains of different fermentation types.

Conflicts of Interest

The authors declared no conflict of interest.

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Supplementary

D vs D-Lp

Metabolite	Significant	Regulate	KEGG Compound ID	Mode	CAS ID	M/Z	Retention time	Formula	Library ID	HMDB Superclass
Dihydrodigoxin	yes	up	–	pos	09-10-5297	824.4849	3.619167	C41H66O14	HMDB0041879	Lipids and lipid-like molecules
Glutaminyl-Proline	yes	down	–	pos	-	244.129	0.801833	C10H17N3O4	HMDB0028805	Organic acids and derivatives
(R)-(+)-2-Pyrrolidone-5-carboxylic acid	yes	up	C02237	pos	-	130.0494	1.048317	C5H7NO3	HMDB0060262; HMDB0000805	Organic acids and derivatives
N-(1-Deoxy-1-fructosyl)leucine	yes	up	–	pos	34393-18-5	294.1546	1.2893	C12H23NO7	HMDB0037840	Organic acids and derivatives
2-Hydroxycinnamic acid	yes	down	C01772	pos	583-17-5	165.0543	0.822833	C9H8O3	HMDB0134028; HMDB0062655; HMDB0002641	Phenylpropanoids and polyketides
Nummularine A	yes	up	C10011	pos	53947-95-8	648.3797	3.39985	C36H49N5O6	HMDB0029336	Organic acids and derivatives
PE(18:4(6Z,9Z,12Z,15Z)/22:6(4Z,7Z,10Z, 13Z,16Z,19Z))	yes	up	–	pos	-	784.4895	3.671333	C45H70NO8P	HMDB0009210; LMGP02010768	Lipids and lipid-like molecules
Poncirin	yes	up	C09830	pos	14941-08-3	617.189	0.761	C28H34O14	LMPK12140333; HMDB0037487	Phenylpropanoids and polyketides
N-(1-Deoxy-1-fructosyl)phenylalanine	yes	up	–	pos	87251-83-0	328.14	2.131817	C15H21NO7	HMDB0037846	Organic acids and derivatives
Tyrosyl-Valine	yes	down	–	pos	-	263.1389	3.036867	C14H20N2O4	HMDB0029118	Organic acids and derivatives
DL-o-Tyrosine	yes	down	–	pos	-	182.0807	1.109483	C9H11NO3	HMDB0006050	Organic acids and derivatives
Lysyl-Proline	yes	up	–	pos	-	226.1549	1.17065	C11H21N3O3	HMDB0028959	Organic acids and derivatives
Hydroxyprolyl-Alanine	yes	up	–	pos	-	185.0912	1.191133	C8H14N2O4	HMDB0028856	Organic acids and derivatives
3-[(3-Methylbutyl)nitrosoamino]-2-butanone	yes	up	–	pos	71016-15-4	151.1225	1.817767	C9H18N2O2	HMDB0033553	Organooxygen compounds
Methionyl-Hydroxyproline	yes	up	–	pos	-	245.0955	2.0884	C10H18N2O4S	HMDB0028974	Organic acids and derivatives
Prolyl-Alanine	yes	up	–	pos	-	169.0966	2.126433	C8H14N2O3	HMDB0029010	Organic acids and derivatives
1,4'-Bipiperidine-1'-carboxylic acid	yes	up	C16836	pos	-	195.1489	2.446067	C11H20N2O2	HMDB0060336	Organoheterocyclic compounds
Linamarin	yes	down	C01594	pos	554-35-8	280.1375	2.780217	C10H17NO6	HMDB0005008; HMDB0033699	Organoheterocyclic compounds
Isoleucyl-Arginine	yes	up	–	pos	-	270.1922	2.831383	C12H25N5O3	HMDB0028901	Organic acids and derivatives
Prednisone	yes	up	C07370	pos	02-03-1953	422.192	3.38985	C21H26O5	LMST02030180; HMDB0014773	Lipids and lipid-like molecules
7,8-Dihydrovomifoliol 9-[apiosyl-(1->6)-glucoside]	yes	up	–	pos	177261-70-0	562.289	3.5565	C24H40O12	HMDB0029771	Lipids and lipid-like molecules
Permetin A	yes	up	–	pos	71888-70-5	562.3438	3.6925	C54H92N12O12	HMDB0030527	Organic acids and derivatives
Phenylalanyl-Gamma-glutamate	yes	up	–	pos	-	316.1288	3.765333	C14H19N3O4	HMDB0029009	Organic acids and derivatives
Norerythromycin	yes	down	–	pos	-	670.4146	3.806817	C35H63NO13	HMDB0061026	Organic oxygen compounds
CL(8:0/18:2(9Z,11Z)/18:2(9Z,11Z)/18:2 (9Z,11Z))	yes	up	–	pos	-	679.4163	3.984317	C71H126O17P2	HMDB0123884	Lipids and lipid-like molecules
14alpha-Hydroxyixocarpanolide	yes	up	–	pos	107221-65-8	521.3085	4.016317	C28H40O7	HMDB0034334	Lipids and lipid-like molecules
Amaranthussaponin II	yes	up	–	pos	139742-10-2	497.2374	4.1308	C48H74O20	HMDB0041352	Lipids and lipid-like molecules
Dimethyl 3-methoxy-4-oxo-5-(8,11,14-pentadecatrienyl)-2-hexenedioate	yes	up	–	pos	-	904.5123	4.622617	C24H36O6	HMDB0032099	Organic acids and derivatives
Gibberellin A88	yes	up	–	pos	146959-87-7	394.1645	5.571217	C19H22O5	HMDB0039240	Lipids and lipid-like molecules
Ovalicin	yes	up	C09674	pos	19683-98-8	360.1803	5.671717	C16H24O5	HMDB0038120	Organooxygen compounds
PE(17:1(9Z)/0:0)	yes	down	–	pos	-	466.2922	8.06525	C22H44NO7P	LMGP02050008	-
CL(a-13:0/i-19:0/18:2(9Z,11Z)/i-20:0)[rac]	yes	up	–	pos	-	739.5023	8.918033	C79H150O17P2	HMDB0076304	Lipids and lipid-like molecules
CL(8:0/10:0/17:0/19:0)	yes	up	–	pos	-	618.4106	8.3409	C63H122O17P2	HMDB0117818	Lipids and lipid-like molecules
CL(18:0/22:5(4Z,7Z,10Z,13Z,16Z)/18:2(9Z,12Z)/22:5(4Z,7Z,10Z,13Z,16Z))	yes	up	–	pos	-	788.5175	8.1769	C89H150O17P2	HMDB0057295	Lipids and lipid-like molecules
Persicaxanthin	yes	up	–	pos	80952-82-5	385.2701	6.971483	C25H36O3	HMDB0034952	Lipids and lipid-like molecules
Cyclic 6-Hydroxymelatonin	yes	up	–	pos	-	211.0871	5.631383	C13H14N2O3	HMDB0060810	Organoheterocyclic compounds
Phenylacetic acid	yes	up	C07086	pos	103-82-2	314.1391	5.631383	C8H8O2	HMDB0000209	Benzenoids
Ganglioside GD1a (d18:0/25:0)	yes	up	–	pos	-	991.0432	4.878433	C92H165N3O39	HMDB0011789	Lipids and lipid-like molecules
Tragopogonsaponin L	yes	up	–	pos	-	897.5081	4.694433	C50H74O15	HMDB0037926	Lipids and lipid-like molecules
Quinquenoside II	yes	down	–	pos	208764-52-7	628.3416	4.48995	C62H104O24	HMDB0032815	Lipids and lipid-like molecules
Ganglioside GM2 (d18:0/24:0)	yes	up	–	pos	-	745.4557	4.05865	C74H134N2O26	HMDB0011904	Lipids and lipid-like molecules
Simonin IV	yes	up	–	pos	151310-53-1	672.4087	3.84815	C68H120O24	HMDB0029977	Organic oxygen compounds

HMDB Class	HMDB Subclass	VIP_pred_ OPLS-DA	VIP_PLS-DA	FC(D_Lp/D)	P_value	FDR	D-Lp1_1	D-Lp1_2	D-Lp1_3	D_1	D_2	D_3
Steroids and steroid derivatives	Steroid lactones	1.4951	1.494	1.2553	8.77E-05	0.001111	4.3313	4.3489	4.4325	3.5544	3.4939	3.3975
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.3408	2.3399	0.2363	5.43E-07	4.85E-05	0.7066	0.6654	0.6386	2.8805	2.8529	2.7739
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.4963	1.4948	1.3741	4.43E-05	0.000708	3.2522	3.2372	3.2978	2.4339	2.398	2.2914
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.7263	1.7257	1.3951	1.38E-05	0.000325	4.1141	4.1526	4.2424	2.9601	3.041	2.9656
Cinnamic acids and derivatives	Hydroxycinnamic acids and derivatives	2.2291	2.2203	0.324	0.01202	0.04243	0.0509	1.4303	1.5993	3.1903	3.2045	3.1166
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.0392	2.0379	1.532	4.99E-06	0.00018	4.6926	4.7149	4.8024	3.1501	3.102	3.0239
Glycerophospholipids	Glycerophosphoethanolamines	1.8972	1.8965	1.5055	3.66E-06	0.000155	4.2	4.2069	4.315	2.7988	2.8447	2.8068
Flavonoids	Flavonoid glycosides	1.1407	1.1413	1.2053	0.001342	0.007784	3.1105	3.1326	3.1078	2.4655	2.5996	2.6933
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.4382	1.4366	1.2301	0.000389	0.003206	4.3998	4.3901	4.5064	3.6657	3.6712	3.4717
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.7645	1.7633	0.7294	3.52E-05	0.000607	3.2984	3.2576	3.43	4.6152	4.5707	4.5052
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.7156	1.7138	0.6847	9.17E-05	0.001143	2.4442	2.5274	2.6588	3.7319	3.7701	3.6388
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.8727	1.8713	2.0637	1.47E-06	9.28E-05	2.6746	2.7462	2.6463	1.3044	1.3171	1.2886
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.8938	1.894	2.2044	5.16E-05	0.000785	2.5769	2.5911	2.6626	1.0764	1.1525	1.3231
Carbonyl compounds	Ketones	2.5545	2.5529	587.5256	1.51E-09	8.35E-06	2.5802	2.563	2.5985	0.0044	0.0045	0.0042
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.8735	1.8715	3.5585	0.008182	0.03152	2.0931	1.7281	2.4227	0.3572	1.0492	0.3479
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.8566	1.8549	1.8013	0.001687	0.009295	3.1523	3.0635	3.272	2.111	1.5796	1.5779
Piperidines	Piperidinecarboxylic acids and derivatives	1.1555	1.156	1.2412	0.000284	0.002541	2.6704	2.7688	2.8208	2.2188	2.2321	2.2023
Benzimidazoles	Sulfinylbenzimidazoles	1.8851	1.8837	0.5873	2.36E-05	0.000461	1.9646	1.9428	2.1045	3.4391	3.4591	3.3386
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.8107	1.8078	2.5792	8.11E-05	0.001058	2.265	2.1197	1.9913	0.8248	0.8362	0.8107
Steroids and steroid derivatives	Hydroxysteroids	2.2516	2.2504	2.9494	3.82E-07	4.10E-05	3.0042	2.9932	3.0907	1.0276	1.0397	1.0125
Fatty Acyls	Fatty acyl glycosides	1.428	1.4298	1.8308	0.001606	0.008956	1.6462	1.8489	2.0256	1.0058	1.0179	0.9908
Peptidomimetics	Depsipeptides	1.2005	1.2006	1.1825	0.00011	0.00128	3.6735	3.7074	3.797	3.1525	3.1658	3.1358
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.1883	2.1864	2.4985	0.001929	0.01025	3.2305	3.2527	3.3198	1.0467	1.8466	1.0316
Organooxygen compounds	Carbohydrates and carbohydrate conjugates	1.413	1.4151	0.2907	0.006133	0.0253	0.2917	0.4591	0.2879	1.2729	1.3996	0.9014
Glycerophospholipids	Glycerophosphoglycerophosphoglycerols	2.5503	2.5488	2.9164	7.33E-08	2.19E-05	3.8734	3.8933	3.9585	1.341	1.3537	1.3251
Steroids and steroid derivatives	Steroid lactones	1.0286	1.0287	1.2667	0.001262	0.007441	2.0267	1.9677	2.1462	1.6173	1.6303	1.6011
Prenol lipids	Terpene glycosides	1.1569	1.1573	1.2055	0.000388	0.0032	3.0862	3.1339	3.2484	2.6192	2.6325	2.6026
Keto acids and derivatives	Beta-keto acids and derivatives	1.2255	1.2257	1.2669	0.000163	0.00168	2.784	2.9029	2.8711	2.2065	2.2943	2.2567
Prenol lipids	Terpene lactones	1.7839	1.7846	2.8456	0.001967	0.01038	2.0561	2.0376	1.9449	0.784	0.3682	0.97
Carbonyl compounds	Ketones	2.174	2.1719	4.6344	7.43E-06	0.000218	2.4002	2.342	2.4068	0.4961	0.6241	0.4223
-	-	1.5309	1.5304	0.2716	0.006922	0.0277	0.6095	0.2584	0.2432	1.2715	1.1578	1.6637
Glycerophospholipids	Glycerophosphoglycerophosphoglycerols	1.7646	1.7615	2.2528	0.00013	0.001432	2.3941	2.1624	2.1222	0.9891	1.0011	0.9742
Glycerophospholipids	Glycerophosphoglycerophosphoglycerols	1.7846	1.7814	2.0059	0.007894	0.03061	3.0732	2.7288	2.2929	1.2645	1.1221	1.6469
Glycerophospholipids	Glycerophosphoglycerophosphoglycerols	1.5002	1.495	1.4876	0.00622	0.02556	3.2098	2.8471	2.5924	1.9393	1.9525	1.9228
Prenol lipids	Diterpenoids	1.3584	1.3603	2.6599	0.002628	0.01297	0.9969	1.3642	1.3091	0.4604	0.4692	0.4496
Indoles and derivatives	Pyrroloindoles	1.9765	1.9762	10.6634	0.00054	0.00404	1.8253	1.7666	1.6171	0.0196	0.0202	0.4486
Benzene and substituted derivatives	-	2.0235	2.0234	12.9736	8.60E-05	0.001097	1.6721	1.6595	1.9684	0.1364	0.1401	0.132
Sphingolipids	Glycosphingolipids	1.4706	1.4711	1.4319	0.000751	0.005062	2.8036	2.9119	2.9888	1.8937	2.1623	2.0231
Prenol lipids	Triterpenoids	1.0553	1.057	1.1272	0.003956	0.01784	4.0013	4.1884	4.2002	3.5863	3.7387	3.6678
Prenol lipids	Triterpenoids	1.0947	1.0923	0.8787	0.003822	0.01734	3.488	3.6431	3.6443	3.9682	4.1866	4.1103
Sphingolipids	Glycosphingolipids	2.6266	2.625	3.2408	2.36E-06	0.000118	3.8991	3.9289	4.0044	1.3379	1.1687	1.145
Organooxygen compounds	Carbohydrates and carbohydrate conjugates	2.905	2.9039	4.0466	9.89E-07	7.21E-05	4.3792	4.4205	4.4922	0.9906	1.0998	1.1934

D vs D-Lp

Metabolite	Significant	Regulate	KEGG Compound ID	Mode	CAS ID	M/Z	Retention time	Formula	Library ID	HMDB Superclass
L-Pyridosine	yes	up	–	pos	31489-08-4	277.118	3.682333	C12H18N2O4	HMDB0029443	Organic acids and derivatives
Capsianside B	yes	down	–	pos	121924-07-0	783.4093	3.661333	C76H124O33	HMDB0030733	Lipids and lipid-like molecules
Glutamylisoleucine	yes	up	–	pos	5879-22-1	243.1336	3.5455	C11H20N2O5	HMDB0028822	Organic acids and derivatives
L-phenylalanyl-L-hydroxyproline	yes	up	–	pos	-	261.1231	3.473183	C14H18N2O4	HMDB0011176	Organic acids and derivatives
Schizonepetoside E	yes	up	C17638	pos	-	349.1846	3.462517	C16H28O8	-	-
Methionyl-Proline	yes	up	–	pos	-	229.1001	3.27585	C10H18N2O3S	HMDB0028981	Organic acids and derivatives
Isoleucyl-Hydroxyproline	yes	up	–	pos	-	227.1386	3.27585	C11H20N2O4	HMDB0028908	Organic acids and derivatives
Mabioside C	yes	down	–	pos	156980-54-0	856.4407	3.24535	C42H64O14	HMDB0040653	Lipids and lipid-like molecules
Vinaginsenoside R1	yes	up	–	pos	156980-41-5	433.2516	3.172367	C44H74O15	HMDB0034624	Lipids and lipid-like molecules
54-Deoxyciguatoxin	yes	up	–	pos	139341-09-6	559.2915	3.088367	C60H86O18	HMDB0029781	Phenylpropanoids and polyketides
Hoduloside X	yes	up	–	pos	154971-14-9	547.2984	3.0677	C53H88O23	HMDB0040662	Lipids and lipid-like molecules
Tyrosyl-Proline	yes	down	–	pos	-	557.2608	2.780217	C14H18N2O4	HMDB0029113	Organic acids and derivatives
Uzarigenin 3-[xylosyl-(1->2)-rhamnoside]	yes	down	–	pos	255861-29-1	338.1704	2.5584	C34H52O12	HMDB0036296	Lipids and lipid-like molecules
Hydroxyprolyl-Proline	yes	up	–	pos	-	211.1072	1.919267	C10H16N2O4	HMDB0028871	Organic acids and derivatives
L-Proline	yes	down	C00148	pos	147-85-3	116.0703	1.807933	C5H9NO2	HMDB0000162	Organic acids and derivatives
Pyridinoline	yes	down	–	pos	63800-01-1	429.1981	1.3408	C18H28N4O8	HMDB0000851	Organic acids and derivatives
L-Isoleucine	yes	down	C00407	pos	73-32-5	132.1016	1.2998	C6H13NO2	HMDB0000172; HMDB0000557	Organic acids and derivatives
Glutamylalanine	yes	up	–	pos	21064-18-6	201.0861	1.258967	C8H14N2O5	HMDB0003764	Organic acids and derivatives
2-Phenylacetamide	yes	down	C02505	pos	103-81-1	136.0756	1.12015	C8H9NO	HMDB0010715	Benzenoids
N-Benzylformamide	yes	down	C15561	pos	-	136.0755	0.833167	C8H9NO	-	-
Rac-4-Hydroxy-4-O-(beta-D-glucuronide)-all-trans-retinyl acetate	yes	down	–	pos	-	559.2347	0.811833	C28H40O9	HMDB0060141	Lipids and lipid-like molecules
Hydroxyprolyl-Hydroxyproline	yes	down	–	pos	-	245.1132	0.811833	C10H16N2O5	HMDB0028864	Organic acids and derivatives
Acetylcholine	yes	up	C01996	pos	51-84-3	146.1172	0.750833	C7H15NO2	HMDB0000895	Organic nitrogen compounds
Vignatic acid A	yes	up	–	neg	181485-19-8	552.2672	3.669033	C30H39N3O7	HMDB0033599	Organic acids and derivatives
Pyroglutamylvaline	yes	up	–	neg	21282-10-0	227.1032	3.10265	C10H16N2O4	HMDB0094651	Organic acids and derivatives
Ganoderenic acid D	yes	up	–	neg	100665-43-8	557.2579	3.834317	C30H40O7	HMDB0036059	Lipids and lipid-like molecules
D-(+)-3-Phenyllactic acid	yes	up	–	neg	-	165.056	3.985167	C9H10O3	-	-
6-Acetyl-2,3-dihydro-2-(hydroxymethyl)-4(1H)-pyridinone	yes	up	–	neg	214218-63-0	190.0538	3.083317	C8H11NO3	HMDB0035178	Organoheterocyclic compounds
Austalide B	yes	up	–	neg	81543-02-4	473.2039	4.85575	C26H34O8	HMDB0030004	Organoheterocyclic compounds
Pyroglutamic acid	yes	up	C01879; C02237	neg	98-79-3	128.035	1.065783	C5H7NO3	HMDB0000267; HMDB0060262; HMDB0000805	Organic acids and derivatives
D-1-Piperideine-2-carboxylic acid	yes	up	C04092	neg	2756-89-0	172.061	1.734133	C6H9NO2	HMDB0001084	Organoheterocyclic compounds
Megestrol	yes	up	C07120	neg	3562-63-8	363.1749	1.792967	C21H28O3	LMST02030177; HMDB0014495	Lipids and lipid-like molecules
4-Oxo-13-cis-retinoate	yes	up	–	neg	-	349.1607	1.851983	C20H26O3	HMDB0012789	Lipids and lipid-like molecules
Jubanine A	yes	down	–	neg	60375-07-7	716.3469	3.073417	C40H49N5O6	HMDB0030205	Organic acids and derivatives
Koryoginsenoside R1	yes	down	–	neg	171674-97-8	903.4839	3.092783	C46H76O15	HMDB0041351	Lipids and lipid-like molecules
Gamma-Glutamylphenylalanine	yes	up	–	neg	7432-24-8	293.1145	3.131967	C14H18N2O5	HMDB0000594	Organic acids and derivatives
2-Isopropylmalic acid	yes	down	C02504	neg	3237-44-3	175.0609	3.238817	C7H12O5	HMDB0000402	Lipids and lipid-like molecules
Licoricesaponin K2	yes	down	–	neg	-	803.3805	3.248317	C42H62O16	HMDB0039250	Lipids and lipid-like molecules
Hoduloside VIII	yes	down	–	neg	154971-12-7	951.469	3.53145	C46H76O18	HMDB0040660	Lipids and lipid-like molecules
Gamma-Glutamyltryptophan	yes	up	–	neg	66471-20-3	314.114	3.786417	C16H19N3O5	HMDB0029160	Organic acids and derivatives

HMDB Class	HMDB Subclass	VIP_pred_ OPLS-DA	VIP_PLS-DA	FC(D_Lp/D)	P_value	FDR	D-Lp1_1	D-Lp1_2	D-Lp1_3	D_1	D_2	D_3
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.5261	1.5255	1.4373	6.30E-06	0.000199	2.9934	3.005	3.0834	2.1066	2.1199	2.0901
Fatty Acyls	Fatty acyl glycosides	1.5491	1.5475	0.7449	0.000205	0.002012	2.6947	2.7983	2.8982	3.8132	3.7849	3.6667
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.2674	2.2679	2.3573	0.000234	0.002181	3.5481	3.5641	3.6168	1.2293	1.5294	1.7938
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.9651	1.9673	2.106	0.00129	0.007536	2.9429	2.9803	3.0795	1.3064	1.17	1.7998
-	-	1.7584	1.758	2.158	7.05E-06	0.000211	2.2074	2.2943	2.3427	1.0582	1.0704	1.0431
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.0574	2.0564	2.4481	3.22E-06	0.000142	2.7851	2.8296	2.8741	1.2281	1.1035	1.1356
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.0222	2.0274	2.3894	0.01532	0.05015	3.0576	3.1386	3.1375	0.9466	0.774	2.1856
Prenol lipids	Terpene glycosides	1.2574	1.2566	0.8618	0.000486	0.003768	3.9219	3.9259	4.0485	4.6603	4.6302	4.5129
Prenol lipids	Terpene glycosides	1.378	1.3784	1.4067	0.007597	0.02973	2.7845	2.7358	2.8738	1.6971	2.2367	2.0333
Ciguatera toxins	-	1.4321	1.4331	2.5644	0.00042	0.003401	1.2022	1.4093	1.4483	0.5283	0.5378	0.5167
Prenol lipids	Triterpenoids	2.4091	2.4075	3.0194	2.91E-07	3.82E-05	3.4309	3.3733	3.4769	1.1272	1.166	1.1119
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.0343	2.0343	0.4709	2.81E-05	0.000525	1.5017	1.4594	1.4262	3.2157	3.1345	2.9659
Steroids and steroid derivatives	Steroid lactones	2.1551	2.1518	0.2026	0.004433	0.01947	0.1867	0.1719	1.1163	2.6634	2.2789	2.3379
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.6788	1.6804	2.0259	0.005983	0.02478	2.4004	1.984	2.6609	1.2944	0.9526	1.2304
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.3947	2.3933	0.1333	1.78E-08	1.43E-05	0.371	0.3464	0.3278	2.5927	2.631	2.6144
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.8602	1.8583	0.6266	0.000257	0.002349	2.2253	2.2091	2.5279	3.7541	3.7445	3.6133
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.4068	1.4057	0.8133	0.000123	0.00138	3.3758	3.4156	3.5053	4.252	4.263	4.1463
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.5164	1.518	1.6589	0.001333	0.007739	2.2847	2.3482	2.4586	1.2261	1.4603	1.5883
Benzene and substituted derivatives	Phenylacetamides	2.3969	2.3956	0.232	1.40E-05	0.000326	0.6373	0.8458	0.5784	3.0226	2.9691	2.8956
-	-	1.9846	1.9842	0.4756	1.15E-05	0.000283	1.4654	1.4635	1.3152	3.0343	2.9564	2.935
Prenol lipids	Terpene glycosides	1.7572	1.7565	0.6599	2.56E-06	0.000121	2.4144	2.3118	2.38	3.6049	3.5883	3.5768
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.5928	1.5918	0.7014	4.54E-06	0.000174	2.3243	2.3969	2.3539	3.3759	3.3883	3.3206
Organonitrogen compounds	Quaternary ammonium salts	1.182	1.1815	1.1804	5.43E-05	0.000811	3.5876	3.6453	3.6604	3.1164	3.066	3.0454
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.1539	2.1509	3.0281	0.000109	0.001493	1.9426	2.0015	2.2346	0.6697	0.6585	0.7125
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.0925	2.0979	1.7731	0.000166	0.001933	3.0025	2.9869	3.0594	1.7786	1.8108	1.5151
Prenol lipids	Triterpenoids	1.8445	1.8457	1.4517	3.48E-05	0.000706	3.2031	3.2266	3.3186	2.3045	2.1851	2.2232
-	-	1.3007	1.3024	1.1228	4.60E-06	0.000201	4.606	4.5772	4.5912	4.1138	4.0754	4.0784
Pyridines and derivatives	Hydropyridines	1.3024	1.2825	1.3431	0.04999	0.1525	2.2709	2.3478	2.4048	1.3202	1.9816	1.9269
Benzopyrans	1-benzopyrans	1.3016	1.3002	1.2423	4.78E-05	0.000861	2.5889	2.5295	2.6044	2.0513	2.0652	2.0988
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.7331	2.7333	3.8747	1.94E-05	0.000479	3.091	2.838	3.0327	0.6956	0.8782	0.739
Pyridines and derivatives	Hydropyridines	1.0683	1.0792	1.1829	0.03046	0.1017	2.5229	2.5638	2.7302	2.355	2.252	2.0007
Steroids and steroid derivatives	Pregnane steroids	1.824	1.8213	1.8595	3.54E-05	0.000708	2.0618	2.1161	2.2148	1.1334	1.1201	1.1834
Prenol lipids	Retinoids	2.5056	2.5028	8.6879	1.64E-05	0.000438	1.9498	2.1893	2.1589	0.2358	0.2298	0.2591
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.1374	1.1304	0.8434	0.03079	0.1027	2.3511	2.1949	2.5938	2.9147	2.8663	2.686
Prenol lipids	Triterpenoids	1.0131	1.0056	0.9291	0.008124	0.03543	4.223	4.1691	4.3106	4.6447	4.5609	4.4659
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.2955	2.3037	2.6647	0.000493	0.004075	2.5054	2.553	2.6131	1.2602	0.7805	0.838
Fatty Acyls	Fatty acids and conjugates	1.083	1.0786	0.9182	0.001071	0.00724	3.9437	3.9277	4.009	4.3661	4.3234	4.251
Prenol lipids	Terpene glycosides	2.1922	2.1951	0.2725	0.000904	0.006305	0.3932	0.3805	0.8675	2.0387	1.9275	2.0546
Prenol lipids	Triterpenoids	1.1057	1.0995	0.8672	0.01685	0.06335	2.5554	2.5212	2.7552	3.0885	3.0737	2.8704
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.8486	2.8472	3.6676	9.80E-08	2.90E-05	3.2724	3.2855	3.3278	0.8836	0.8711	0.9405

D vs D-Lp

Metabolite	Significant	Regulate	KEGG Compound ID	Mode	CAS ID	M/Z	Retention time	Formula	Library ID	HMDB Superclass
5,9,11-trihydroxyprosta-6E,14Z-dien-1-oate	yes	up	–	neg	80998-07-8	584.2476	3.824967	C30H37NO8	HMDB0062413	Lipids and lipid-like molecules
2-Hepteneoylglycine	yes	down	–	neg	-	206.0815	4.118383	C9H15NO3	HMDB0094728	Organic acids and derivatives
Lucidenic acid N	yes	up	–	neg	364622-33-3	495.2539	4.539633	C27H40O6	HMDB0038352	Lipids and lipid-like molecules
Neuromedin N	yes	up	C15868	neg	102577-25-3	654.3275	5.166483	C32H51N5O7	HMDB0013022	Organic acids and derivatives
Simvastatin	yes	up	–	neg	79902-63-9	463.2665	5.389917	C25H38O5	HMDB0005007	Organoheterocyclic compounds
1-(beta-D-Glucopyranosyloxy)-3-octanone	yes	up	–	neg	194919-40-9	341.1343	5.56715	C14H26O7	HMDB0031315	Lipids and lipid-like molecules
Homocapsaicin	yes	up	C20215	neg	58493-48-4	364.2116	6.375883	C19H29NO3	HMDB0036329	Benzenoids
3-trans-Caffeoyltormentic acid	yes	up	–	neg	144604-16-0	671.3502	7.393667	C39H54O8	HMDB0040650	Lipids and lipid-like molecules
PS(18:4(6Z,9Z,12Z,15Z)/18:4(6Z,9Z,12Z,15Z))	yes	up	–	neg	-	796.4245	5.059	C42H66NO10P	HMDB0112496; LMGP03010441	Lipids and lipid-like molecules
Pubesenolide	yes	up	–	neg	98569-64-3	493.2765	5.01	C28H42O5	HMDB0033728	Lipids and lipid-like molecules
Neoporrigenin B	yes	up	–	neg	196607-74-6	481.2757	4.980767	C27H42O5	HMDB0032680	Lipids and lipid-like molecules
Ganoderic acid C2	yes	up	–	neg	98296-48-1	555.2682	4.95125	C30H46O7	HMDB0035304	Lipids and lipid-like molecules
Esculentoside E	yes	up	–	neg	65649-36-7	649.356	4.597833	C35H54O11	HMDB0034635	Lipids and lipid-like molecules
Dihydrozeatin-9-N-glucoside-O-glucoside	yes	up	–	neg	-	580.2053	4.386333	C22H35N5O11	HMDB0012212	Lipids and lipid-like molecules
Tyramine glucuronide	yes	up	C03033	neg	27972-85-6	294.0979	4.17725	C14H19NO7	HMDB0010328	Organic oxygen compounds
3-hydroxyhexanoyl carnitine	yes	up	–	neg	-	334.1862	3.937833	C14H27NO5	HMDB0061633	Lipids and lipid-like molecules
Blasticidin S	yes	up	C02010	neg	2079-00-7	403.1829	3.862633	C17H26N8O5	HMDB0030452	Organooxygen compounds
Acetyl-DL-Leucine	yes	up	C02710	neg	-	172.0976	3.824967	C8H15NO3	HMDB0011756	Organic acids and derivatives
N-(1-Deoxy-1-fructosyl)histidine	yes	up	–	neg	25020-13-7	338.0981	3.688683	C12H19N3O7	HMDB0037839	Organic acids and derivatives
PS(MonoMe(11,3)/MonoMe(9,5))	yes	down	–	neg	-	876.4386	3.669033	C44H74NO12P	HMDB0061605	Lipids and lipid-like molecules
Ganosporelactone A	yes	down	–	neg	138008-04-5	547.2438	3.610117	C30H40O7	HMDB0036406	Lipids and lipid-like molecules
Bilastine	yes	up	–	neg	202189-78-4	498.2543	3.4824	C28H37N3O3	HMDB0240232	Organoheterocyclic compounds
Saponin E	yes	down	–	neg	85191-73-7	817.4346	3.472767	C42H68O14	HMDB0035958	Lipids and lipid-like molecules
Digitoxin	yes	down	C06955	neg	71-63-6	785.4064	3.364417	C41H64O13	HMDB0015468; LMST01120018	Lipids and lipid-like molecules
Benzoquinoneacetic acid	yes	down	–	neg	10275-07-7	165.0189	3.3453	C8H6O4	HMDB0002334	Organic oxygen compounds
Testosterone sulfate	yes	up	–	neg	651-45-6	413.1664	3.287133	C19H28O5S	LMST05020032; HMDB0002833	Lipids and lipid-like molecules
Isosakuranin	yes	up	–	neg	491-69-0	447.1327	3.277517	C22H24O10	LMPK12140336; HMDB0029481	Phenylpropanoids and polyketides
Glycinoeclepin B	yes	up	–	neg	103847-17-2	593.2555	3.10265	C31H42O9	HMDB0037035	Lipids and lipid-like molecules
3,4',5-Trihydroxy-3',7-dimethoxyflavanone	yes	down	–	neg	37971-67-8	331.085	3.005333	C17H16O7	HMDB0037503	Phenylpropanoids and polyketides
CDP-DG(16:0/20:4(8Z,11Z,14Z,17Z))	yes	down	–	neg	-	982.4909	3.005333	C48H81N3O15P2	HMDB0006974; LMGP13010009	Lipids and lipid-like molecules
(4S,6R)-p-Mentha-1,8-diene-6,7-diol 7-glucoside	yes	up	–	neg	402593-54-8	365.1343	2.900617	C16H26O7	HMDB0039056	Lipids and lipid-like molecules
Alliospiroside D	yes	down	–	neg	114317-58-7	775.3863	2.880733	C39H62O14	HMDB0030915	Lipids and lipid-like molecules
(S)-Nerolidol 3-O-[a-L-rhamnopyranosyl-(1->2)-b-D-glucopyranoside]	yes	down	–	neg	130466-30-7	575.3047	2.8711	C27H46O10	HMDB0040844	Lipids and lipid-like molecules
Methylprednisolone	yes	down	–	neg	83-43-2	395.1809	2.790333	C22H30O5	HMDB0015094	Lipids and lipid-like molecules
Prolylhydroxyproline	yes	down	–	neg	18684-24-7	273.1086	2.170683	C10H16N2O4	HMDB0006695; HMDB0029018	Organic acids and derivatives
Beta-D-3-Ribofuranosyluric acid	yes	down	C05513	neg	2124-54-1	299.0627	2.0088	C10H12N4O7	HMDB0029920	Organoheterocyclic compounds
Blumenol C glucoside	yes	up	–	neg	62512-23-6	393.1867	1.4248	C19H32O7	HMDB0040668	Lipids and lipid-like molecules
N-(1-Deoxy-1-fructosyl)isoleucine	yes	up	–	neg	87304-79-8	292.1396	1.321317	C12H23NO7	HMDB0039780	Organic acids and derivatives
L-4-Hydroxyglutamate semialdehyde	yes	up	C05938	neg	-	128.0352	0.842117	C5H9NO4	HMDB0006556	Organic acids and derivatives
Neuraminic acid	yes	down	C06469	neg	114-04-5	266.0873	0.690617	C9H17NO8	HMDB0000830	Organic oxygen compounds

HMDB Class	HMDB Subclass	VIP_pred_ OPLS-DA	VIP_PLS-DA	FC(D_Lp/D)	P_value	FDR	D-Lp1_1	D-Lp1_2	D-Lp1_3	D_1	D_2	D_3
Prenol lipids	Diterpenoids	1.4643	1.4619	1.3326	0.000529	0.004261	2.5539	2.4917	2.6945	1.9232	1.9091	1.9763
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.4209	1.4174	0.7634	9.60E-05	0.001387	1.9924	1.8785	1.9291	2.57	2.5094	2.5164
Prenol lipids	Triterpenoids	1.6388	1.6405	1.4966	0.000135	0.001703	2.3381	2.4699	2.4516	1.6916	1.5852	1.5735
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.1004	2.0996	3.2233	1.62E-05	0.000436	1.9077	1.8016	1.9764	0.578	0.5675	0.6181
Lactones	Delta valerolactones	1.455	1.4593	1.4331	0.000433	0.003715	2.1423	2.0349	2.1852	1.5246	1.5158	1.401
Fatty Acyls	Fatty acyl glycosides	1.4073	1.412	1.6109	0.00037	0.003366	1.6446	1.4872	1.6031	1.0254	0.9778	0.9357
Benzene and substituted derivatives	Phenols and derivatives	2.1982	2.2078	13.5415	0.000469	0.003903	1.8003	1.5042	1.4442	0.2895	0.0245	0.0367
Prenol lipids	Triterpenoids	1.4254	1.4156	1.5181	0.002405	0.01325	1.7878	1.7775	1.8599	1.02	1.2552	1.2987
Glycerophospholipids	Glycerophosphoserines	1.9715	1.9705	2.4226	1.19E-06	0.000104	1.9633	1.924	1.9809	0.7962	0.7842	0.8417
Steroids and steroid derivatives	Steroid lactones	2.6952	2.7014	13.8826	8.65E-05	0.001302	2.3153	2.305	2.4063	0.4284	0.0103	0.0675
Steroids and steroid derivatives	Steroidal glycosides	1.671	1.6688	1.4031	1.95E-05	0.000479	2.8433	2.8501	2.9323	2.0361	2.0219	2.0893
Prenol lipids	Triterpenoids	1.0168	1.0158	1.1689	0.004287	0.02085	2.2285	2.1625	2.3128	1.8728	1.9799	1.884
Prenol lipids	Triterpenoids	1.3948	1.3936	2.1993	0.01112	0.04515	0.9617	1.1297	1.3647	0.4995	0.67	0.4018
Fatty Acyls	Fatty acyl glycosides	2.886	2.8852	1197471	2.89E-07	4.82E-05	2.4131	2.4388	2.5331	0	0	0
Organooxygen compounds	Carbohydrates and carbohydrate conjugates	1.4016	1.3934	1.3821	0.001126	0.007448	2.1231	2.1737	2.1059	1.4223	1.5538	1.6562
Fatty Acyls	Fatty acid esters	1.4319	1.434	1.9226	0.005216	0.02438	1.553	1.3166	1.1678	0.643	0.7027	0.7548
Carbohydrates and carbohydrate conjugates	Sugar acids and derivatives	2.3564	2.3564	2.0505	1.43E-06	0.000114	3.1634	3.1813	3.2749	1.5853	1.5573	1.5484
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.7136	1.7158	1.4545	5.13E-05	0.000911	2.7937	2.7455	2.8619	1.9504	1.9661	1.8586
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.6615	1.6596	1.5237	2.72E-05	0.00059	2.3603	2.3325	2.4396	1.5471	1.5331	1.5993
Glycerophospholipids	Glycerophosphoserines	1.7314	1.7307	0.7391	0.000448	0.003801	2.4338	2.5388	2.6877	3.4979	3.495	3.3695
Steroids and steroid derivatives	Steroid lactones	1.0779	1.0751	0.8818	0.000322	0.003079	2.5717	2.5633	2.6296	2.9746	2.93	2.9002
Benzimidazoles	-	1.51	1.5066	1.4619	0.000159	0.001892	2.0717	2.1399	2.2266	1.4555	1.4417	1.5074
Prenol lipids	Terpene glycosides	1.2408	1.2348	0.884	0.003464	0.01771	3.5561	3.5715	3.6866	4.1567	4.1277	3.9504
Steroids and steroid derivatives	Steroid lactones	1.4548	1.4556	0.6764	0.000221	0.002344	1.3395	1.3577	1.2773	1.977	1.876	2.0235
Organooxygen compounds	Carbonyl compounds	1.3285	1.3175	0.7763	0.02584	0.08993	2.3755	1.8591	1.9169	2.7421	2.5801	2.6035
Steroids and steroid derivatives	Sulfated steroids	1.9307	1.929	2.17	1.79E-05	0.00046	2.0267	1.9872	2.1286	0.9321	0.9195	0.9798
Flavonoids	Flavonoid glycosides	1.8457	1.8499	1.6189	0.001425	0.00892	2.6704	2.7776	2.7436	1.6956	1.9058	1.4584
Prenol lipids	Sesquiterpenoids	2.4735	2.4709	4.3932	9.77E-06	0.000315	2.2342	2.3639	2.4293	0.4865	0.5895	0.5234
Flavonoids	O-methylated flavonoids	1.4637	1.4663	0.6884	0.002065	0.01166	1.5929	1.4174	1.3629	1.9946	2.1827	2.1764
Glycerophospholipids	CDP-glycerols	1.2999	1.2964	0.8995	0.000659	0.004948	4.5024	4.5324	4.6071	5.1062	5.0879	4.9699
Prenol lipids	Terpene glycosides	2.0815	2.0857	1.878	0.000252	0.002545	2.7043	2.7781	2.8783	1.6668	1.3602	1.4254
Steroids and steroid derivatives	Steroidal glycosides	1.4143	1.4088	0.7734	0.006459	0.02927	2.0915	2.0064	2.3444	2.8794	2.7913	2.656
Fatty Acyls	Fatty acyl glycosides	1.5405	1.5371	0.8351	0.000823	0.005881	3.5403	3.5937	3.7266	4.4044	4.3773	4.2238
Steroids and steroid derivatives	Hydroxysteroids	2.4689	2.4661	0.1791	1.39E-06	0.000114	0.4451	0.3498	0.3826	2.2438	2.184	2.1479
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.9978	2.004	0.4076	0.007823	0.03439	0.4302	0.895	1.3149	2.2082	2.1923	2.0758
Imidazopyrimidines	Purines and purine derivatives	2.4684	2.4687	0.4457	0.001708	0.01019	1.4209	1.1131	1.9636	3.3853	3.3751	3.33
Fatty Acyls	Fatty acyl glycosides	1.1048	1.104	1.2195	0.001114	0.007399	2.0926	2.0985	1.9777	1.6732	1.6591	1.7258
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.2313	1.2259	1.1846	0.002629	0.01421	2.8518	3.0778	3.0018	2.5004	2.4861	2.5539
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.7671	1.764	1.5088	0.002259	0.01259	2.6927	2.7827	3.0377	1.7889	2.0633	1.7907
Organooxygen compounds	Carbohydrates and carbohydrate conjugates	1.3763	1.3707	0.7939	0.001492	0.009202	2.1582	2.284	2.1911	2.867	2.8274	2.6593

D vs D-Lf

Metabolite	Significant	Regulate	KEGG Compound ID	Mode	CAS ID	M/Z	Retention time	Formula	Library ID	HMDB Superclass
Dihydrodigoxin	yes	up	–	pos	09-10-5297	824.4849	3.619167	C41H66O14	HMDB0041879	Lipids and lipid-like molecules
Glutaminyl-Proline	yes	down	–	pos	-	244.129	0.801833	C10H17N3O4	HMDB0028805	Organic acids and derivatives
(R)-(+)-2-Pyrrolidone-5-carboxylic acid	yes	up	C02237	pos	-	130.0494	1.048317	C5H7NO3	HMDB0060262; HMDB0000805	Organic acids and derivatives
N-(1-Deoxy-1-fructosyl)leucine	yes	up	–	pos	34393-18-5	294.1546	1.2893	C12H23NO7	HMDB0037840	Organic acids and derivatives
2-Hydroxycinnamic acid	yes	down	C01772	pos	583-17-5	165.0543	0.822833	C9H8O3	HMDB0134028; HMDB0062655; HMDB0002641	Phenylpropanoids and polyketides
Nummularine A	yes	up	C10011	pos	53947-95-8	648.3797	3.39985	C36H49N5O6	HMDB0029336	Organic acids and derivatives
PE(18:4(6Z,9Z,12Z,15Z)/22:6(4Z,7Z,10Z, 13Z,16Z,19Z))	yes	up	–	pos	-	784.4895	3.671333	C45H70NO8P	HMDB0009210; LMGP02010768	Lipids and lipid-like molecules
N-(1-Deoxy-1-fructosyl)phenylalanine	yes	up	–	pos	87251-83-0	328.14	2.131817	C15H21NO7	HMDB0037846	Organic acids and derivatives
PG(18:1(9Z)/0:0)	yes	up	–	pos	-	533.2836	9.961467	C24H47O9P	LMGP04050006	-
Tyrosyl-Valine	yes	down	–	pos	-	263.1389	3.036867	C14H20N2O4	HMDB0029118	Organic acids and derivatives
DL-o-Tyrosine	yes	down	–	pos	-	182.0807	1.109483	C9H11NO3	HMDB0006050	Organic acids and derivatives
Lysyl-Proline	yes	up	–	pos	-	226.1549	1.17065	C11H21N3O3	HMDB0028959	Organic acids and derivatives
Hydroxyprolyl-Alanine	yes	up	–	pos	-	185.0912	1.191133	C8H14N2O4	HMDB0028856	Organic acids and derivatives
3-[(3-Methylbutyl)nitrosoamino]-2-butanone	yes	up	–	pos	71016-15-4	151.1225	1.817767	C9H18N2O2	HMDB0033553	Organooxygen compounds
Methionyl-Hydroxyproline	yes	up	–	pos	-	245.0955	2.0884	C10H18N2O4S	HMDB0028974	Organic acids and derivatives
Prolyl-Alanine	yes	up	–	pos	-	169.0966	2.126433	C8H14N2O3	HMDB0029010	Organic acids and derivatives
1,4'-Bipiperidine-1'-carboxylic acid	yes	up	C16836	pos	-	195.1489	2.446067	C11H20N2O2	HMDB0060336	Organoheterocyclic compounds
Linamarin	yes	down	C01594	pos	554-35-8	280.1375	2.780217	C10H17NO6	HMDB0005008; HMDB0033699	Organoheterocyclic compounds
Isoleucyl-Arginine	yes	up	–	pos	-	270.1922	2.831383	C12H25N5O3	HMDB0028901	Organic acids and derivatives
Prednisone	yes	up	C07370	pos	02-03-1953	422.192	3.38985	C21H26O5	LMST02030180; HMDB0014773	Lipids and lipid-like molecules
7,8-Dihydrovomifoliol 9-[apiosyl-(1->6)-glucoside]	yes	up	–	pos	177261-70-0	562.289	3.5565	C24H40O12	HMDB0029771	Lipids and lipid-like molecules
Permetin A	yes	up	–	pos	71888-70-5	562.3438	3.6925	C54H92N12O12	HMDB0030527	Organic acids and derivatives
Trigoneoside XIIIa	yes	up	–	pos	290348-13-9	614.3097	3.7445	C57H94O28	HMDB0038313	Lipids and lipid-like molecules
Phenylalanyl-Gamma-glutamate	yes	up	–	pos	-	316.1288	3.765333	C14H19N3O4	HMDB0029009	Organic acids and derivatives
Ganglioside GM3 (d18:0/14:0)	yes	up	–	pos	-	575.3512	3.899983	C55H102N2O21	HMDB0011914	Lipids and lipid-like molecules
CL(8:0/18:2(9Z,11Z)/18:2(9Z,11Z)/18:2 (9Z,11Z))	yes	up	–	pos	-	679.4163	3.984317	C71H126O17P2	HMDB0123884	Lipids and lipid-like molecules
Amaranthussaponin II	yes	up	–	pos	139742-10-2	497.2374	4.1308	C48H74O20	HMDB0041352	Lipids and lipid-like molecules
Dimethyl 3-methoxy-4-oxo-5-(8,11,14-pentadecatrienyl)-2-hexenedioate	yes	up	–	pos	-	904.5123	4.622617	C24H36O6	HMDB0032099	Organic acids and derivatives
PA(22:5(4Z,7Z,10Z,13Z,16Z)/24:1(15Z))	yes	down	–	pos	-	874.6345	4.673767	C49H85O8P	HMDB0115375	Lipids and lipid-like molecules
Gibberellin A88	yes	up	–	pos	146959-87-7	394.1645	5.571217	C19H22O5	HMDB0039240	Lipids and lipid-like molecules
Ovalicin	yes	up	C09674	pos	19683-98-8	360.1803	5.671717	C16H24O5	HMDB0038120	Organooxygen compounds
PE(17:1(9Z)/0:0)	yes	down	–	pos	-	466.2922	8.06525	C22H44NO7P	LMGP02050008	-
CL(18:0/22:5(4Z,7Z,10Z,13Z,16Z)/18:2(9Z,12Z)/22:5(4Z,7Z,10Z,13Z,16Z))	yes	up	–	pos	-	788.5175	8.1769	C89H150O17P2	HMDB0057295	Lipids and lipid-like molecules
Cyclic 6-Hydroxymelatonin	yes	up	–	pos	-	211.0871	5.631383	C13H14N2O3	HMDB0060810	Organoheterocyclic compounds
Phenylacetic acid	yes	up	C07086	pos	103-82-2	314.1391	5.631383	C8H8O2	HMDB0000209	Benzenoids
Ganglioside GD1a (d18:0/25:0)	yes	up	–	pos	-	991.0432	4.878433	C92H165N3O39	HMDB0011789	Lipids and lipid-like molecules
Tragopogonsaponin L	yes	up	–	pos	-	897.5081	4.694433	C50H74O15	HMDB0037926	Lipids and lipid-like molecules
Quinquenoside II	yes	down	–	pos	208764-52-7	628.3416	4.48995	C62H104O24	HMDB0032815	Lipids and lipid-like molecules
Ganglioside GM2 (d18:0/24:0)	yes	up	–	pos	-	745.4557	4.05865	C74H134N2O26	HMDB0011904	Lipids and lipid-like molecules
Simonin IV	yes	up	–	pos	151310-53-1	672.4087	3.84815	C68H120O24	HMDB0029977	Organic oxygen compounds
L-Pyridosine	yes	up	–	pos	31489-08-4	277.118	3.682333	C12H18N2O4	HMDB0029443	Organic acids and derivatives
Capsianside B	yes	down	–	pos	121924-07-0	783.4093	3.661333	C76H124O33	HMDB0030733	Lipids and lipid-like molecules

HMDB Class	HMDB Subclass	VIP_pred_ OPLS-DA	VIP_PLS-DA	FC(D_Lf/D)	P_value	FDR	D-Lf_1	D-Lf_2	D-Lf_3	D_1	D_2	D_3
Steroids and steroid derivatives	Steroid lactones	1.5687	1.5618	1.2886	3.85E-05	0.000555	4.448	4.525	4.4879	3.5544	3.4939	3.3975
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.6562	1.6563	0.5956	0.000592	0.004155	1.6192	1.5408	1.9081	2.8805	2.8529	2.7739
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.4187	1.4088	1.3467	0.000186	0.001751	3.2306	3.1083	3.252	2.4339	2.398	2.2914
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.7862	1.7781	1.4343	3.22E-06	9.16E-05	4.241	4.2991	4.3197	2.9601	3.041	2.9656
Cinnamic acids and derivatives	Hydroxycinnamic acids and derivatives	1.3972	1.3955	0.7454	0.000184	0.001746	2.4597	2.2724	2.3577	3.1903	3.2045	3.1166
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.0635	2.0547	1.5602	1.58E-06	5.78E-05	4.7965	4.8367	4.8392	3.1501	3.102	3.0239
Glycerophospholipids	Glycerophosphoethanolamines	1.9462	1.9389	1.547	5.41E-07	2.81E-05	4.3207	4.3991	4.3553	2.7988	2.8447	2.8068
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.491	1.4827	1.2528	0.000186	0.001751	4.4986	4.5544	4.4896	3.6657	3.6712	3.4717
-	-	1.0575	1.054	1.1827	0.000746	0.004947	3.0061	3.0357	3.0085	2.649	2.5098	2.494
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.6757	1.6695	0.7487	3.98E-05	0.000566	3.3482	3.5118	3.3914	4.6152	4.5707	4.5052
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.332	1.3341	0.7989	0.000831	0.005389	3.0124	2.8239	3.0637	3.7319	3.7701	3.6388
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.9358	1.927	2.1696	3.12E-06	8.96E-05	2.7768	2.7987	2.9067	1.3044	1.3171	1.2886
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.82	1.8132	2.1554	0.000474	0.00353	2.6488	2.3351	2.6725	1.0764	1.1525	1.3231
Carbonyl compounds	Ketones	2.5453	2.5355	601.1837	7.30E-07	3.34E-05	2.549	2.6556	2.7177	0.0044	0.0045	0.0042
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.8893	1.8717	3.5841	0.003625	0.01749	2.2559	2.0626	1.9687	0.3572	1.0492	0.3479
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.9648	1.9585	1.9186	0.000927	0.005817	3.2751	3.4267	3.4047	2.111	1.5796	1.5779
Piperidines	Piperidinecarboxylic acids and derivatives	1.2435	1.2369	1.2858	0.000177	0.001701	2.7749	2.8453	2.9352	2.2188	2.2321	2.2023
Benzimidazoles	Sulfinylbenzimidazoles	1.8916	1.8853	0.5689	0.000218	0.001959	1.7524	2.1306	1.9412	3.4391	3.4591	3.3386
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.9816	1.9759	2.9457	7.18E-06	0.000159	2.4134	2.5247	2.3441	0.8248	0.8362	0.8107
Steroids and steroid derivatives	Hydroxysteroids	2.2282	2.2189	2.9649	8.95E-08	8.26E-06	3.0301	3.0179	3.0858	1.0276	1.0397	1.0125
Fatty Acyls	Fatty acyl glycosides	1.8876	1.8806	2.4567	0.000153	0.001534	2.2604	2.5673	2.5807	1.0058	1.0179	0.9908
Peptidomimetics	Depsipeptides	1.2467	1.2419	1.2009	1.68E-06	6.02E-05	3.7694	3.8073	3.7758	3.1525	3.1658	3.1358
Steroids and steroid derivatives	Steroidal glycosides	1.2317	1.2284	1.2278	0.000898	0.005673	3.339	3.5031	3.4088	2.8844	2.762	2.7026
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.2083	2.1883	2.5535	0.001667	0.009325	3.3302	3.3134	3.375	1.0467	1.8466	1.0316
Sphingolipids	Glycosphingolipids	1.006	0.9998	1.1405	8.31E-05	0.000975	3.3233	3.3179	3.3919	2.9327	2.946	2.9161
Glycerophospholipids	Glycerophosphoglycerophosphoglycerols	2.5453	2.5355	2.9657	2.00E-09	9.85E-07	3.9593	3.9797	3.9841	1.341	1.3537	1.3251
Prenol lipids	Terpene glycosides	1.2458	1.2401	1.2414	8.39E-06	0.000178	3.2126	3.2564	3.2809	2.6192	2.6325	2.6026
Keto acids and derivatives	Beta-keto acids and derivatives	1.1605	1.153	1.2624	0.01026	0.04007	3.0514	2.614	2.864	2.2065	2.2943	2.2567
Glycerophospholipids	Glycerophosphates	1.2621	1.2507	0.5382	0.03404	0.1045	0.9068	0.7412	1.0035	1.9788	1.21	1.737
Prenol lipids	Terpene lactones	1.7262	1.7316	2.799	0.00218	0.01156	2.032	1.9106	1.9972	0.784	0.3682	0.97
Carbonyl compounds	Ketones	2.151	2.1384	4.6558	1.49E-05	0.000278	2.4225	2.304	2.4556	0.4961	0.6241	0.4223
-	-	1.3493	1.3236	0.3648	0.04196	0.1232	0.268	0.9984	0.2263	1.2715	1.1578	1.6637
Glycerophospholipids	Glycerophosphoglycerophosphoglycerols	1.0107	1.0047	1.2549	0.04237	0.1242	2.707	2.1284	2.4599	1.9393	1.9525	1.9228
Indoles and derivatives	Pyrroloindoles	1.7086	1.7091	8.7039	0.004103	0.01943	1.6261	1.1097	1.5138	0.0196	0.0202	0.4486
Benzene and substituted derivatives	-	1.7705	1.7589	10.6608	0.0023	0.01209	1.6909	1.0757	1.589	0.1364	0.1401	0.132
Sphingolipids	Glycosphingolipids	1.5333	1.5243	1.4793	0.00054	0.003871	2.8863	3.0328	3.0731	1.8937	2.1623	2.0231
Prenol lipids	Triterpenoids	1.1136	1.1064	1.1416	0.001618	0.009134	4.0877	4.1938	4.2681	3.5863	3.7387	3.6678
Prenol lipids	Triterpenoids	1.1483	1.1454	0.863	0.002665	0.01366	3.4274	3.6163	3.5408	3.9682	4.1866	4.1103
Sphingolipids	Glycosphingolipids	2.629	2.6193	3.3131	1.46E-06	5.45E-05	4.0024	4.0516	4.0407	1.3379	1.1687	1.145
Organooxygen compounds	Carbohydrates and carbohydrate conjugates	2.8747	2.865	4.0731	6.38E-07	3.14E-05	4.4283	4.4682	4.4823	0.9906	1.0998	1.1934
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.5222	1.5149	1.4482	1.89E-05	0.000331	2.9869	3.0407	3.121	2.1066	2.1199	2.0901
Fatty Acyls	Fatty acyl glycosides	1.4321	1.4292	0.7747	0.00029	0.002416	2.8067	2.9978	2.9214	3.8132	3.7849	3.6667

D vs D-Lf

Metabolite	Significant	Regulate	KEGG Compound ID	Mode	CAS ID	M/Z	Retention time	Formula	Library ID	HMDB Superclass
Glutamylisoleucine	yes	up	–	pos	5879-22-1	243.1336	3.5455	C11H20N2O5	HMDB0028822	Organic acids and derivatives
L-phenylalanyl-L-hydroxyproline	yes	up	–	pos	-	261.1231	3.473183	C14H18N2O4	HMDB0011176	Organic acids and derivatives
Schizonepetoside E	yes	up	C17638	pos	-	349.1846	3.462517	C16H28O8	-	-
Normetanephrine	yes	down	C05589	pos	97-31-4	225.1229	3.316683	C9H13NO3	HMDB0000819	Benzenoids
Methionyl-Proline	yes	up	–	pos	-	229.1001	3.27585	C10H18N2O3S	HMDB0028981	Organic acids and derivatives
Isoleucyl-Hydroxyproline	yes	up	–	pos	-	227.1386	3.27585	C11H20N2O4	HMDB0028908	Organic acids and derivatives
Mabioside C	yes	down	–	pos	156980-54-0	856.4407	3.24535	C42H64O14	HMDB0040653	Lipids and lipid-like molecules
Vinaginsenoside R1	yes	up	–	pos	156980-41-5	433.2516	3.172367	C44H74O15	HMDB0034624	Lipids and lipid-like molecules
Mocimycin	yes	up	–	pos	50935-71-2	819.4104	3.088367	C43H60N2O12	HMDB0030465	Organooxygen compounds
54-Deoxyciguatoxin	yes	up	–	pos	139341-09-6	559.2915	3.088367	C60H86O18	HMDB0029781	Phenylpropanoids and polyketides
Hoduloside X	yes	up	–	pos	154971-14-9	547.2984	3.0677	C53H88O23	HMDB0040662	Lipids and lipid-like molecules
Kinetensin 4-7	yes	up	–	pos	138482-56-1	604.3166	2.841383	C26H37N9O6	HMDB0012986	Organic acids and derivatives
Tyrosyl-Proline	yes	down	–	pos	-	557.2608	2.780217	C14H18N2O4	HMDB0029113	Organic acids and derivatives
Hydroxyprolyl-Proline	yes	up	–	pos	-	211.1072	1.919267	C10H16N2O4	HMDB0028871	Organic acids and derivatives
Gamma-Glutamylarginine	yes	up	–	pos	31106-03-3	286.1512	1.8381	C11H21N5O5	HMDB0029143	Organic acids and derivatives
L-Proline	yes	down	C00148	pos	147-85-3	116.0703	1.807933	C5H9NO2	HMDB0000162	Organic acids and derivatives
Pyridinoline	yes	down	–	pos	63800-01-1	429.1981	1.3408	C18H28N4O8	HMDB0000851	Organic acids and derivatives
L-Isoleucine	yes	down	C00407	pos	73-32-5	132.1016	1.2998	C6H13NO2	HMDB0000172; HMDB0000557	Organic acids and derivatives
Glutamylalanine	yes	up	–	pos	21064-18-6	201.0861	1.258967	C8H14N2O5	HMDB0003764	Organic acids and derivatives
2-Phenylacetamide	yes	down	C02505	pos	103-81-1	136.0756	1.12015	C8H9NO	HMDB0010715	Benzenoids
N-Acetyl-L-glutamic acid	yes	up	C00624	pos	-	190.0706	1.109483	C7H11NO5	HMDB0001138	Organic acids and derivatives
N-Benzylformamide	yes	down	C15561	pos	-	136.0755	0.833167	C8H9NO	-	-
2-amino-6-hydroxyhexanoic acid	yes	up	–	pos	-	148.0963	0.833167	C6H13NO3	HMDB0142963	Organic acids and derivatives
Rac-4-Hydroxy-4-O-(beta-D-glucuronide)-all-trans-retinyl acetate	yes	down	–	pos	-	559.2347	0.811833	C28H40O9	HMDB0060141	Lipids and lipid-like molecules
Hydroxyprolyl-Hydroxyproline	yes	down	–	pos	-	245.1132	0.811833	C10H16N2O5	HMDB0028864	Organic acids and derivatives
Vignatic acid A	yes	up	–	neg	181485-19-8	552.2672	3.669033	C30H39N3O7	HMDB0033599	Organic acids and derivatives
Pyroglutamylvaline	yes	up	–	neg	21282-10-0	227.1032	3.10265	C10H16N2O4	HMDB0094651	Organic acids and derivatives
Ganoderenic acid D	yes	up	–	neg	100665-43-8	557.2579	3.834317	C30H40O7	HMDB0036059	Lipids and lipid-like molecules
D-(+)-3-Phenyllactic acid	yes	up	–	neg	-	165.056	3.985167	C9H10O3	-	-
Austalide B	yes	up	–	neg	81543-02-4	473.2039	4.85575	C26H34O8	HMDB0030004	Organoheterocyclic compounds
Pyroglutamic acid	yes	up	C01879; C02237	neg	98-79-3	128.035	1.065783	C5H7NO3	HMDB0000267; HMDB0060262; HMDB0000805	Organic acids and derivatives
4-Oxo-13-cis-retinoate	yes	up	–	neg	-	349.1607	1.851983	C20H26O3	HMDB0012789	Lipids and lipid-like molecules
Suxibuzone	yes	up	–	neg	27470-51-5	473.151	2.4556	C24H26N2O6	HMDB0042019	Lipids and lipid-like molecules
Glaucarubolone 15-O-beta-D-glucopyranoside	yes	up	–	neg	89202-76-6	537.1949	2.7704	C26H36O13	HMDB0035036	Lipids and lipid-like molecules
Gamma-Glutamylphenylalanine	yes	up	–	neg	7432-24-8	293.1145	3.131967	C14H18N2O5	HMDB0000594	Organic acids and derivatives
2-Isopropylmalic acid	yes	down	C02504	neg	3237-44-3	175.0609	3.238817	C7H12O5	HMDB0000402	Lipids and lipid-like molecules
Licoricesaponin K2	yes	down	–	neg	-	803.3805	3.248317	C42H62O16	HMDB0039250	Lipids and lipid-like molecules
Mevalonolactone	yes	down	–	neg	503-48-0	129.056	3.502	C6H10O3	HMDB0006024	Organoheterocyclic compounds
Hoduloside VIII	yes	down	–	neg	154971-12-7	951.469	3.53145	C46H76O18	HMDB0040660	Lipids and lipid-like molecules
4-Hydroxybenzaldehyde	yes	down	C00633	neg	123-08-0	121.0296	3.61965	C7H6O2	HMDB0011718	Organic oxygen compounds
Gamma-Glutamyltryptophan	yes	up	–	neg	66471-20-3	314.114	3.786417	C16H19N3O5	HMDB0029160	Organic acids and derivatives

HMDB Class	HMDB Subclass	VIP_pred_ OPLS-DA	VIP_PLS-DA	FC(D_Lf/D)	P_value	FDR	D-Lf_1	D-Lf_2	D-Lf_3	D_1	D_2	D_3
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.2819	2.2778	2.4166	0.000194	0.001799	3.6565	3.6882	3.6522	1.2293	1.5294	1.7938
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.9354	1.9393	2.1095	0.001225	0.007272	3.0347	2.9465	3.0376	1.3064	1.17	1.7998
-	-	1.6865	1.6803	2.1041	0.000101	0.001126	2.0748	2.3059	2.2924	1.0582	1.0704	1.0431
Phenols	Methoxyphenols	1.0815	1.0793	0.818	0.001876	0.0102	2.3509	2.1233	2.2009	2.7169	2.7015	2.741
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.042	2.0353	2.4697	1.77E-06	6.21E-05	2.8332	2.879	2.8532	1.2281	1.1035	1.1356
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.0311	2.0497	2.4485	0.01324	0.04935	3.2063	3.1757	3.1828	0.9466	0.774	2.1856
Prenol lipids	Terpene glycosides	1.2174	1.2164	0.8661	0.000531	0.003834	3.9061	4.0412	4.0089	4.6603	4.6302	4.5129
Prenol lipids	Terpene glycosides	1.4252	1.4169	1.4449	0.007109	0.03019	2.7294	2.9819	2.9098	1.6971	2.2367	2.0333
Carbohydrates and carbohydrate conjugates	Glycosyl compounds	1.1262	1.1334	1.4137	0.04956	0.1406	1.7616	2.5491	2.2721	1.5526	1.5656	1.5364
Ciguatera toxins	-	1.9228	1.9147	3.8874	0.000273	0.00232	1.8072	2.1152	2.2307	0.5283	0.5378	0.5167
Prenol lipids	Triterpenoids	2.4631	2.4536	3.1753	7.56E-07	3.43E-05	3.5183	3.661	3.6332	1.1272	1.166	1.1119
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.1672	1.1629	1.2254	0.009569	0.0379	3.0975	3.3782	3.3107	2.8044	2.7042	2.4761
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.0463	2.0424	0.4467	4.68E-05	0.000634	1.4799	1.2928	1.3872	3.2157	3.1345	2.9659
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.9417	1.9386	2.3425	0.000165	0.00163	2.6365	2.7206	2.7883	1.2944	0.9526	1.2304
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.8923	1.8828	3.2737	6.29E-05	0.000795	1.9625	2.1287	2.2268	0.6672	0.6837	0.5791
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.3036	2.2934	0.1731	8.07E-06	0.000173	0.5322	0.518	0.3068	2.5927	2.631	2.6144
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.6933	1.694	0.6768	0.00046	0.003449	2.4911	2.3357	2.6954	3.7541	3.7445	3.6133
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.7186	1.717	0.7121	6.36E-05	0.000799	3.0149	2.8978	3.1015	4.252	4.263	4.1463
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.3465	1.3418	1.5677	0.01368	0.05067	2.4353	1.9162	2.351	1.2261	1.4603	1.5883
Benzene and substituted derivatives	Phenylacetamides	1.633	1.633	0.6232	0.000629	0.004366	1.9305	1.6305	1.9783	3.0226	2.9691	2.8956
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.631	1.6056	2.41	0.01933	0.06745	2.2523	1.695	2.1799	1.0977	1.1221	0.3221
-	-	1.3665	1.3617	0.7439	3.28E-05	0.00049	2.1713	2.2433	2.2232	3.0343	2.9564	2.935
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.1557	1.1518	1.1912	1.43E-06	5.42E-05	3.3667	3.4041	3.397	2.851	2.8385	2.8455
Prenol lipids	Terpene glycosides	1.8111	1.8049	0.627	1.11E-05	0.000224	2.348	2.1935	2.2116	3.6049	3.5883	3.5768
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.6115	1.607	0.6847	2.95E-06	8.67E-05	2.3396	2.2804	2.2859	3.3759	3.3883	3.3206
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.0649	2.0402	3.2383	7.20E-06	0.000225	2.2578	2.2442	2.1082	0.6697	0.6585	0.7125
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.929	1.9006	1.7866	0.000152	0.00167	3.0375	3.0051	3.073	1.7786	1.8108	1.5151
Prenol lipids	Triterpenoids	1.7239	1.7013	1.4741	1.29E-05	0.000335	3.2925	3.2667	3.3367	2.3045	2.1851	2.2232
-	-	1.3551	1.3399	1.1607	4.62E-05	0.000747	4.7904	4.6829	4.7657	4.1138	4.0754	4.0784
Benzopyrans	1-benzopyrans	1.1527	1.1476	1.2355	0.001507	0.009166	2.6528	2.4435	2.5826	2.0513	2.0652	2.0988
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.1922	2.1691	3.2456	0.00027	0.00253	2.6794	2.2428	2.5827	0.6956	0.8782	0.739
Prenol lipids	Retinoids	2.142	2.1285	8.1043	0.004064	0.02031	2.2084	1.3807	2.285	0.2358	0.2298	0.2591
Fatty Acyls	Fatty acid esters	1.2272	1.2086	1.3219	0.006088	0.02801	2.1568	2.5162	2.3745	1.7232	1.8042	1.8038
Prenol lipids	Terpene lactones	1.7446	1.7234	2.7206	1.77E-06	9.26E-05	1.7452	1.7255	1.6788	0.6209	0.6101	0.6623
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.1557	2.127	2.7543	0.000383	0.003249	2.619	2.6278	2.6823	1.2602	0.7805	0.838
Fatty Acyls	Fatty acids and conjugates	1.2521	1.2374	0.8697	8.07E-05	0.001057	3.7459	3.7432	3.7649	4.3661	4.3234	4.251
Prenol lipids	Terpene glycosides	1.9483	1.9245	0.303	0.00132	0.008307	0.537	0.3556	0.9318	2.0387	1.9275	2.0546
Lactones	Delta valerolactones	1.5081	1.4862	0.7055	3.34E-05	0.000607	2.0051	1.8958	1.9431	2.8084	2.7419	2.7325
Prenol lipids	Triterpenoids	1.0521	1.0421	0.8602	0.005609	0.02641	2.6008	2.5283	2.6403	3.0885	3.0737	2.8704
Organooxygen compounds	Carbonyl compounds	1.3255	1.3084	0.7602	0.0271	0.09294	2.547	1.9577	2.3894	3.2384	2.8274	3.0018
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	2.6016	2.5685	3.6821	1.37E-07	2.76E-05	3.3319	3.2671	3.3248	0.8836	0.8711	0.9405

D vs D-Lf

Metabolite	Significant	Regulate	KEGG Compound ID	Mode	CAS ID	M/Z	Retention time	Formula	Library ID	HMDB Superclass
5,9,11-trihydroxyprosta-6E,14Z-dien-1-oate	yes	up	–	neg	80998-07-8	584.2476	3.824967	C30H37NO8	HMDB0062413	Lipids and lipid-like molecules
2-Hepteneoylglycine	yes	down	–	neg	-	206.0815	4.118383	C9H15NO3	HMDB0094728	Organic acids and derivatives
Lucidenic acid N	yes	up	–	neg	364622-33-3	495.2539	4.539633	C27H40O6	HMDB0038352	Lipids and lipid-like molecules
Neuromedin N	yes	up	C15868	neg	102577-25-3	654.3275	5.166483	C32H51N5O7	HMDB0013022	Organic acids and derivatives
7alpha-Hydroxy-3-oxo-4-cholestenoate	yes	up	C17337	neg	115538-85-7	465.28	5.379717	C27H42O4	HMDB0012458	Lipids and lipid-like molecules
Simvastatin	yes	up	–	neg	79902-63-9	463.2665	5.389917	C25H38O5	HMDB0005007	Organoheterocyclic compounds
Homocapsaicin	yes	up	C20215	neg	58493-48-4	364.2116	6.375883	C19H29NO3	HMDB0036329	Benzenoids
3-Oxohexadecanoic acid	yes	down	–	neg	-	315.2168	7.82605	C16H30O3	HMDB0010733	Lipids and lipid-like molecules
Prostaglandin F1a	yes	down	C06475	neg	745-62-0	355.2475	5.8559	C20H36O5	HMDB0002685	Lipids and lipid-like molecules
2-(1-Pentenyl)furan	yes	up	–	neg	81677-78-3	181.0865	5.729017	C9H12O	HMDB0039782	Organoheterocyclic compounds
PS(18:4(6Z,9Z,12Z,15Z)/18:4(6Z,9Z,12Z,15Z))	yes	up	–	neg	-	796.4245	5.059	C42H66NO10P	HMDB0112496; LMGP03010441	Lipids and lipid-like molecules
Pubesenolide	yes	up	–	neg	98569-64-3	493.2765	5.01	C28H42O5	HMDB0033728	Lipids and lipid-like molecules
Neoporrigenin B	yes	up	–	neg	196607-74-6	481.2757	4.980767	C27H42O5	HMDB0032680	Lipids and lipid-like molecules
Ganoderic acid C2	yes	up	–	neg	98296-48-1	555.2682	4.95125	C30H46O7	HMDB0035304	Lipids and lipid-like molecules
P-Salicylic acid	yes	up	C00156	neg	-	137.0245	4.687783	C7H6O3	HMDB0000500	Benzenoids
Esculentoside E	yes	up	–	neg	65649-36-7	649.356	4.597833	C35H54O11	HMDB0034635	Lipids and lipid-like molecules
PS(20:4(5Z,8Z,11Z,14Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	yes	up	–	neg	-	890.471	4.5786	C48H74NO10P	LMGP03010978; HMDB0012439	Lipids and lipid-like molecules
Dihydrozeatin-9-N-glucoside-O-glucoside	yes	up	–	neg	-	580.2053	4.386333	C22H35N5O11	HMDB0012212	Lipids and lipid-like molecules
Durupcoside B	yes	up	–	neg	121521-92-4	907.4613	4.051683	C47H74O18	HMDB0030976	Lipids and lipid-like molecules
Blasticidin S	yes	up	C02010	neg	2079-00-7	403.1829	3.862633	C17H26N8O5	HMDB0030452	Organooxygen compounds
Acetyl-DL-Leucine	yes	up	C02710	neg	-	172.0976	3.824967	C8H15NO3	HMDB0011756	Organic acids and derivatives
N-(1-Deoxy-1-fructosyl)histidine	yes	up	–	neg	25020-13-7	338.0981	3.688683	C12H19N3O7	HMDB0037839	Organic acids and derivatives
PS(MonoMe(11,3)/MonoMe(9,5))	yes	down	–	neg	-	876.4386	3.669033	C44H74NO12P	HMDB0061605	Lipids and lipid-like molecules
Bilastine	yes	up	–	neg	202189-78-4	498.2543	3.4824	C28H37N3O3	HMDB0240232	Organoheterocyclic compounds
Digitoxin	yes	down	C06955	neg	71-63-6	785.4064	3.364417	C41H64O13	HMDB0015468; LMST01120018	Lipids and lipid-like molecules
Benzoquinoneacetic acid	yes	down	–	neg	10275-07-7	165.0189	3.3453	C8H6O4	HMDB0002334	Organic oxygen compounds
Testosterone sulfate	yes	up	–	neg	651-45-6	413.1664	3.287133	C19H28O5S	LMST05020032; HMDB0002833	Lipids and lipid-like molecules
Isosakuranin	yes	up	–	neg	491-69-0	447.1327	3.277517	C22H24O10	LMPK12140336; HMDB0029481	Phenylpropanoids and polyketides
Glycinoeclepin B	yes	up	–	neg	103847-17-2	593.2555	3.10265	C31H42O9	HMDB0037035	Lipids and lipid-like molecules
3,4',5-Trihydroxy-3',7-dimethoxyflavanone	yes	down	–	neg	37971-67-8	331.085	3.005333	C17H16O7	HMDB0037503	Phenylpropanoids and polyketides
CDP-DG(16:0/20:4(8Z,11Z,14Z,17Z))	yes	down	–	neg	-	982.4909	3.005333	C48H81N3O15P2	HMDB0006974; LMGP13010009	Lipids and lipid-like molecules
(4S,6R)-p-Mentha-1,8-diene-6,7-diol 7-glucoside	yes	up	–	neg	402593-54-8	365.1343	2.900617	C16H26O7	HMDB0039056	Lipids and lipid-like molecules
Methylprednisolone	yes	down	–	neg	83-43-2	395.1809	2.790333	C22H30O5	HMDB0015094	Lipids and lipid-like molecules
Prolylhydroxyproline	yes	down	–	neg	18684-24-7	273.1086	2.170683	C10H16N2O4	HMDB0006695; HMDB0029018	Organic acids and derivatives
Beta-D-3-Ribofuranosyluric acid	yes	down	C05513	neg	2124-54-1	299.0627	2.0088	C10H12N4O7	HMDB0029920	Organoheterocyclic compounds
Dicaffeoylputrescine	yes	down	–	neg	60422-23-3	447.1347	0.955617	C22H24N2O6	HMDB0033467	Phenylpropanoids and polyketides
2-Hydroxyglutaric acid lactone	yes	up	–	neg	-	175.0239	0.85135	C5H6O4	HMDB0059743	Organoheterocyclic compounds
L-4-Hydroxyglutamate semialdehyde	yes	up	C05938	neg	-	128.0352	0.842117	C5H9NO4	HMDB0006556	Organic acids and derivatives
Maleic acid	yes	down	C00122; C01384	neg	110-16-7	115.004	0.842117	C4H4O4	HMDB0000176	Organic acids and derivatives

HMDB Class	HMDB Subclass	VIP_pred_ OPLS-DA	VIP_PLS-DA	FC(D_Lf/D)	P_value	FDR	D-Lf_1	D-Lf_2	D-Lf_3	D_1	D_2	D_3
Prenol lipids	Diterpenoids	1.3882	1.3697	1.3554	9.46E-06	0.000265	2.5996	2.6253	2.6456	1.9232	1.9091	1.9763
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.0235	1.0074	0.8507	0.00029	0.002655	2.2031	2.1172	2.1429	2.57	2.5094	2.5164
Prenol lipids	Triterpenoids	1.4169	1.4056	1.4527	0.00086	0.005967	2.4717	2.2203	2.3565	1.6916	1.5852	1.5735
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.9578	1.9304	3.322	2.51E-06	0.000117	1.8912	1.9824	1.9866	0.578	0.5675	0.6181
Steroids and steroid derivatives	Bile acids, alcohols and derivatives	1.0294	1.0155	1.2705	8.82E-05	0.001121	1.7595	1.8004	1.7952	1.3924	1.3787	1.444
Lactones	Delta valerolactones	1.3996	1.3784	1.4757	0.000123	0.001427	2.1358	2.2263	2.19	1.5246	1.5158	1.401
Benzene and substituted derivatives	Phenols and derivatives	1.6455	1.6473	9.9487	0.008581	0.03704	1.525	0.8364	1.1277	0.2895	0.0245	0.0367
Fatty Acyls	Fatty acids and conjugates	1.9667	1.9388	0.2236	0.01064	0.04431	0.864	0.2033	0.2284	1.7967	1.5795	2.4189
Fatty Acyls	Eicosanoids	1.0805	1.0691	0.7159	0.0101	0.04219	1.1515	0.9971	1.3144	1.5355	1.6665	1.634
Heteroaromatic compounds	-	1.065	1.0743	1.265	0.01324	0.05327	2.2351	1.9988	2.1537	1.6116	1.5973	1.8411
Glycerophospholipids	Glycerophosphoserines	1.8578	1.8316	2.5229	7.10E-06	0.000225	1.9733	2.0385	2.1003	0.7962	0.7842	0.8417
Steroids and steroid derivatives	Steroid lactones	2.4635	2.4322	13.9182	7.91E-05	0.00104	2.3806	2.3191	2.3432	0.4284	0.0103	0.0675
Steroids and steroid derivatives	Steroidal glycosides	1.5647	1.5451	1.4261	3.77E-06	0.000142	2.9239	2.8969	2.9438	2.0361	2.0219	2.0893
Prenol lipids	Triterpenoids	1.0299	1.012	1.2035	0.001504	0.009162	2.2272	2.3297	2.3448	1.8728	1.9799	1.884
Benzene and substituted derivatives	Benzoic acids and derivatives	1.1946	1.1882	1.2394	0.001437	0.008838	2.7845	2.5965	2.7436	2.1262	2.1829	2.2463
Prenol lipids	Triterpenoids	1.3328	1.3277	2.3769	0.02407	0.08546	1.444	0.8687	1.4219	0.4995	0.67	0.4018
Glycerophospholipids	Glycerophosphoserines	1.0277	1.0109	1.2692	0.01434	0.0567	1.9324	1.973	1.9912	1.5875	1.6986	1.3623
Fatty Acyls	Fatty acyl glycosides	2.6787	2.6425	1242218	4.91E-08	1.73E-05	2.5208	2.539	2.6011	0	0	0
Prenol lipids	Terpene glycosides	1.2157	1.1969	1.7331	0.02833	0.09647	1.3637	1.5652	1.3987	0.4923	0.9823	1.0233
Carbohydrates and carbohydrate conjugates	Sugar acids and derivatives	2.1481	2.1201	2.0505	1.37E-06	7.95E-05	3.2197	3.1422	3.2586	1.5853	1.5573	1.5484
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.5156	1.4949	1.4291	0.000244	0.002353	2.7711	2.6429	2.8398	1.9504	1.9661	1.8586
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.5808	1.5591	1.5718	8.85E-06	0.000252	2.4085	2.4904	2.4558	1.5471	1.5331	1.5993
Glycerophospholipids	Glycerophosphoserines	1.2769	1.2676	0.8292	0.000395	0.003327	2.8039	2.919	2.8693	3.4979	3.495	3.3695
Benzimidazoles	-	1.5093	1.4887	1.5538	2.71E-05	0.000537	2.2468	2.252	2.3448	1.4555	1.4417	1.5074
Steroids and steroid derivatives	Steroid lactones	1.1367	1.1322	0.7417	0.01089	0.04503	1.2637	1.4719	1.6221	1.977	1.876	2.0235
Organooxygen compounds	Carbonyl compounds	1.2997	1.2846	0.7676	0.000577	0.004429	1.9883	1.9973	2.0991	2.7421	2.5801	2.6035
Steroids and steroid derivatives	Sulfated steroids	1.8157	1.7918	2.2484	1.17E-05	0.000315	2.1008	2.1994	2.0654	0.9321	0.9195	0.9798
Flavonoids	Flavonoid glycosides	1.1811	1.1612	1.3242	0.01336	0.05367	2.2346	2.2227	2.245	1.6956	1.9058	1.4584
Prenol lipids	Sesquiterpenoids	2.3161	2.2881	4.5883	8.51E-06	0.000247	2.5424	2.3392	2.4566	0.4865	0.5895	0.5234
Flavonoids	O-methylated flavonoids	1.467	1.447	0.6322	0.000289	0.002655	1.3671	1.2949	1.3561	1.9946	2.1827	2.1764
Glycerophospholipids	CDP-glycerols	1.1694	1.157	0.9021	0.000352	0.003074	4.554	4.5466	4.5794	5.1062	5.0879	4.9699
Prenol lipids	Terpene glycosides	1.9028	1.8782	1.8787	0.000159	0.001716	2.7874	2.7639	2.8123	1.6668	1.3602	1.4254
Steroids and steroid derivatives	Hydroxysteroids	2.2731	2.243	0.1605	4.70E-07	4.62E-05	0.3679	0.3263	0.3614	2.2438	2.184	2.1479
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.6294	1.6138	0.4854	0.0291	0.09831	1.3084	0.3904	1.4444	2.2082	2.1923	2.0758
Imidazopyrimidines	Purines and purine derivatives	2.7684	2.7266	0.1719	0.000602	0.004549	1.0458	0.0682	0.6207	3.3853	3.3751	3.33
Cinnamic acids and derivatives	Hydroxycinnamic acids and derivatives	1.5561	1.5372	0.7491	1.13E-06	7.23E-05	2.5478	2.5857	2.6073	3.4371	3.4525	3.441
Lactones	Gamma butyrolactones	1.3513	1.329	1.437	0.001075	0.007088	2.095	2.2323	2.2429	1.5414	1.6227	1.4072
Carboxylic acids and derivatives	Amino acids, peptides, and analogues	1.5835	1.5614	1.4843	0.000668	0.004962	2.846	2.7715	2.7571	1.7889	2.0633	1.7907
Carboxylic acids and derivatives	Dicarboxylic acids and derivatives	1.3811	1.3644	0.7835	5.63E-05	0.00085	2.4717	2.4444	2.5106	3.1179	3.225	3.1378