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Discovery of taste-active metabolites in *Lactobacillus plantarum*-fermented chili sauce via web-based computational analysis

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ABSTRACT: The utilization of *Lactobacillus plantarum* (LP) in chili sauce production is well-known for its capacity to enhance product quality and sensory attributes. However, there is still limited knowledge regarding the taste-active metabolites in the sauce. To bridge this gap, our study employed metabolomics and web-based computational tools to investigate the dynamic changes of taste-active metabolites during chili sauce fermentation. By leveraging the advantages of the feature-based molecular network (FBMN), we conducted a rapid annotation of metabolites, successfully identifying 205 metabolites, a considerable portion of which were previously unreported. Through the utilization of the VirtualTaste tool, we identified dihydrosphingosine, lactic acid, isoleucine, phytosphingosine, and gluconic acid as potential taste indicators for quality control. Pathway enrichment analysis further supported their primary involvement in key biochemical pathways, including amino acid tRNA biosynthesis, phenylalanine, tyrosine, tryptophan biosynthesis, and sphingolipid metabolism. This investigation provides valuable insights into the underlying mechanisms contributing to the distinctive flavor profile of chili sauce.

Keywords: Chili sauce, *Lactobacillus plantarum*, Feature-based molecular network,Metabolomics, Taste

1. Introduction

Chili sauce, commonly referred to as hot sauce, is a popular condiment admired for its appealing color and spicy taste[1]. However, traditional production methods involving diverse naturally occurring microorganisms pose challenges in maintaining consistent product quality[2]. *Lactobacillus plantarum* (LP), a widely distributed species of lactic acid bacteria (LAB), has gained significant attention in the food industry due to its versatile functional properties[3]. These properties include enhanced flavor, probiotic activity, antimicrobial effects, and reduction of undesirable components[3-4]. Therefore, utilizing a single LP strain in the production of chili sauce ensures consistent product quality and excellent sensory attributes, while addressing the aforementioned concerns.

The taste attributes of food components, including sweetness, bitterness, acidity, umami, and saltiness, play a significant role in shaping consumer preferences and purchasing behavior[5]. Sweetness enhances the

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overall enjoyment of food, while sourness adds a tangy taste that enhances the flavor of chili sauce. Excessive acidity can overpower other flavors, resulting in a harsh sharpness on the palate, and bitterness can negatively impact food acceptance, despite its protective function against toxic substances in humans. Given the complexity of chili sauce, evaluating the taste properties of these metabolites solely through experimental methods is labor-intensive and costly in terms of time and resources. Conversely, the integration of web-based computing tools and food science has introduced innovative methodologies in flavor research that facilitate the exploration of metabolite structural characteristics and enable early detection of potential key flavor indicators in conjunction with omics technologies. Non-targeted metabolomics techniques, particularly ultra-high-performance liquid chromatography coupled with time-of-flight mass spectrometry (UHPLC-Q-TOF-MS), have recently gained significant popularity for identifying essential food metabolites[6-9]. Metabolomics studies typically rely on in-house or public databases for metabolite annotation. It is essential, however, to recognize the inherent limitations of this approach. These limitations include the database's inability to encompass all potential metabolites and the risk of incorrect matches or search failures[10]. The primary challenge, therefore, is to achieve comprehensive coverage and accurate identification of metabolites within the given analytical framework..

The molecular network (MN) is a crucial component of the Global Molecular Network for the Natural Products Society (GNPS) platform. It utilizes MS^1 data to visualize and explore the relationships between metabolites[11-12]. An upgraded version of MN is Feature-Based Molecular Networks (FBMN), which incorporates retention time and MS^2 data to investigate unidentified constituents. FBMN provides a more comprehensive understanding of metabolite structures and relationships, making it easier to identify specific substructures or chemical modifications[13-16]. Additionally, VirtualTaste, a web-based platform, employs machine learning algorithms to predict the three fundamental taste sensations of compounds—sweet, bitter, and sour[17]. This data-driven methodology holds considerable promise as a rapid tool for analyzing the flavor profile of food and beverage products.

This study employed an untargeted metabolomics approach based on ultra-high-performance liquid chromatography coupled with UHPLC-Q-TOF in conjunction with the FBMN tool to identify the non-volatile metabolites present in chili sauce during different fermentation durations (0-day, 3-day, 5-day). Subsequently, the taste activity of the identified key metabolites was evaluated using the machine learning-based VirtualTaste tool. This integrated approach, combining metabolomics and computational tools, offers several advantages, including improved efficiency in screening taste-active metabolites and facilitating a systematic and comparative analysis of the metabolites involved in the fermentation process. The findings of this investigation provide valuable insights and robust data support for ensuring the quality control of chili sauce processing.

2. Materials and methods

2.1. Materials and reagents

The HPLC-grade formic acid was procured from Kermel Chemical Reagent Co., Ltd. (Tianjin, China), while the ammonium acetate was obtained from Krohne Chemical Reagent Co., Ltd. (Chengdu, China). LCMS-grade acetonitrile was purchased from FTSCI Corporation (Wuhan, China), and methanol used for extraction was provided by Chron Chemical Co., Ltd. (Chengdu, China). Ultrapure water with a resistivity of 18.2 MΩ·cm was obtained using a Milli-Q system (Millipore, Bedford, USA).

2.2. Fermentation and extraction of chili peppers

Chili peppers (*Capsicum annuum* var.) were procured from supermarkets in Chengdu, China. The LP B5 strain (NCBI accession number: OP782666) was activated by incubating it in a sterile MRS liquid medium at 37 ℃ for 8 h. Subsequently, the medium components were eliminated using sterile saline. The concentration of the seed solution was adjusted to $OD_{600\ nm} = 0.8$. The pepper samples were washed, crushed, and subjected to sterilization at 60 ℃ for 30 min. After cooling to room temperature, 3.0% NaCl was added, and the mixture was homogenized. The homogenized pepper samples were divided into 15 portions and transferred to sterile Petri dishes, with each dish containing a 25 g sample. Inoculation was performed by adding 1.5 mL of the LP B5 strain solution to each Petri dish. The samples were incubated in a dedicated incubator at a constant temperature of 35 ℃ for a duration of 5 days. Freeze-drying was carried out on samples collected at 0, 3, and 5 days of fermentation ($n = 5$) to obtain freeze-dried samples.

The freeze-dried samples were subjected to extraction using a modified version of a previously described method[18]. To initiate the extraction process, 1.0 g of each sample was placed in a 15 mL centrifuge tube, followed by the addition of 10 mL of methanol. The resulting mixture was vortexed for 1 min and subjected to ultrasonication at room temperature for 20 min. Subsequently, the extract was filtered through a 0.22 μm membrane to facilitate further UHPLC-Q-TOF analysis.

2.3. UHPLC-Q-TOFmethod

The X500 UHPLC-Q-TOF system (SCIEX Co., Framingham, MA, USA) equipped with an ESI source was utilized in this study. Chromatographic separation was carried out on an HSS T3 C_{18} column(10 cm × 2.1 mm, 1.8 μm) (Waters, Shanghai, China).The mobile phase A consisted of acetonitrile, while mobile phase B consisted of ultrapure water with 0.1% ammonium acetate. The injection volume was set to 4 μL, and the flow rate was 250 μL/min. The column temperature was maintained at 40 ℃. The gradient elution program was as follows: (0−2) min 5% A, (2−13) min from 5% to 100% A, (13−16) min 100% A, followed by a return to 5% A in (16−16.5) min, and finally (16.5−20) min 5% A. Samples were analyzed in both positive and negative ionization modes using MS precursor ion scanning from 70 Da to 600 Da and MS/MS product ion scanning from 50 Da to 600 Da. The information dependent acquisition (IDA) function was utilized for data acquisition.

The ion source was TurboSpray with parameter settings as follows: ion source gas 1 (GS1), 55 psi; ion source gas 2 (GS2), 55 psi; curtain gas (CUR), 35 psi; temperature, 400 ℃; ionspray voltage, +5 500 V for positive and −4 500 V for negative ionization modes; declustering potential (DP), 80 V; collision energy (CE), 10 eV. Dynamic background subtraction (DBS) to exclude multiply charged ions and isotopes were invoked during the data acquisition. In order to monitor the stability of the system and ensure the accuracy of the data

acquired by the developed method, the calibration delivery system (CDS, APCI calibration solution) was run every 5 samples injections.

2.4. Data processing and statistical analysis

The massdata files were initially imported into MS-DIAL 4.60 softwarefor metabolite name and species annotation in the 6 sample sets, consisting of three sets each for positive and negative ion modes[19]. The resulting dataset was then subjected to principal component analysis (PCA) using unity variance scale, partial least squares discriminant analysis (PLS-DA), and variable importance in projection (VIP) scoring using SIMCA 14.1. Differential metabolites were screened using ANOVA (VIP > 1.0, $P < 0.05$) in SPSS25. Heat map visualization analysis was performed using OmicStudio tools (https://www.omicstudio.cn/tool). Metabolic pathway enrichment analysis was conducted using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/) with Arabidopsis thaliana as the background in order to identify functional annotations and perform enrichment analysis on chili sauce at different fermentation times. Additionally, the VirtualTaste tool (https://insilico-cyp.charite.de/VirtualTaste/) was employed to identify potential flavor markers for quality control purposes.

2.5. FBMN analysis

The clustering process in GNPS involves the following steps: 1) FBMN analysis: The metabolomics data obtained from UHPLC-Q-TOF are processed using msdial 4.60 to extract features that represent individual metabolites. These features include *m/z* values, retention times, and intensities. 2) Spectral similarity scoring: The mass spectra of the features are compared using cosine similarity scoring algorithms. Features with similar spectra, indicating potential structural similarity, receive higher similarity scores. 3) Network construction: Based on the similarity scores, the features are connected to form clusters in a network representation. Each cluster represents a group of metabolites that share similar spectral characteristics.The precursor ion mass tolerance and the MS^2 fragment ion were set to 0.01 Da. Edges were formed if the minimum cosine score of 0.7 was exceeded with > 6 matched mass peaks. 4) Visualization and analysis: The network of clustered metabolites is visualized in GNPS, allowing users to explore the relationships between metabolites. The network can be further analyzed to identify substructures, chemical families, or related biosynthetic pathways.

Spectra in the network were searched across GNPS spectral databases. Substructure annotation was performed using MS^2LDA interface in GNPS. The resulting data were then imported into Cytoscape 3.9.1 for visualization analysis[20]. The task results for the FBMN analysis can be accessed at https://gnps.ucsd.edu/ProteoSAFe/result.jsp?task=b0ebf8ee8b3248669480ee7179fc635e&view=network_components, and https://gnps.ucsd.edu/ProteoSAFe/result.jsp?task=4abbac2684a748948688b5de5c863ee0&view=network_components.

3. Results

3.1. Global FBMN analysis

Untargeted metabolomics was employed in this study to analyze chili sauce at different fermentation durations (0, 3, and 5 days). Chromatographic separation was achieved within 20 min (Fig. S1), and the MS^2 data were analyzed using the FBMN tool. The FBMN analysis enabled the visualization of metabolite families based on their similarity in MS^2 fragmentation patterns (Fig. 1). A total of 13517 positive precursor ions($[M + H]^+$, $[M + 2H]^{2+}$, $[M + Na]^+$ and $[M + NH_4]^+$) and 8 472 negative precursor ions ($[M-H]^-$ and $[M-2H]^{2-}$) were organized into MN with 101 and 61 clusters (nodes ≥ 2), respectively. These clusters were comprised of 569 and 273 nodes, respectively. The nodes in the MN were color-coded to represent the relative content of metabolites at the three different fermentation times. Self-linked points at the bottom of the network represented spectra not classified into molecular families.

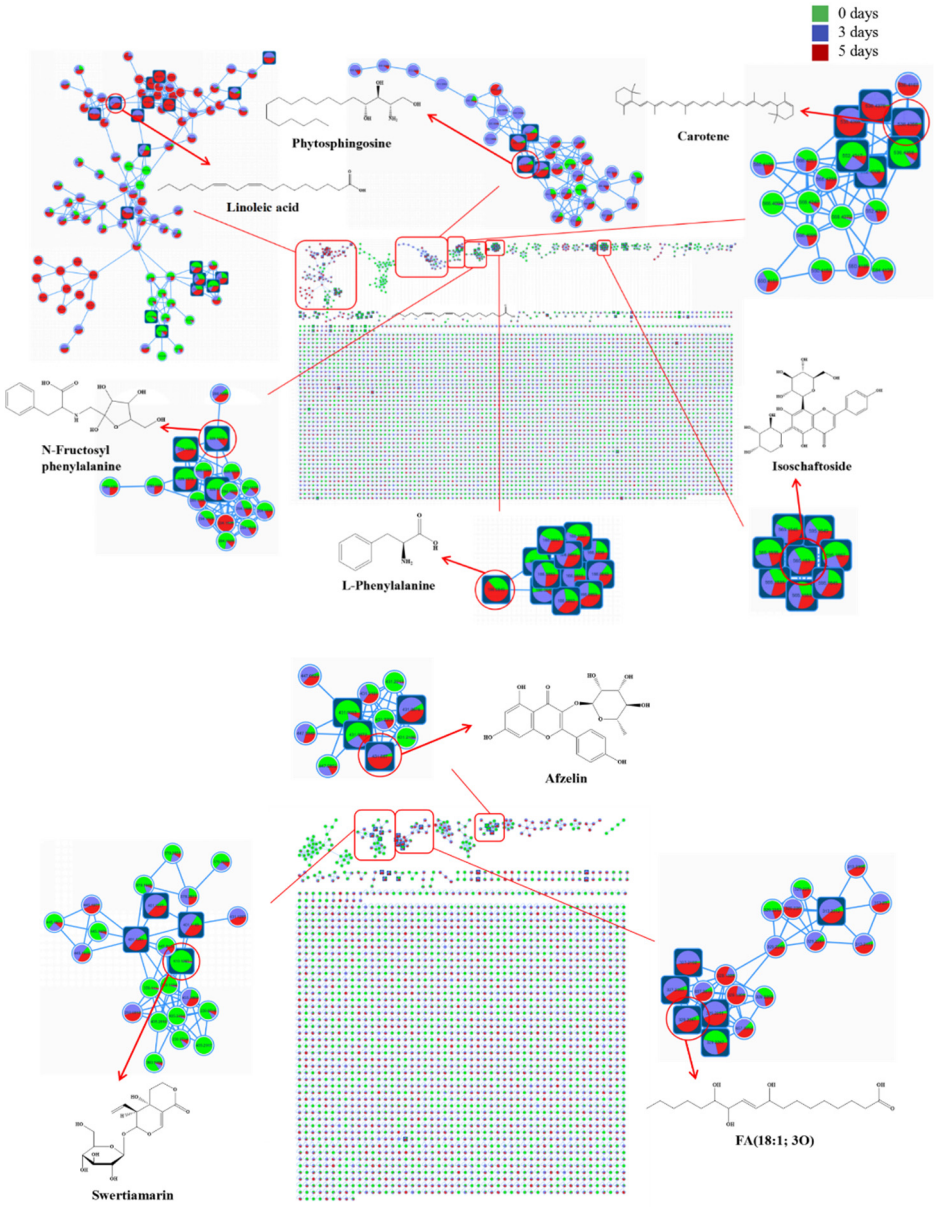


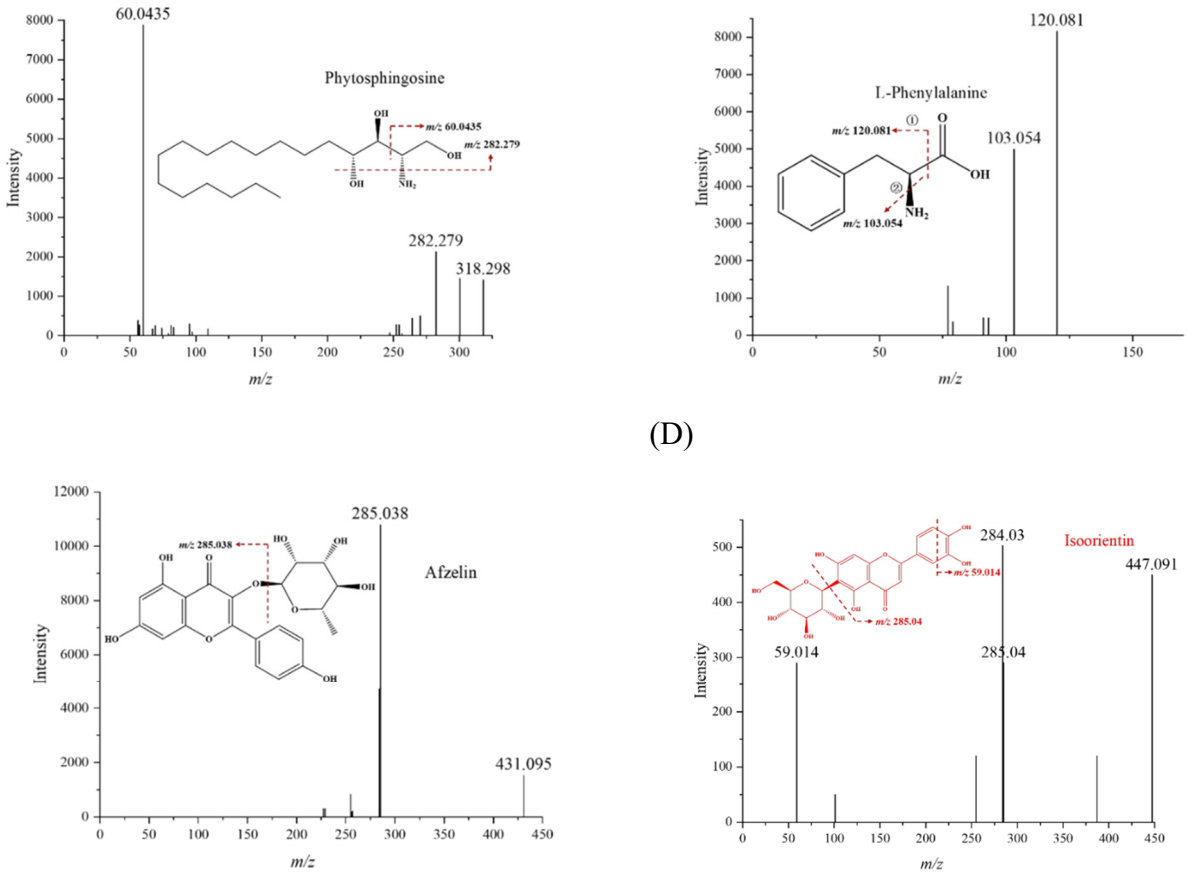
Fig. 1.FBMNanalysis of fermented chili sauce extracts using liquid chromatography-tandem mass spectrometry: identification and highlighting of major metabolite classes.
ESI^+:Fatty acids and derivatives (A), sphingolipids (B), carotenes (C), *n*-fructosyl amino acids (D), amino acids (E), and flavonoids (F);ESI^-:glycosides (G), oxidized fatty acids (H), andflavonoids (I).

Node color ratios represent the relative abundance of substances in the three sets of samples. Round-rectangle nodes indicate matched substances in the MS-DIAL database.

In the positive electrospray ionization (ESI^+) mode, the molecular families identified primarily consisted of fatty acids, sphingolipids, carotenoids, and amino acids. Conversely, the negative electrospray ionization (ESI^-) mode revealed molecular families predominantly composed of glycosides, flavonoids, and oxidized fatty acids. Following manual de-duplication and merging, a total of 205 metabolites were annotated using the GNPS database. These metabolites were categorized into 12 major groups, including amino acids, phenolics, alkaloids, acids, terpenoids, flavonoids, lipids, ketones, esters, sugars, vitamins, and other classified constituents (Table S1). Notably, many of these metabolites had not been previously reported in chili sauce. Nevertheless, it is crucial to mention that approximately 70% of the nodes remain unidentified, underscoring the intricate nature of the metabolic composition within fermented chili sauce.

To evaluate the reliability of the identified molecular families, manual analysis was performed on the MS^2 fragments obtained from the FBMN database search results (Fig. 2). The essential characteristic information of these components can be found in Table S2.

(A) (B)



(C) (D)

Fig. 2. Molecular family and structure deduction based on MS^2 spectrometry. (A) Phytosphingosine; (B) *L*-Phenylalanine; (C) Afzelin;(D)Isoorientin.

Within the sphingolipid family, the parent ion of phytosphingosine has an *m/z* value of 318.298 (Fig. 2A). Cleavage of the amide bond, followed by hydroxylation-amination reactions, generates fragment ions with

m/z values of 60.0435 and 282.279. In the amino acid family, the fragment ion of *L*-phenylalanine is observed at *m/z* 120.081 (Fig. 2B). This fragment undergoes decarboxylation and subsequent loss of one amino group, resulting in a fragment ion at *m/z* 103.054. As an example in the flavonoid family (Fig. 2C), afzelin produces a fragment ion with an *m/z* value of 285.038 after the elimination of one molecule of rhamnose. Similarly, isoorientin (Fig. 2D) with a parent ion *m/z* value of 477.091 was identified based on the similarity of the secondary mass spectra in the flavonoid family. It undergoes glycoside loss, forming a fragment ion with an *m/z* value of 285.04, or an ion fragment peak with an *m/z* value of 59.014 due to ring breakage.

The identification of metabolites with taste activity is crucial for enhancing the taste and flavor characteristics of chili sauce. However, annotating unknown metabolites presents a significant challenge in metabolomics. Previous investigations on chili sauce have mainly focused on a limited number of non-volatile compounds, such as capsaicinoids, amino acids, and organic acids[21]. In this study, we employed the FBMN tool, which utilizes powerful cloud computing capabilities, to annotate highly complex metabolomics data. This approach facilitated the efficient clustering of metabolites and eliminated redundant information, thereby improving the exploration and annotation efficiency of unknown components in chili sauce.

3.2. Multivariate statistical analysis

PCA is a commonly used multivariate analysis method in metabolomics, which allows for the identification of significant differences between sample groups. The PCA results obtained in this study reveal a clear clustering and separation among the three different fermentation times of chili sauce, indicating distinct metabolic compositions for each time point (Fig. 3). The first two principal components account for a total combined variance of 77.8%, suggesting that the model retains a substantial amount of information from the original data. Additionally, the PLS-DA model was employed to further screen and identify 33 differentiated markers representing 8 major categories of metabolites ($R^2X = 0.737$, $R^2Y = 0.948$, and $Q^2 = 0.874$; Fig. 3B). These findings demonstrate the effectiveness of both PCA and PLS-DA models in detecting differences in the metabolic composition of chili sauce at different fermentation times.

(A) (B)

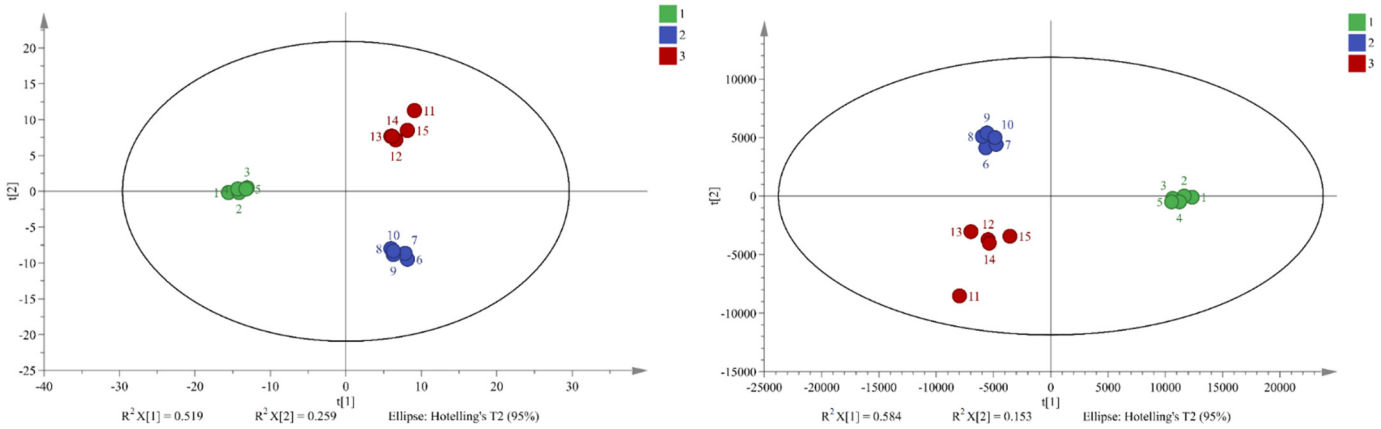


Fig.3. Chemometricanalysis of metabolites in chili sauce during different fermentation times. PCA score plots (A),PLS-DA score plot(B). Group 1−3 represent 0, 3, and 5 days of fermentation, respectively.

A semi-quantitative analysis of the 33 variability markers was conducted, using dihydrocapsaicin as a standard for reference (Table 1). The results revealed a significant increase in the lipid component, rising from 24.1 μg/g to 307.7 μg/g. The content of organic acids initially rose from 0 μg/g to 327.5 μg/g, before slightly decreasing. Amino acids also displayed a slight increase, from 160.0 μg/g to 199.6 μg/g. Among the aromatic amino acids (AAA), Phe exhibited the most substantial increase, reaching 60.57 μg/g, while the contents of Tyr and Try were relatively low, amounting to less than one-third of the Phe content.

To visualize the dynamic changes in these components, a heat map was generated (Fig. 4), utilizing a color gradient (from blue to red) to represent the relative content change from low to high. To gain further insights, the ClassyFire tool was employed, which is a web-based application facilitating rule-based structural classification of chemical entities[22]. By utilizing ClassyFire, the differential components were rapidly classified into eight distinct groups. This classification and compound description not only enhances our understanding of chemistry but also strengthens the correlation between chemistry, flavor formation, and biological activity.

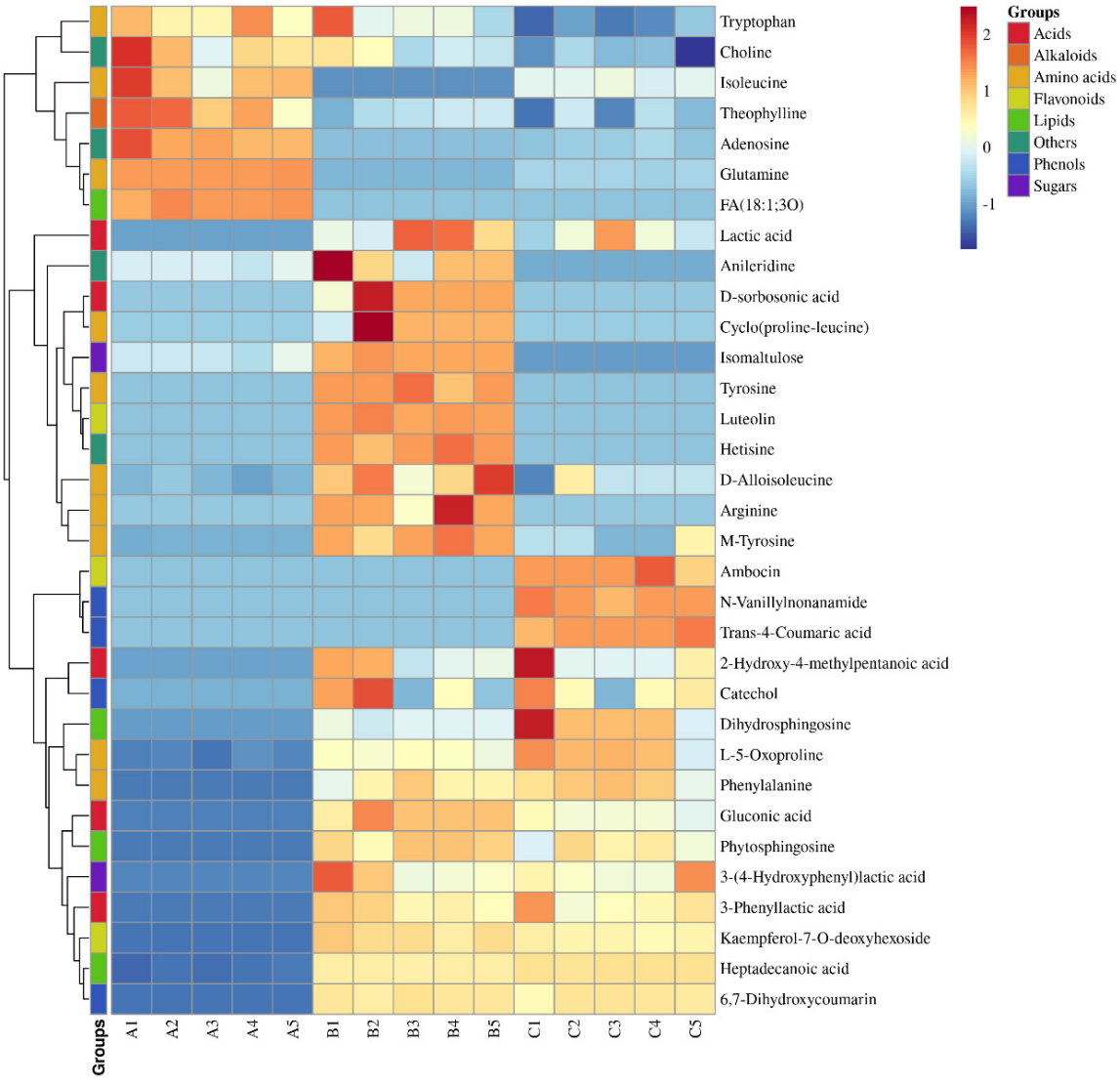


Fig. 4. Heat map comparison of differential metabolites in chili sauce during different fermentation times. The color-coded squares on the left indicate group classification information.

Table 1. Taste traits and contents of metabolite markers during LP fermented chili sauce.

RT(min)	*m/z*	VIP scores	Formula	Compounds	Tastetrait	0 day(mg/g)	3 days (mg/g)	5 days (mg/g)
4.037	131.0672	1.851	$C_6H_{12}O_3$	2-Hydroxy-4-methylpentanoic acid	Sour (0.918)	ND	40.85	43.79
5.486	165.0507	1.846	$C_9H_{10}O_3$	3-Phenyllactic acid	Sour (0.969)	ND	32.85	32.59
1.460	193.0310	1.446	$C_6H_{10}O_7$	*D*-Sorbosonicacid	Sour (0.999)	ND	6.29	ND
1.440	195.0464	2.517	$C_6H_{12}O_7$	Gluconic acid	Sour (0.968)	ND	47.36	30.43
1.709	89.0216	4.500	$C_3H_6O_3$	Lactic acid	Sour (0.980)	ND	185.24	117.54
2.911	181.0461	1.191	$C_9H_{10}O_4$	3-(4-Hydroxyphenyl)lactic acid	Sour (0.941)	ND	14.92	13.66
1.594	203.0520	2.427	$C_7H_8N_4O_2$	Theophylline	Bitter (0.999)	106.70	51.18	38.17
1.619	175.1188	1.717	$C_6H_{14}N_4O_2$	Arginine	Sweet (0.893)	ND	9.73	ND
7.272	211.1428	1.058	$C_{11}H_{18}N_2O_2$	Cyclo(proline-leucine)	Bitter (0.940)	ND	4.00	ND
2.757	132.1007	1.994	$C_6H_{13}NO_2$	*D*-Alloisoleucine	Sweet (0.813)	15.15	35.33	20.43
1.763	145.0588	2.192	$C_5H_{10}N_2O_3$	Glutamine	Sweet (0.964)	44.31	ND	5.66
2.436	132.1008	2.629	$C_6H_{13}NO_2$	Isoleucine	Sweet (0.813)	47.36	ND	24.15
1.622	128.0313	2.189	$C_5H_7NO_3$	*L*-5-Oxoproline	Sweet (0.648)	23.77	55.06	67.82
2.487	180.0619	1.429	$C_9H_{11}NO_3$	*m*-Tyrosine	ND	0.27	8.50	2.25
4.686	164.0662	2.462	$C_9H_{11}NO_2$	Phenylalanine	Sweet (0.931)	ND	53.46	60.57
5.822	203.0766	1.447	$C_{11}H_{12}N_2O_2$	Tryptophan	Sweet (0.971)	29.16	26.52	18.72
2.367	182.0801	1.078	$C_9H_{11}NO_3$	Tyrosine	Bitter (0.507)	ND	3.61	ND
8.223	565.1525	2.129	$C_{26}H_{28}O_{14}$	Ambocin	Sweet (0.641)	ND	ND	14.81
8.269	431.0876	1.353	$C_{21}H_{20}O_{10}$	Kaempferol-7-*O*-deoxyhexoside	Sweet (0.505)	ND	16.90	14.63
8.476	287.0539	1.276	$C_{15}H_{10}O_6$	Luteolin	Bitter (1.000)	ND	5.03	ND
13.859	302.3032	5.618	$C_{18}H_{39}NO_2$	Dihydrosphingosine	Sweet (0.575)	ND	107.95	233.32
8.590	329.2247	1.490	$C_{18}H_{34}O_5$	Fatty acids (18:1;3O)	Sour (0.776)	20.50	ND	ND
15.542	269.2432	1.371	$C_{17}H_{34}O_2$	Heptadecanoic acid	Sour (1.000)	3.63	20.05	21.59
12.940	318.2981	2.550	$C_{18}H_{39}NO_3$	Phytosphingosine	Sweet (0.547)	ND	66.03	52.86
5.278	268.1034	1.581	$C_{10}H_{13}N_5O_4$	Adenosine	Bitter (0.814)	26.33	ND	1.51
9.155	353.2274	2.441	$C_{22}H_{28}N_2O_2$	Anileridine	Bitter (0.930)	8.13	20.73	ND
1.573	104.1069	1.236	$C_5H_{14}NO$	Choline	Bitter (0.751)	29.50	23.61	17.00
11.983	330.2037	1.052	$C_{20}H_{27}NO_3$	Hetisine	Bitter (0.865)	ND	3.43	ND
6.916	177.0148	2.142	$C_9H_6O_4$	6,7-Dihydroxycoumarin	Bitter (0.656)	ND	42.66	41.77
6.647	109.0265	1.016	$C_6H_6O_2$	Catechol	Sweet (0.685)	ND	14.98	15.34
11.422	294.2067	1.067	$C_{17}H_{27}NO_3$	*N*-Vanillylnonanamide	ND	ND	ND	3.68
3.678	163.0353	1.819	$C_9H_8O_3$	*trans*-4-Coumaric acid	Sour (0.654)	ND	ND	9.63
1.985	360.1497	1.287	$C_{12}H_{22}O_{11}$	Isomaltulose	Sweet (0.996)	1.73	4.93	ND

Note: *Values in parentheses indicate probabilities, and ND stands for not detected.

Chili sauce lipids are prone to oxidation and degradation due to microbial metabolism, leading to the generation of a diverse range of aroma compounds, including alcohols, aldehydes, ketones, acids, and esters. Prior investigations into chili sauce have predominantly concentrated on free amino acids (FAAs), organic acids, and volatile aroma components[23-24], with inadequate attention given to lipid composition. Hence, the objective of this study is to investigate the chemical properties and dynamics of lipids in LP, aiming to enhance comprehension of the formation of chili sauce flavor.

3.3. Identification of taste-active metabolites using VirtualTaste

In this study, the taste attributes of the 33 differential components were assessed using the VirtualTaste tool. The results, presented in Table 1, identified 13 metabolites related to sweetness, including arginine, *D*-alloisoleucine, glutamine, isoleucine, *L*-5-oxoproline, phenylalanine, ambocin, kaempferol-7-*O*-deoxyhexoside, dihydrosphingosine, phytosphingosine, catechol, and isomaltulose. Additionally, 9 metabolites related to sour taste were identified, namely 2-hydroxy-4-methylpentanoic acid,

3-phenyllactic acid, *D*-sorbosonic acid, gluconic acid, lactic acid, 3-(4-hydroxyphenyl)-lactic acid, fatty acids, heptadecanoic acid, and *trans*-4-coumaric acid. Finally, 9 metabolites related to bitterness were identified, including theophylline, cyclo(proline-leucine), tyrosine, luteolin, adenosine, anileridine, choline, hetisine, and 6,7-dihydroxycoumarin.

Fermented chili sauce is widely consumed and highly regarded as a popular condiment. However, despite its widespread popularity, our understanding of its flavor profile and the specific taste-active metabolites responsible for its sensory attributes remains limited. In this study, our objective was to address these knowledge gaps and identify key taste-active metabolites in fermented chili sauce, with a particular emphasis on exploring the potential improvements offered by the utilization of LP in its production.

While traditional sensory evaluation methods involving trained experts play a crucial role in assessing the quality of fermented chili sauce, they are often time-consuming, expensive, and carry potential risks associated with direct taste assessment. Consequently, there is an urgent need for an effective and efficient method to predict the taste characteristics of compounds present in fermented chili sauce. Machine learning modeling has emerged as a promising approach for taste prediction in the realm of food ingredients. A noteworthy instance is the VirtualTaste online tool, meticulously crafted for this purpose. It has showcased remarkable performance, reporting a 96% accuracy in predicting sweet and bitter tastes using an independent test set[25].

The utilization of LP in chili sauce production offers specific improvements in product quality and sensory attributes. LP, a probiotic lactic acid bacterium, is known for enhancing the flavor and texture of fermented foods. One notable improvement is the development of a more complex and well-rounded flavor profile. Through our analysis, we successfully identified the top 5 main taste-active metabolites in fermented chili sauce: dihydrosphingosine, isoleucine, phytosphingosine, lactic acid, and gluconic acid. Dihydrosphingosine, isoleucine, and phytosphingosine contribute to the sauce's sweet taste properties, while lactic acid and gluconic acid are associated with its sour taste properties. Our findings provide crucial insights into the taste markers that play a significant role in chili sauce production, allowing for potential adjustments to enhance and refine the overall taste qualities of the sauce according to diverse consumer preferences.

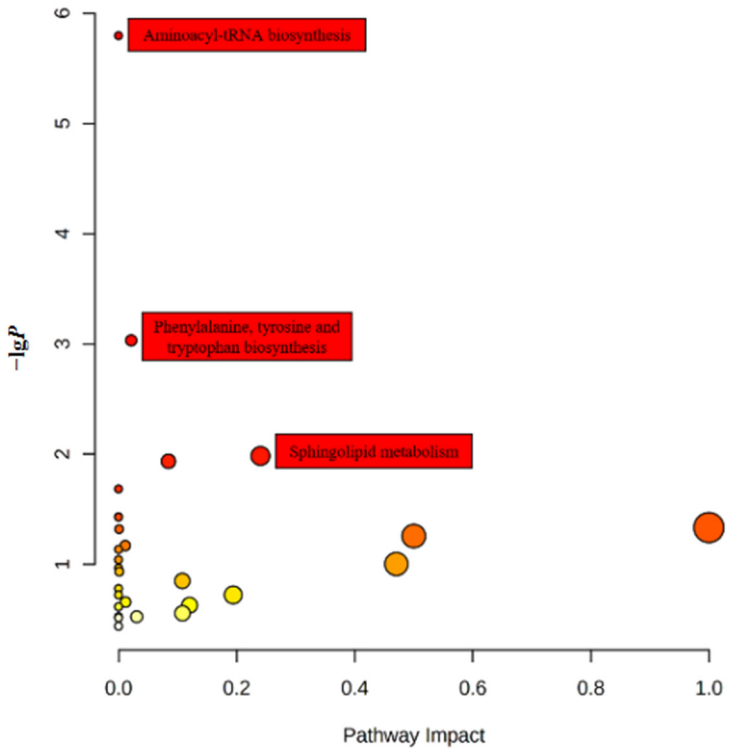
Furthermore, the unique flavor of chili sauce is mainly attributed to their alkaloids, especially capsaicinoids. In our study, we observed that the fermentation process involving LP did not have a significant impact on the total content of capsaicinoids in chili peppers, suggesting that it does not affect the spiciness of the sauce. This finding adds to our understanding of LP fermented chili sauce, its flavor profile, and the specific taste-active metabolites that play a significant role in its sensory attributes.

3.4 KEGG annotation and interpretation of pathway

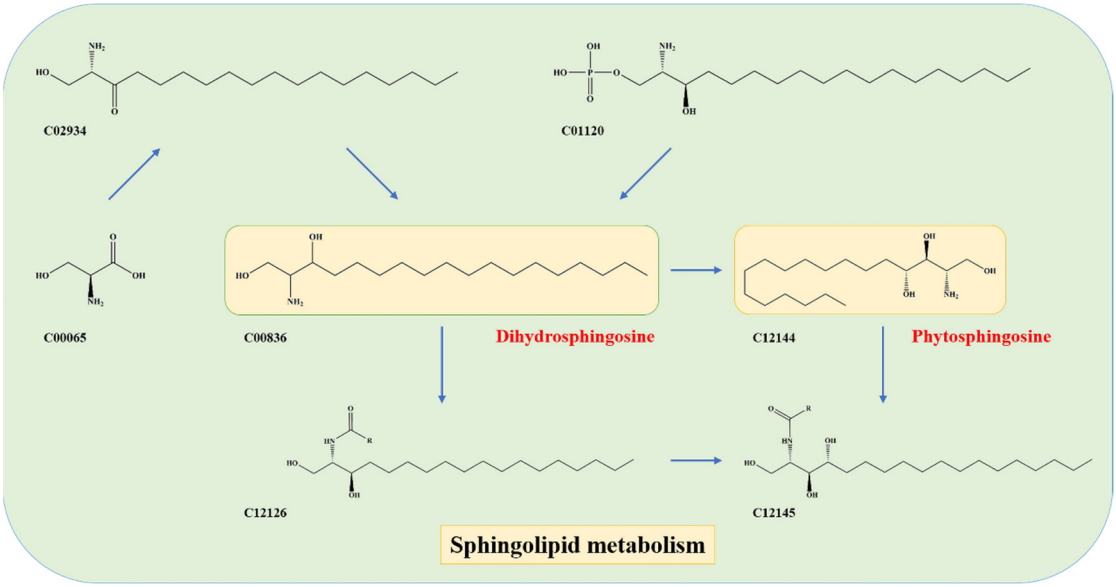
Pathway enrichment analysis, a widely used bioinformatics method for detecting enriched metabolic or signaling pathways using metabolomics data, was performed in this study. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis successfully identified 33 differential metabolites associated with 29 metabolic pathways. The findings of the study are visually presented in Fig. 5A, which demonstrates the

significance of each pathway using both *P*-values and impact factors. Notably, pathways with greater significance are represented by larger and darker bubbles in the figure. A rigorous screening process was conducted to identify significant pathways, including those involved in amino acid tRNA, phenylalanine, tyrosine, and tryptophan biosynthesis, as well as sphingolipid metabolism. This screening process involved setting a *P*-value threshold of less than 0.05 and an influence factor threshold of greater than 0.1, or alternatively, a *P*-value threshold of less than 0.001.

A



B



C

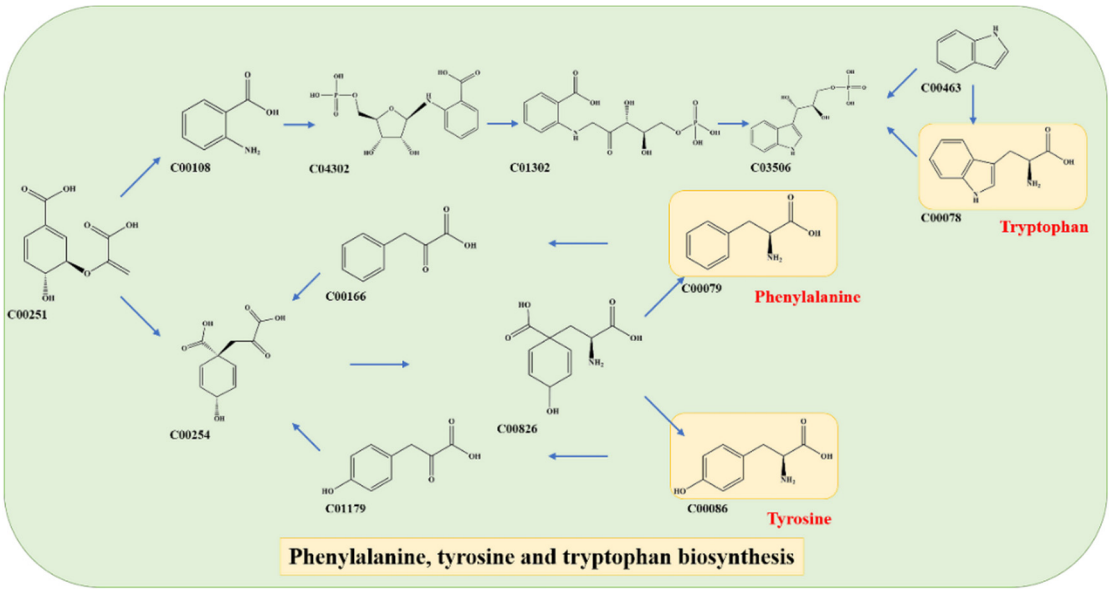


Fig. 5. Pathway analysis of differential metabolites in fermented chili sauces.(A) Identification of key metabolic pathways and enrichment analysis; (B)Sphingolipid metabolism;(C) Phenylalanine, tyrosine, and tryptophan biosynthesis.

The present study highlights the crucial role of serine (C00065) as a precursor in the biosynthesis of dihydrosphingosine (C00836) and phytosphingosine (C12144) (Fig. 5B), 2 vital sphingolipids involved in the early stages of sphingolipid biosynthesis. These sphingolipids are essential for cellular functions such as signal transduction, cell growth, and apoptosis. The biosynthesis of sphingolipids involves enzymatic reactions with various precursor molecules, and serine plays a particularly significant role in this process. Therefore, the availability of serine significantly impacts the efficiency of sphingolipid biosynthesis, which in turn influences taste perception[26]. Additionally, two other metabolic pathways were identified as key pathways of AAA metabolism, including phenylalanine, tryptophan, and tyrosine metabolism. Chorismate (C00251) was identified as a crucial precursor material that is converted to anthranilate (C00108) and prephenate (C00254) (Fig. 5C), ultimately leading to AAA formation[27]. AAAs have been shown to be essential precursors of monoamine neurotransmitters, playing a crucial role in regulating behavior, emotional function, and antioxidant biosynthesis[28-29]. Therefore, the physiological modulatory effects of fermented chili sauce are well recognized, providing valuable insights into the metabolic pathways underlying its taste development and potential implications for enhancing flavor and nutritional properties. Modulating the proportions of these precursor metabolites could be a promising approach to optimize the taste profile of chili sauce.

4. Conclusion

In conclusion, the integration of metabolomics techniques with network computing tools has significantly advanced the identification of taste-active components in chili sauce, facilitating more effective prediction and understanding of the taste characteristics of compounds in fermented chili sauce. In this study, we successfully identified dihydrosphingosine, isoleucine, phytosphingosine, lactic acid, and gluconic acid as key taste-active metabolites in fermented chili sauce. These metabolites hold immense potential as taste markers, providing opportunities to optimize the taste qualities of chili sauce by adjusting their

proportions.Furthermore, pathway enrichment analysis has further supported our findings by uncovering the primary involvement of the identified taste-active metabolites in essential biochemical pathways, such as amino acid tRNA biosynthesis, phenylalanine, tyrosine, tryptophan biosynthesis, and sphingolipid metabolism. These insights deepen our understanding of the underlying biochemical processes associated with the taste characteristics of chili sauce. The findings presented in this study pave the way for further research and development in the field of chili sauce production, opening up new possibilities for tailored flavor profiles and improved sensory experiences.

Declaration of interest statement

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

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Reference

[1]X. Li, X.Q. Cheng, J. Yang, et al., Unraveling the difference in physicochemical properties, sensory, and volatile profiles of dry chili sauce and traditional fresh dry chili sauce fermented by *Lactobacillus plantarum* PC8 using electronic nose and HS-SPME-GC-MS, Food Biosci. 50 (2022) 102057. https://doi.org/10.1016/j.fbio.2022.102057.

[2]Y. Zheng, J. Chen, L. Chen, et al., Analysis and control of microbial gas production in fermented chili paste, J. Food Process Preserv. 44 (2020) 10. https://doi.org/10.1111/jfpp.14806.

[3]N. Echegaray, B. Yilmaz, H. Sharma, et al., A novel approach to *Lactiplantibacillus plantarum*: from probiotic properties to the omics insights, Microbiol. Res. 268 (2023) 127289. https://doi.org/10.1016/j.micres.2022.127289.

[4]Y.Y. Hu, L. Zhang, R.X. Wen, et al., Role of lactic acid bacteria in flavor development in traditional Chinese fermented foods: a review, Crit. Rev. Food Sci. Nutr. 62 (2022) 2741-2755. https://doi.org/10.1080/10408398.2020.1858269.

[5]P. Besnard, P. Passilly-Degrace, N.A. Khan, Taste of fat: a sixth taste modality?, Physiol. Rev. 96 (2016) 151-176. https://doi.org/10.1152/physrev.00002.2015.

[6]B. Avula, K. Katragunta, Y.H. Wang, et al., Chemical profiling and UHPLC-QToF analysis for the simultaneous determination of anthocyanins and flavonoids in *Sambucus* berries and authentication and detection of adulteration in elderberry dietary supplements using UHPLC-PDA-MS, J. Food Compost. Anal. 110 (2022)104584. https://doi.org/10.1016/j.jfca.2022.104584.

[7]K. Barathikannan, R. Chelliah, S.J. Yeon, et al., Untargeted metabolomics of fermented onion (*Allium cepa* L) using UHPLC Q-TOF MS/MS reveals anti-obesity metabolites and *in vivo* efficacy in Caenorhabditis elegans, Food Chem. 404 (2022) 134710. https://doi.org/10.1016/j.foodchem.2022.134710.

[8]V. Dadwal, R. Joshi, M. Gupta, A comparative metabolomic investigation in fruit sections of *Citrus medica* L. and *Citrus maxima* L. detecting potential bioactive metabolites using UHPLC-QTOF-IMS, Food Res. Int. 157 (2022) 111486. https://doi.org/10.1016/j.foodres.2022.111486.

[9]V. Dadwal, R. Joshi, M. Gupta, Comparative metabolomics of Himalayan crab apple (*Malus baccata*) with commercially utilized apple (*Malus domestica*) using UHPLC-QTOF-IMS coupled with multivariate analysis, Food Chem. 402 (2023) 134529. https://doi.org/10.1016/j.foodchem.2022.134529.

[10]M. Vinaixa, E.L. Schymanski, S. Neumann, et al., Mass spectral databases for LC/MS- and GC/MS-based metabolomics: state of the field and future prospects, Trends Analyt. Chem. 78 (2016) 23-35. https://doi.org/10.1016/j.trac.2015.09.005.

[11]L.F. Nothias, D. Petras, R. Schmid, et al., Feature-based molecular networking in the GNPS analysis environment, Nat. Methods 17 (2020) 905-908. https://doi.org/10.1038/s41592-020-0933-6.

[12]F. Olivon, G. Grelier, F. Roussi, et al., MZmine 2 data-preprocessing to enhance molecular networking reliability, Anal. Chem. 89 (2017) 7836-7840. https://doi.org/10.1021/acs.analchem.7b01563.

[13]W. Li, S. Mei, H. Zhou, et al., Metabolite fingerprinting of the ripening process in Pixian douban using a feature-based molecular network and metabolomics analysis, Food Chem. 418 (2023) 135940. https://doi.org/10.1016/j.foodchem.2023.135940.

[14]W. Li, J. Wang, C. Zhang, et al., Using an integrated feature-based molecular network and lipidomics approach to reveal the differential lipids in yak shanks and flanks, Food Chem. 403 (2023) 134352. https://doi.org/10.1016/j.foodchem.2022.134352.

[15]Y. Zhang, X. Bian, G. Yan, et al., Discovery of novel ascorbic acid derivatives and other metabolites in fruit of *Rosa roxburghii* Tratt through untargeted metabolomics and feature-based molecular networking, Food Chem. 405 (2022) 134807. https://doi.org/10.1016/j.foodchem.2022.134807.

[16]X. Zhao, E. Hengchao, H. Dong, et al., Combination of untargeted metabolomics approach and molecular networking analysis to identify unique natural components in wild *Morchella* sp. by UPLC-Q-TOF-MS, Food Chem. 366 (2022) 130642. https://doi.org/10.1016/j.foodchem.2021.130642.

[17]F. Fritz, R. Preissner, P. Banerjee, VirtualTaste: a web server for the prediction of organoleptic properties of chemical compounds, Nucleic. Acids Res. 49 (2021) W679-W684. https://doi.org/10.1093/nar/gkab292.

[18]A. Tobolka, T. Škorpilová, Z. Dvořáková, et al., Determination of capsaicin in hot peppers (*Capsicum* spp.) by direct analysis in real time (DART) method, J. Food Compost. Anal. 103 (2021) 104074. https://doi.org/10.1016/j.jfca.2021.104074.

[19]H. Tsugawa, K. Ikeda, M. Takahashi, et al., A lipidome atlas in MS-DIAL 4, Nat. Biotechnol. 38 (2020) 1159-1163. https://doi.org/10.1038/s41587-020-0531-2.

[20]D. Otasek, J.H. Morris, J. Boucas, et al., Cytoscape automation: empowering workflow-based network analysis, Genome Biol. 20 (2019) 185. https://doi.org/10.1186/s13059-019-1758-4.

[21]J. Blasko, Z. Niznanska, R. Kubinec, et al., Simple, fast method for the sample preparation of major capsaicinoids in ground peppers, in potato chips and chilli sauces and their analysis by GC-MS, J. Food Compost. Anal. 114 (2022) 104733. https://doi.org/10.1016/j.jfca.2022.104733.

[22]Y. Djoumbou Feunang, R. Eisner, C. Knox, et al., ClassyFire: automated chemical classification with a comprehensive, computable taxonomy, J. Cheminform. 8 (2016) 61. https://doi.org/10.1186/s13321-016-0174-y.

[23]Q. Shi, H. Tang, Y. Mei, et al., Effects of endogenous capsaicin stress and fermentation time on the microbial succession and flavor compounds of chili paste (a Chinese fermented chili pepper), Food Res. Int. 168 (2023) 112763. https://doi.org/10.1016/j.foodres.2023.112763.

[24]Z. Ye, Z. Shang, M. Li, et al., Effect of ripening and variety on the physiochemical quality and flavor of fermented Chinese chili pepper (Paojiao), Food Chem. 368 (2022) 130797. https://doi.org/10.1016/j.foodchem.2021.130797.

[25]P. Banerjee, R. Preissner, BitterSweet forest: a random forest based binary classifier to predict bitterness and sweetness of chemical compounds, Front. Chem. 6 (2018) 93. https://doi.org/10.3389/fchem.2018.00093.

[26]C.R. Ferreira, S.M.I. Goorden, A. Soldatos, et al., Deoxysphingolipid precursors indicate abnormal sphingolipid metabolism in individuals with primary and secondary disturbances of serine availability, Mol. Genet Metab. 124 (2018) 204-209. https://doi.org/10.1016/j.ymgme.2018.05.001.

[27]M. Cao, M. Gao, M. Suastegui, et al., Building microbial factories for the production of aromatic amino acid pathway derivatives: from commodity chemicals to plant-sourced natural products, Metab. Eng. 58 (2020) 94-132. https://doi.org/10.1016/j.ymben.2019.08.008.

[28]A. Maroli, V. Nandula, S. Duke, et al., Stable isotope resolved metabolomics reveals the role of anabolic and catabolic processes in glyphosate-induced amino acid accumulation in *Amaranthus palmeri* biotypes, J. Agric. Food Chem. 64 (2016) 7040-7048. https://doi.org/10.1021/acs.jafc.6b02196.

[29]X. Zhang, K. Yoshihara, N. Miyata, et al., Dietary tryptophan, tyrosine, and phenylalanine depletion induce reduced food intake and behavioral alterations in mice, Physiol. Behav. 244 (2022) 113653. https://doi.org/10.1016/j.physbeh.2021.113653.