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# Exploring the influence of garlic on microbial diversity and metabolite dynamics during kimchi fermentation

Ha-Young Jang<sup>a,1</sup>, Min Ji Kim<sup>a,b,1</sup>, Ji Young Jeong<sup>a</sup>, In Min Hwang<sup>a,\*\*</sup>, Jong-Hee Lee<sup>a,\*</sup>

<sup>a</sup> Fermentation Regulation Research Group, Technology Innovation Research Division, World Institute of Kimchi, Gwangju 61755, Republic of Korea <sup>b</sup> Division of Food and Nutrition, Chonnam National University, Gwangju 61186, Republic of Korea

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## ABSTRACT

Garlic (Allium sativum) is a key ingredient in Korean cuisine, particularly in the preparation of kimchi, contributing to its flavor and taste. Garlic has been a potential resource for lactic acid bacteria (LAB) in kimchi. However, the mechanism by which it influences microbial diversity and metabolite production is unclear. This study investigated the effect of garlic on the bacterial composition of and metabolite changes in kimchi. To achieve this, four separate batches of kimchi were prepared with varying garlic concentrations (w/w): 0 %, 1 %, 2 %, and 4 %, and the bacterial communities and metabolite production were monitored. In the early stages of fermentation, the count of LAB, operational taxonomic units (OTUs), and Shannon index increased linearly with the increase in garlic content. This indicated that garlic is a rich resource and contributes to the diversity of LAB during kimchi fermentation. Compared with the kimchi samples with a lower garlic content, those with a high garlic content ( $\geq 2$  %) exhibited increased abundance of Lactobacillus and Leuconostoc as well as noticeable differences in functional diversity, including carbohydrate, amino acid, and energy metabolisms. Correlation analysis between sugars, organic acids, and predominant LAB in the garlic-containing kimchi samples suggested that in kimchi samples with high garlic content, LAB played a significant role in the fermentation process by metabolizing sugars and producing organic acids. Overall, this study demonstrated that the addition of garlic has a positive impact on the bacterial diversity and metabolite production during kimchi fermentation, potentially affecting the fermentation process and flavor profile of kimchi.

#### 1. Introduction

Kimchi is a representative traditional fermented food in Korea. It comprises a variety of vegetables, including salted kimchi cabbage, garlic, leek, ginger, and dried red pepper powder, as well as other regional and seasonal ingredients [1]. It is a non-sterilized fermented food, and its quality and taste are influenced by a diverse range of microorganisms. Fermentation plays a crucial role in kimchi preservation as it prevents the growth of harmful bacteria and promotes the production of phytochemicals and probiotic lactic

\* Corresponding author.

\*\* Corresponding author.

<sup>1</sup> Both authors contributed equally to this manuscript.

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E-mail addresses: imhwang@wikim.re.kr (I.M. Hwang), leejonghee@wikim.re.kr (J.-H. Lee).

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#### Abbreviations

γ-aminobutyric acid (GABA)
hydroxyisocaproic acid (HICA)
lactic acid bacteria (LAB)
liquid chromatography-mass spectrometry (LC-MS)
de Man, Rogosa, and Sharpe (MRS)
operational taxonomic units (OTUs)
principal component analysis (PCA)
phenyllactic acid (PLA)
arginine deiminase (ADI)

acid bacteria (LAB) [2]. Low temperature, high salinity, and hypoxic conditions are favorable for kimchi fermentation. Notably, the LAB found in kimchi are either psychrophilic or psychrotrophic, facultative anaerobic, salt- and acid-tolerant organisms, and they are the dominant bacterial species during the fermentation process [3–6]. Moreover, the production of organic acids by LAB increases the acidity of kimchi and prevents the growth of non-LAB [4].

Studies on bacteria in kimchi have revealed the genera *Lactobacillus, Leuconostoc*, and *Weissella* as the dominant LAB. The raw ingredients used for the production of kimchi are the major sources of microorganisms and also are the nutritional resources for bacterial growth [7]. Notably, recent studies have demonstrated that kimchi ingredients influence the ontogeny of LAB. For example, red pepper has been identified as a source of *Weissella*, including *Weissella* cibaria and *Weissella koreensis* [8]. The addition of red pepper powder was shown to increase the abundance of *Weissella* species, which possess strong arginine deiminase (ADI) activity, resulting in increased levels of ornithine and nitrogen cycle metabolites [9]. Another study indicated that *W. koreensis* is associated with sorbitol, thiamine, and folate production in kimchi [10]. These findings demonstrated that the raw ingredients used for the production of kimchi serve as bacterial resources and play significant roles in metabolite production beyond their function as precursors.

Garlic is an essential seasoning ingredient in kimchi and a nutritional resource rich in carbohydrates, organosulfur compounds, proteins, free amino acids, fiber, vitamins, and minerals [11–13]. Studies on the bacterial origins of kimchi have indicated that garlic is an abundant source of LAB [7,14,15]. In particular, the addition of garlic influences the bacterial composition of kimchi during the early stages of fermentation [15]. Therefore, garlic has been suggested to play a key role in the dynamics of bacterial composition and metabolite production during kimchi fermentation. Although previous studies have shown that garlic is a potential resource for LAB in kimchi, the specific mechanisms by which it influences metabolite production remain unclear. The present study addressed this gap by investigating how garlic contributes to the metabolic pathways during kimchi fermentation, specifically focusing on the bacterial composition and related changes in metabolites. To investigate this, we prepared various batches of kimchi with different garlic concentrations and analyzed the bacterial taxa and metabolites using pyrosequencing and liquid chromatography-mass spectrometry (LC-MS), respectively.

## 2. Materials and methods

# 2.1. Kimchi preparation and physiological properties

To investigate the effect of garlic on kimchi fermentation, different batches of kimchi with varying garlic concentrations (0 %, 1 %, 2 %, and 4 %, w/w designated as kimchi A0, A1, A2, A4, respectively) were prepared. Kimchi was prepared using the following raw materials obtained from a local market: salted kimchi cabbage (84 %), red pepper powder (3 %), radish (3 %), ginger (0.8 %), onion (2.5 %), salted shrimp (1.5 %), glutinous rice paste (0.8 %), water (4.4 %), and garlic (0 %, 1 %, 2 %, and 4 %).

To analyze its physiological properties, kimchi was homogenized using a hand blender (Philips, Eindhoven, Netherlands) and then filtered through sterilized gauze. The obtained filtrate was used to measure the physiological properties, bacterial content, titratable acidity, and pH. Titratable acidity was determined using a pH meter and the filtrate was titrated to pH 8.3 using 0.1 M NaOH [16]. The total bacterial and LAB contents were measured using Petrifilm AC and LAB plates, respectively (3 M, St Paul, MN, USA). One milliliter of kimchi filtrate was spread on Petrifilm and incubated at 30 °C for 24 h.

#### 2.2. Analysis of bacterial composition

The bacterial community analysis was performed by CJ-Bioscience (Seoul, Korea) using 16S rDNA pyrosequencing with total DNA extracted from kimchi. Total DNA extraction was carried out following the manufacturer's instructions using the Fast DNA Spin kit (MP Biomedicals, CA, USA). A 16S rDNA sequencing library was prepared using the 16S metagenomic sequencing library preparation protocol (Illumina, San Diego, CA, USA). The V3 and V4 hypervariable regions of the 16S rRNA gene were amplified and purified using the KAPA HiFi HotStart ReadyMix kit (KAPA Biosystems, Wilmington, MA, USA) and Agencourt AMPure XP system (Beckman Coulter, Brea, CA, USA).

Quality assessment and product size analyses were performed on a Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA) using a DNA

7500 chip. The amplified fragments were pooled, and sequencing was conducted on an Illumina MiSeq Sequencing System (Illumina, CA, USA) following the manufacturer's instructions. Raw reads were processed by initially checking the quality and filtering out lowquality reads (<Q25) using Trimmomatic ver. 0.321. After the reads passed the quality check, the paired-end sequences were merged using the fastq\_mergepairs command of VSEARCH version 2.13.42 with the default parameters. Taxonomic assignments were made using the EzBioCloud 16S rRNA database 5. Chimeric reads with <97 % similarity were removed using the UCHIME algorithm 6 and non-chimeric 16S rRNA database from EzBioCloud. Reads that could not be identified at the species level (with <97 % similarity) in the EzBioCloud database were compiled, and the cluster fast command 2 was used to perform de novo clustering and generate additional operational taxonomic units (OTUs). Secondary analysis, including diversity calculations (ACE and Shannon indices), was conducted using in-house programs developed by CJ Bioscience, Inc. (Seoul, Korea).

#### 2.3. Metabolite analysis using UHPLC-Q-orbitrap MS

Standard reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA). Formic acid (98 % purity) was purchased from Fluka (Buchs, Switzerland). All LC-MS solvents were purchased from J. T. Baker (Phillipsburg, NJ, USA). Ultrapure water was obtained using the Milli-Q Plus and Milli-Q systems (Millipore, MA, USA). A water/methanol mixture (80/20, v/v) containing 0.1 % formic acid was used to optimize the extraction solvent. Approximately 2 g of the homogenized kimchi sample were weighed and placed in a polypropylene tube. Subsequently, 20 mL of the extraction solvent was added, and the mixture was shaken for 5 min. Ultrasonic extraction was performed for 20 min. The extracted sample was centrifuged at  $3200 \times g$  for 10 min, and the supernatant was filtered through a 0.22 µm syringe filter for direct injection in ultra-high-pressure liquid chromatography (UHPLC). The solution was transferred and diluted using the extraction solvent for the identification and quantitative analysis of metabolites. A quadrupole-Orbitrap mass spectrometer coupled with a UHPLC Dionex Ultimate 3000 (Thermo Scientific, Bremen, Germany) was used for metabolite quantification. The analytical conditions were as follows: solvent A, distilled water containing 0.1 % formic acid; solvent B, acetonitrile containing 0.1 % formic acid; injection volume, 2 µL; flow rate, 0.3 mL/min; spray voltage, 4 kV; source temperature, 300 °C. The transition of the metabolites was as reported previously [17]. The transition list is provided in Supplementary Table 1.

#### 2.4. Statistical analysis

The data are presented as means  $\pm$  standard deviations. The statistical analysis of metagenome data was performed using a twosample comparison with a two-sided Fisher's exact test in the STAMP software [16]. Statistical significance was set at p < 0.05.

#### 3. Results

#### 3.1. Physicochemical properties of kimchi

The kimchi samples were stored at 4 °C and collected weekly to monitor the progress of kimchi fermentation. After one week of fermentation, significant changes in the overall physicochemical properties were observed (Fig. 1). The initial acidity of kimchi A was approximately 0.3 at week 0 and did not vary depending on the garlic content. However, at week 1, compared with kimchi A0, kimchi A4 exhibited a significant increase in acidity (p < 0.05) (Fig. 1A). The LAB content also showed a significant difference (p < 0.05) between kimchi A0 and the other kimchi samples at week 1 (Fig. 1B). The initial LAB contents of kimchi A0, A1, A2, and A4 were 4.56  $\pm$  0.31, 4.77  $\pm$  0.21, 4.48  $\pm$  0.44, and 4.95  $\pm$  0.05 log CFU/mL, respectively. At week 1, the LAB content of A0, A1, A2, and A4 increased to 6.35  $\pm$  0.06, 5.67  $\pm$  0.35, 6.99  $\pm$  0.18, and 7.47  $\pm$  0.02 log CFU/mL, respectively (Fig. 1B). No significant differences in the total bacterial count were observed among the kimchi samples during fermentation (Fig. 1C).

#### 3.2. Bacterial community analysis

The bacterial community was analyzed by pyrosequencing technology and processed using EzBioCloud by CJ Bioscience (Seoul, Korea). Bacterial diversity and richness were determined using OTU and Shannon indices, respectively. Compared with kimchi samples with garlic contents of 0 % and 1 %, those with garlic contents of 2 % and 4 % exhibited higher OTU and Shannon indices at one week of fermentation, which was consistent with the higher LAB count in A2 and A4 than that in A0 and A1 (Table 1).

A total of 56 LAB species belonging to four genera, namely *Weissella*, *Leuconostoc*, *Lactococcus*, and *Lactobacillus*, were identified in the kimchi samples (Fig. 2A). Among them, 15 species accounted for more than 0.1 % of the total bacterial population; their relative proportions and count are shown in Fig. 1 and Supplementary Fig. 1, respectively. Notably, the identified bacterial taxa showed distinct differences depending on the kimchi's garlic content. After one week of fermentation, significant changes in the LAB composition were observed. Kimchi samples with low garlic content (A0 and A1) were predominantly composed of *Weissella* species (89–92 %), with the highest abundance of *Weissella kandleri* and *Weissella diestrammenae* (Fig. 2B).

In contrast, A2 and A4 exhibited a more diverse LAB composition, including *Lactobacillus* (36–37 %), *Leuconostoc* (20–21 %), and *Weissella* (23–26 %) (Fig. 2A). They had higher abundances of LAB species such as *Leuconostoc carnosum*, *Latilactobacillus sakei*, *Leuconostoc gilidum*, *Leuconostoc mesenteroides*, and *Dellaglioa algida*. Moreover, the abundances of *Weissella ghanesis*, *Weissella paramesenteroides*, and *Weissella confusa* were slightly higher in A2 and A4 than in A0 and A1 (Fig. 2B).

To examine the functional differences in bacterial composition, a PICRUSt analysis was performed based on the KEGG database [18]. Fig. 3 presents all significantly different (p < 0.05) functions in terms of their abundances. The analysis revealed two distinct

metabolic pathways between A0 and A1 at one week of fermentation (Fig. 3A). Furthermore, eight metabolic pathways, including carbohydrate metabolism and energy metabolism, differed between the bacterial taxa of A2 and A4 and those of A0 at one week (Fig. 3B and C). These results suggested that an increase in garlic concentration in kimchi increases the complexity of metabolic



**Fig. 1.** Physicochemical properties of kimchi fermented with varying garlic contents. Changes in (A) acidity (%), (B) lactic acid bacteria count (log CFU/mL), and (C) total bacteria count (log CFU/mL) of kimchi samples during fermentation with different garlic concentrations. Groups: A0, 0 % garlic; A1, 1 % garlic; A2, 2 % garlic; and A4, 4 % garlic. Error bars represent the standard deviation among the three replicates.

Table 1			
Summary of pyrosequencing	and statistical ana	alysis results for	kimchi samples.

Sample name	Garlic content (%)	Fermentation time (weeks)	Target reads	OTUs	Shannon
A0_0W	0	0	10270	491	4.41
A0_1W	0	1	29117	196	0.71
A0_2W	0	2	26529	159	1.54
A0_3W	0	3	31084	104	0.45
A0_4W	0	4	38251	85	0.29
A1_0W	1	0	16035	570	4.41
A1_1W	1	1	28478	202	0.62
A1_2W	1	2	33080	112	1.07
A1_3W	1	3	33106	85	0.56
A1_4W	1	4	30576	94	0.42
A2_0W	2	0	14708	544	4.43
A2_1W	2	1	16649	257	2.33
A2_2W	2	2	29115	122	1.12
A2_3W	2	3	38792	91	0.42
A2_4W	2	4	38475	253	1.22
A4_0W	4	0	13840	519	4.32
A4_1W	4	1	30266	372	2.49
A4_2W	4	2	36588	184	1.55
A4_3W	4	3	29606	70	0.61
A4_4W	4	4	35163	86	0.48

Summary of sequencing results, operational taxonomic units (OTUs), and microbial alpha-diversity (Shannon) indices for kimchi samples with varying garlic content. Sample names A0–A4 represent garlic content (0 %, 1 %, 2 %, and 4 %, respectively) at different time points (week 0–4) during the fermentation period.

activities within the bacterial community.

#### 3.3. Metabolite analysis

The composition of kinchi metabolites depending on the garlic content was investigated. Changes in metabolites, including sugars, amino acids, and organic acids, were quantified using LC-MS. A statistical analysis of these metabolites was performed using the SPSS Statistical Package for Social Sciences Software Version 20 (IBM, NY, USA), and the detailed data are presented in Table 2.

During fermentation, the hexose (glucose + fructose) and sucrose content significantly decreased. The hexose content was approximately 44.7%–50.3 % in A0 and A1 and 26.5%–35.7 % in A2 and A4 after three weeks of fermentation. The sucrose content ranged from 11.2 % to 14.7 % in A0 and A1 and from 2.6 % to 3.6 % in A2 and A4 after three weeks of fermentation. The sugar alcohols, namely mannitol and sorbitol, were not detected initially but were detected earlier in A2 and A4 after one week of fermentation, with concentrations of 12.50  $\pm$  1.45 mM and 14.18  $\pm$  2.70 mM, respectively, in A4.

Initially, the citric acid concentration was slightly higher in A2 and A4 ( $2.94 \pm 0.27 \text{ mM}-4.25 \pm 0.38 \text{ mM}$ ) than in A0 and A1 ( $2.75 \pm 0.29 \text{ mM}-2.76 \pm 0.33 \text{ mM}$ ). However, as fermentation progressed, the citric acid content decreased to a range of  $2.25 \pm 0.20 \text{ mM}$  to  $1.02 \pm 0.08 \text{ mM}$  for A0 and A1 and to  $0.28 \pm 0.03 \text{ mM}$  and undetectable in A2 and A4, respectively. The malic acid content initially ranged from  $7.13 \pm 0.20 \text{ mM}$  to  $10.09 \pm 0.49 \text{ mM}$ , but it decreased to a range of  $6.98 \pm 0.41 \text{ mM}$  to  $6.78 \pm 0.89 \text{ mM}$  in A0 and A1 and was completely depleted in A2 and A4. The succinic acid content increased slightly throughout the fermentation period but was lower in A2 and A4 than in A0 and A1. The initial lactic acid content ranged from  $0.82 \pm 0.07 \text{ mM}$  to  $1.31 \pm 0.14 \text{ mM}$ , but significantly increased after one week of fermentation in A2 and A4, reaching  $17.94 \pm 1.61 \text{ mM}$  and  $29.85 \pm 2.45 \text{ mM}$ , respectively. After one week of fermentation, the lactic acid contents of A0 and A1 were  $6.37 \pm 0.61 \text{ mM}$  and  $2.60 \pm 0.22 \text{ mM}$ , respectively. The higher lactic acid content was consistent with the higher acidity observed in A2 and A4 after one week of fermentation (Fig. 1A).

Of the 16 amino acids measured, glutamine was the most abundant, with the highest amount detected at the initial time point ranging from  $65.22 \pm 5.23$  mM to  $84.11 \pm 7.54$  mM. The glutamine content slightly decreased during fermentation, but no significant changes were observed depending on the garlic content or the fermentation progress. The addition of garlic resulted in a linear increase in the arginine content at the initial time point, with concentrations of  $1.93 \pm 0.27$  mM,  $2.02 \pm 0.24$  mM,  $2.24 \pm 0.24$  mM, and  $2.86 \pm 0.31$  mM in A0, A1, A2, and A4 samples, respectively. Furthermore, the content of known secondary metabolites produced during kimchi fermentation were investigated [19–21]. Hydroxyisocaproic acid (HICA), phenyllactic acid (PLA), and  $\gamma$ -aminobutyric acid (GABA) were quantified and their levels increased slightly during the fermentation process, but no significant changes were observed with the addition of garlic (Table 2).

Principal component analysis (PCA) was performed to investigate the changes in metabolites depending on the garlic concentration. The PCA score plot and loading showed that the garlic content primarily affected the content of metabolites, with kimchi samples with a high garlic content clustering along PC1 (Fig. 4). Notable divergence was observed between high garlic and low garlic kimchi samples over four weeks of fermentation (Fig. 4A–E). PC1 accounted for 89.63 %, 76.46 %, 71.83 %, and 67.36 % of the total variance for week 1, 2, 3 and 4, respectively. The biplot indicated that organic acids and sugars were the contributing factors to the divergence between kimchi samples with different concentrations of kimchi. Mannitol, sorbitol, and lactic acid showed the same trend as A2 and A4 groups, while hexose, inositol, and malic acid showed the opposite trend. These findings indicated that garlic promotes



Fermentation Time (weeks)

**Fig. 2.** Analysis of bacterial communities in kimchi fermented with varying garlic contents. Distribution of bacterial communities at the (A) genera (%), and (B) species level (%). The major LAB groups of *Lactobacillus, Leuconostoc, Weissella*, and *Lactococcus* genera are represented in Fig. 2A. The indicated bacteria at the species level, which accounted for more than 0.1 % of the total bacterial population, are represented in Fig. 2B. The bacteria not included in this category are represented as the ETC group. The relative abundance of bacterial communities at the genera and species level (%) depending on the garlic content over four weeks of fermentation is shown. Groups: A0, 0 % garlic; A1, 1 % garlic; A2, 2 % garlic; and A4, 4 % garlic. Sample names A0–A4 represent the garlic contents (0 %, 1 %, 2 %, and 4 %, respectively) at different time points (week 0–4) during the fermentation period.



**Fig. 3.** Functional characterization of different groups based on the PICRUSt analysis. Comparison of PICRUSt-predicted functional pathways was performed using a two-sided Welch's *t*-test and filtered for false discoveries using the Benjamini Hochberg method. The bar plots on the left side display the mean proportion of each KEGG pathway. The dot plots on the right side show the differences in mean proportions between the two indicated groups with corresponding *p*-values. Items with *p*-values <0.05 are shown in the figure. The extended error bars method in STAMP was used to display the relative difference in pathways for comparisons of kimchi samples with 0 %, 1 %, 2 %, and 4 % garlic at week 1. A0, 0 % garlic; A1, 1 % garlic; A2, 2 % garlic; and A4, 4 % garlic. Comparisons are shown as (A) A0 and A1, (B) A0 and A2, and (C) A0 and A4, respectively.

the functional differences in bacterial composition, particularly by enhancing the carbohydrate metabolic processes.

The correlations between bacteria and metabolites were analyzed using Spearman's rank correlation coefficient. The results indicated that *D. algida, L. sakei, L. carnosum, L. gelidum*, and *L. mesenteroides*, which were highly abundant in A2 and A4, were negatively correlated with malic acid, inositol, hexose, and sucrose, and positively correlated with lactic acid, mannitol, and sorbitol in garlic-containing kimchi (Fig. 5).

#### 4. Discussion

Garlic is a widely used seasoning in Korean cuisine. In 2017, Korea was the leading producer of garlic, followed by China, India, and Bangladesh [22]. Garlic is rich in amino acids, carbohydrates, flavonoids such as anthocyanin, vitamins, and allyl sulfur-containing compounds [11,23,24].

Garlic serves as an important ingredient in kimchi, contributing to its flavor and inhibiting the growth of harmful bacteria [25]. Recent studies have revealed that garlic also serves as an abundant source of LAB during kimchi fermentation [7]. Therefore, it is intriguing to investigate the effects of garlic on kimchi fermentation, particularly regarding LAB and metabolite production.

Studies on the dominant bacterial taxa in the raw ingredients used for kimchi preparation suggested that leek, ginger, kimchi cabbage, and garlic are sources of LAB, with garlic being a major source [7,15,26]. Garlic samples exhibit variations in LAB species; for example, *L. mesenteroides, Leuconostoc citreum*, and *W. cibaria* differed among garlic samples. One study reported that *Leuconostoc* accounted for 77 % of total LAB content, followed by *Weissella* and *Lactobacillus* [27]. Omitting garlic from kimchi preparation resulted in a reduction in the abundance of *L. citreum* [15]. Moreover, another study showed that the gamma-ray irradiation of raw materials (ginger, red pepper powder, and cabbage), except for garlic, contributed to abundance of *Leuconostoc* and *Weissella* during kimchi fermentation [26].

In the present study, we obtained detailed information on the bacterial composition of kimchi through pyrosequencing analysis, which revealed that the major LAB groups in kimchi fermentation were *Weissella*, *Leuconostoc*, and *Lactobacillus*. The A2 and A4 groups showed an increase in the abundance of *Leuconostoc* (*L. carnosum*, *L. gelidum*, *L. mesenteroides*) and *Weissella* species (*W. ghanensis*, *W. paramesenteroides*, and *W. confusa*). These results were consistent with a previous report and suggested that garlic is an especially rich source of LAB, in particular *Leuconostoc* and *Weissella* [26,27].

In addition, the abundance of L. sakei was higher in A2 and A4 than in A0 and A1. L. sakei is the most common LAB species in

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Metabolites (mM)	A0 A1								A2					<u>A4</u>						
	0W	1W	2W	3W	4W	0W	1W	2W	3W	4W	0W	1W	2W	3W	4W	0W	1W	2W	3W	4W
Sugar and Sugar alcohol																				
Mannitol	N.D.	N.D.	$\begin{array}{c} 24.79 \ \pm \\ 2.80^{e} \end{array}$	$\begin{array}{c} 34.24 \\ \pm \ 2.39^d \end{array}$	$\begin{array}{c} 52.42 \\ \pm \ 4.19^a \end{array}$	N.D.	N.D.	$34.06 \pm 3.51^{d}$	$40.38 \pm 3.52^{\circ}$	$\begin{array}{c} 49.88 \\ \pm \ 6.24^a \end{array}$	N.D.	N.D.	$\begin{array}{c} 35.58 \\ \pm \ 2.09^d \end{array}$	${\begin{array}{c} {\rm 44.76} \pm \\ {\rm 1.36^{b}} \end{array}}$	$\begin{array}{c} 42.49 \pm \\ 3.50^{bc} \end{array}$	N.D.	$\begin{array}{c} 12.50 \pm \\ 1.45^{\mathrm{f}} \end{array}$	$\begin{array}{c} \textbf{26.77} \pm \\ \textbf{2.84}^{e} \end{array}$	$36.86 \pm 2.65^{d}$	${\begin{array}{c} {\rm 45.73} \pm \\ {\rm 1.54^b} \end{array}}$
Sorbitol	N.D.	N.D.	$\begin{array}{c} 29.29 \pm \\ 6.08^{f} \end{array}$	$\begin{array}{c} 39.52 \\ \pm \ 2.55^e \end{array}$	$59.21 \pm 4.50^{a}$	N.D.	N.D.	$\begin{array}{c} 39.32 \\ \pm \ 3.76^{\rm e} \end{array}$	46.17 ± 3.24 <sup>cd</sup>	$\begin{array}{c} 56.46 \\ \pm \ 4.80^a \end{array}$	N.D.	$\begin{array}{c} 1.96 \pm \\ 0.40^h \end{array}$	$\begin{array}{l} 40.97 \\ \pm \ 2.24^e \end{array}$	$\begin{array}{l} 49.12 \pm \\ 2.87^{bc} \end{array}$	$\begin{array}{l} 48.46 \ \pm \\ 3.75^{bc} \end{array}$	N.D.	${\begin{array}{c} 14.18 \ \pm \\ 2.70^{g} \end{array}}$	$\begin{array}{c} 31.43 \pm \\ 6.09^{f} \end{array}$	$\begin{array}{l} 42.35 \pm \\ 3.70^{de} \end{array}$	${\begin{array}{c} 51.96 \pm \\ 1.70^{b} \end{array}}$
Inositol	$\begin{array}{c} 101.53 \\ \pm \ 2.94^{\rm a} \end{array}$	$\begin{array}{c} 86.19 \ \pm \\ 3.13^{d} \end{array}$	$\begin{array}{l} 67.21 \ \pm \\ 2.04^{e} \end{array}$	$\begin{array}{c} 45.42 \\ \pm \ 1.31^{\text{g}} \end{array}$	$\begin{array}{c} 39.96 \\ \pm \ 2.50^h \end{array}$	92.34 ± 6.65°	$96.51 \pm 6.99^{b}$	$\begin{array}{c} 54.94 \\ \pm \ 1.97^{\rm f} \end{array}$	$\begin{array}{c} 46.46 \\ \pm \ 1.58^{\rm g} \end{array}$	$46.99 \pm 3.36^{g}$	98.62 $\pm$ 2.85 <sup>ab</sup>	$\begin{array}{l} 91.62 \pm \\ 5.94^{c} \end{array}$	$\begin{array}{c} \textbf{38.26} \\ \pm \textbf{2.48}^{h} \end{array}$	$\begin{array}{c} \textbf{26.17} \pm \\ \textbf{0.76}^i \end{array}$	$\begin{array}{c} 28.99 \pm \\ 0.90^i \end{array}$	${75.80} \pm \\ {2.58}^{e}$	${\begin{array}{c} 55.54 \ \pm \\ 1.60^{f} \end{array}}$	${\begin{array}{c} {\rm 44.86} \pm \\ {\rm 1.30^g} \end{array}}$	$\begin{array}{c} \textbf{27.10} \pm \\ \textbf{2.32}^i \end{array}$	$\begin{array}{c} 24.97 \pm \\ 0.97^i \end{array}$
Hexose	$\begin{array}{c} 101.53 \\ \pm \ 2.94^{a} \end{array}$	$\begin{array}{c} 86.19 \pm \\ 3.13^{d} \end{array}$	$67.21 \pm 2.04^{e}$	$45.42 \pm 1.31^{g}$	$\begin{array}{l} 39.96 \\ \pm \ 2.50^{\rm h} \end{array}$	92.34 ± 7.66°	$96.51 \pm 6.99^{b}$	$\begin{array}{c} 54.94 \\ \pm \ 1.97^{\rm f} \end{array}$	$\begin{array}{c} \textbf{46.46} \\ \pm \ \textbf{1.58}^{\textbf{g}} \end{array}$	$\begin{array}{c} \textbf{46.99} \\ \pm \textbf{3.88}^{\textbf{g}} \end{array}$	2.85 98.62 ± 2.85 <sup>ab</sup>	$\begin{array}{l} 91.62 \pm \\ 5.94^c \end{array}$	$\begin{array}{c} \textbf{38.26} \\ \pm \textbf{ 2.48}^{h} \end{array}$	$\begin{array}{c} 26.17 \pm \\ 0.76^i \end{array}$	$\begin{array}{c} \textbf{28.99} \pm \\ \textbf{0.90}^i \end{array}$	${75.80} \pm \\ {2.58}^{e}$	${\begin{array}{c} 55.54 \pm \\ 1.60^{f} \end{array}}$	$\begin{array}{l} 44.86 \pm \\ 1.30^g \end{array}$	$\begin{array}{c} \textbf{27.10} \pm \\ \textbf{2.32}^i \end{array}$	$\begin{array}{c} \textbf{24.97} \pm \\ \textbf{0.97}^i \end{array}$
Sucrose	$\begin{array}{c} 0.80 \ \pm \\ 0.05^c \end{array}$	$\begin{array}{c} 0.42 \pm \\ 0.02^i \end{array}$	$\begin{array}{c} 0.69 \ \pm \\ 0.03^d \end{array}$	$\begin{array}{c} 0.09 \ \pm \\ 0.00^k \end{array}$	$\begin{array}{c} 0.04 \ \pm \\ 0.00^l \end{array}$	$\begin{array}{c} 0.88 \pm \\ 0.05^b \end{array}$	$\begin{array}{c} 0.48 \pm \\ 0.02^h \end{array}$	$\begin{array}{c} 0.46 \ \pm \\ 0.02^h \end{array}$	$\begin{array}{c} 0.13 \pm \\ 0.01^{j} \end{array}$	$\begin{array}{c} 0.05 \ \pm \\ 0.00^{kl} \end{array}$	$\begin{array}{c} 1.12 \pm \\ 0.06^a \end{array}$	$\begin{array}{c} 0.53 \ \pm \\ 0.03^{g} \end{array}$	$\begin{array}{c} 0.40 \ \pm \\ 0.02^{j} \end{array}$	$\begin{array}{c} 0.03 \pm \\ 0.00^l \end{array}$	$\begin{array}{c} 0.03 \pm \\ 0.00^l \end{array}$	$\begin{array}{c} 1.10 \ \pm \\ 0.07^a \end{array}$	$\begin{array}{c} \textbf{0.64} \pm \\ \textbf{0.04}^{e} \end{array}$	$\begin{array}{c} 0.59 \ \pm \\ 0.04^{f} \end{array}$	$\begin{array}{c} 0.04 \pm \\ 0.00^l \end{array}$	$\begin{array}{c} 0.02 \pm \\ 0.00^l \end{array}$
Organic acids																				
Malic acid	$\begin{array}{c} 8.02 \pm \\ 0.22^b \end{array}$	${\begin{array}{c} {\rm 6.98} \pm \\ {\rm 0.41}^{\rm cd} \\ \end{array}}$	N.D.	N.D.	N.D.	$\begin{array}{c} 7.13 \pm \\ 0.20^c \end{array}$	$6.78 \pm 0.89^{d}$	N.D.	N.D.	N.D.	$\begin{array}{c} \textbf{7.81} \pm \\ \textbf{0.21}^{b} \end{array}$	N.D.	N.D.	N.D.	N.D.	$\begin{array}{c} 10.09 \pm \\ 0.49^a \end{array}$	N.D.	N.D.	N.D.	N.D.
Lactic acid	$\begin{array}{c} 0.85 \pm \\ 0.07^i \end{array}$	$\begin{array}{c} \textbf{6.37} \pm \\ \textbf{0.61}^i \end{array}$	${\begin{array}{c} 38.91 \pm \\ 3.25^{f} \end{array}}$	54.59 ± 4 11 <sup>cd</sup>	$66.42 \pm 4.94^{b}$	$\begin{array}{c} 1.08 \pm \\ 0.13^i \end{array}$	$\begin{array}{c} 2.60 \pm \\ 0.22^i \end{array}$	$47.51 \pm 5.10^{e}$	55.74 ± 4.85 <sup>cd</sup>	59.79 ± 4.88 <sup>c</sup>	$\begin{array}{c} 0.82 \pm \\ 0.07^i \end{array}$	${17.94} \pm {1.61}^{\rm h}$	59.63 ± 5.36 <sup>c</sup>	${\begin{array}{c} 68.91 \pm \\ 5.32^{b} \end{array}}$	$\begin{array}{l} 68.08 \ \pm \\ 6.98^{b} \end{array}$	$\begin{array}{c} 1.31 \pm \\ 0.14^i \end{array}$	${29.85\ \pm}\\{2.45^{g}}$	$\begin{array}{l} 52.08 \pm \\ 5.61^{de} \end{array}$	${\begin{array}{c} 71.15 \pm \\ 5.28^{b} \end{array}}$	$\begin{array}{l} 82.46 \ \pm \\ 8.15^{a} \end{array}$
Succinic acid	$\begin{array}{c} 0.78 \pm \\ 0.02^c \end{array}$	$\begin{array}{c} 0.99 \pm \\ 0.03^{ab} \end{array}$	$\begin{array}{c} 0.81 \ \pm \\ 0.05^c \end{array}$	$0.93 \pm 0.05^{b}$	$\begin{array}{c} 1.03 \pm \\ 0.03^a \end{array}$	$\begin{array}{c} 0.44 \pm \\ 0.02^h \end{array}$	$\begin{array}{c} 0.63 \pm \\ 0.02^{efg} \end{array}$	$\begin{array}{c} 1.00 \ \pm \\ 0.03^a \end{array}$	0.83 ± 0.06 <sup>c</sup>	$\begin{array}{c} 0.70 \ \pm \\ 0.01^d \end{array}$	$\begin{array}{c} 0.38 \pm \\ 0.02^{hi} \end{array}$	$\begin{array}{c} 0.67 \pm \\ 0.04^{def} \end{array}$	$\begin{array}{c} 0.67 \pm \\ 0.06^{de} \end{array}$	$\begin{array}{c} 0.62 \pm \\ 0.06^{efg} \end{array}$	$\begin{array}{c} 0.67 \pm \\ 0.10^{def} \end{array}$	$\begin{array}{c} 0.36 \pm \\ 0.01^i \end{array}$	$\begin{array}{c} 0.54 \pm \\ 0.05^g \end{array}$	$\begin{array}{c} 0.61 \pm \\ 0.05^{efg} \end{array}$	$\begin{array}{c} 0.58 \pm \\ 0.02^{\mathrm{fg}} \end{array}$	$\begin{array}{c} 0.61 \pm \\ 0.03^{efg} \end{array}$
Citric acid	$\begin{array}{c} 2.75 \pm \\ 0.29^{e} \end{array}$	$2.92 \pm 0.31^{ m de}$	$\begin{array}{c} 2.35 \pm \\ 0.30^{f} \end{array}$	$\begin{array}{c} \textbf{2.25} \pm \\ \textbf{0.20}^{fg} \end{array}$	$\begin{array}{c} 1.23 \pm \\ 0.10^{hi} \end{array}$	$\begin{array}{c} \textbf{2.76} \pm \\ \textbf{0.33}^{e} \end{array}$	$\begin{array}{c} 3.22 \pm \\ 0.35^{bcd} \end{array}$	$1.98~\pm$ $0.19^{ m g}$	$\begin{array}{c} 1.02 \pm \\ 0.08^i \end{array}$	$\begin{array}{c} 0.36 \pm \\ 0.04^{j} \end{array}$	$\begin{array}{c} \textbf{2.94} \pm \\ \textbf{0.27}^{cde} \end{array}$	$3.46 \pm 0.43^{ m b}$	$\begin{array}{c} 1.43 \pm \\ 0.13^h \end{array}$	$\begin{array}{c} 0.28 \pm \\ 0.03^{j} \end{array}$	$\begin{array}{c} 0.34 \pm \\ 0.04^{j} \end{array}$	$\begin{array}{c} 4.25 \pm \\ 0.38^{a} \end{array}$	$\begin{array}{c} 3.26 \ \pm \\ 0.38^{bc} \end{array}$	$\begin{array}{c} 0.99 \pm \\ 0.11^i \end{array}$	N.D.	N.D.
Amino acids																				
Glutamic acid	10.17 $\pm$ $0.35^{cde}$	$\begin{array}{c} 9.05 \pm \\ 0.83^g \end{array}$	$\begin{array}{l} 9.80 \pm \\ 0.57^{defg} \end{array}$	$^{\pm11.21}_{\pm1.01^{ m ab}}$	$\begin{array}{c} 11.76 \\ \pm \ 0.72^a \end{array}$	$\begin{array}{l} 9.83 \pm \\ 0.34^{defg} \end{array}$	$11.44 \pm 0.43^{ab}$	${}^{9.20~\pm}_{0.72^{fg}}$	$\begin{array}{l} 9.41 \pm \\ 0.45^{efg} \end{array}$	10.11 $\pm$ 0.81 <sup>gdef</sup>	$\begin{array}{l} 9.82 \pm \\ 0.47^{defg} \end{array}$	$\begin{array}{l} 9.854 \pm \\ 0.43^{defg} \end{array}$	$\begin{array}{c} 10.56 \\ \pm \\ 0.42^{bcd} \end{array}$	$\begin{array}{l} 10.98 \pm \\ 0.65^{abc} \end{array}$	$\begin{array}{c} 11.29 \pm \\ 0.93^{ab} \end{array}$	$\begin{array}{l} \textbf{7.98} \pm \\ \textbf{0.69}^{h} \end{array}$	$\begin{array}{l} 9.58 \pm \\ 0.53^{efg} \end{array}$	$\begin{array}{l} 8.94 \pm \\ 0.83^g \end{array}$	$\begin{array}{l} 9.79 \pm \\ 0.35^{defg} \end{array}$	$\begin{array}{l} 9.55 \pm \\ 0.35^{efg} \end{array}$
Asparagine(L)	$\begin{array}{l} 8.33 \pm \\ 1.06^{efgh} \end{array}$	$\begin{array}{l} 9.18 \pm \\ 0.96^{cdefgh} \end{array}$	$\begin{array}{l} 8.96 \pm \\ 1.08^{defgh} \end{array}$	$^{\pm11.01}_{\pm1.28^{ m ab}}$	$^{\pm11.03}_{\pm1.13^{ m ab}}$	$\begin{array}{c} 7.71 \ \pm \\ 0.85^h \end{array}$	$\begin{array}{c} 8.19 \pm \\ 1.07^{fgh} \end{array}$	$10.65 \pm 1.14^{abc}$	$10.80 \pm 1.15^{ab}$	10.50 ± 1.11 <sup>abcd</sup>	$\begin{array}{c} 8.00 \ \pm \\ 0.91^{gh} \end{array}$	$\begin{array}{l} 9.49 \pm \\ 0.98^{bcdefg} \end{array}$	10.61 ± 1.19 <sup>abc</sup>	$\begin{array}{c} 10.60 \pm \\ 1.08^{abc} \end{array}$	${11.15} \pm \\ {1.34}^{a}$	$\begin{array}{l} 9.71 \pm \\ 0.98^{abcdef} \end{array}$	$\begin{array}{c} 9.04 \pm \\ 0.91^{cdefgh} \end{array}$	$\begin{array}{l} 9.83 \pm \\ 1.08^{abcde} \end{array}$	$\begin{array}{c} 9.19 \pm \\ 1.00^{cdefgh} \end{array}$	$\begin{array}{c} 9.79 \pm \\ 1.00^{abcde} \end{array}$
Alanine(DL)	${3.46} \pm {0.13}^{ab}$	$\begin{array}{c} \textbf{2.90} \pm \\ \textbf{0.16}^{cdef} \end{array}$	$\begin{array}{c} 2.94 \ \pm \\ 0.20^{bcdef} \end{array}$	3.28 ± 0.50 <sup>abcd</sup>	$3.29 \pm 0.25^{abcd}$	$2.49 \pm 0.39^{\rm f}$	$2.99 \pm 0.39^{bcdef}$	2.76 ±	$2.77 \pm 0.29^{\text{def}}$	3.20 ± 0.31 <sup>abcd</sup>	$2.76 \pm 0.33^{def}$	$\begin{array}{c} \textbf{2.97} \pm \\ \textbf{0.62}^{bcdef} \end{array}$	$3.22 \pm 0.41^{abcd}$	$\begin{array}{c} 2.93 \pm \\ 0.61^{bcdef} \end{array}$	3.07 0.511 <sup>bcde</sup>	$2.65 \pm 0.25^{ef}$	$\begin{array}{c} 3.00 \pm \\ 0.20^{bcdef} \end{array}$	$\begin{array}{c} 2.96 \pm \\ 0.26^{bcdef} \end{array}$	$3.61 \pm 0.22^{\rm a}$	$\begin{array}{c} 3.39 \pm \\ 0.29^{abc} \end{array}$
Arginine(L)	$\begin{array}{c} 1.93 \ \pm \\ 0.27^d \end{array}$	$\begin{array}{c} 1.58 \pm \\ 0.20^{e} \end{array}$	$\begin{array}{c} 0.08 \pm \\ 0.01^{\rm f} \end{array}$	$0.11 \\ 0.01^{\rm f}$	$\begin{array}{c} 0.07 \ \pm \\ 0.00^{\rm f} \end{array}$	$\begin{array}{c} \textbf{2.02} \pm \\ \textbf{0.24}^{cd} \end{array}$	$\begin{array}{c} 2.36 \ \pm \\ 0.26^{b} \end{array}$	$\begin{array}{c} 0.08 \pm \\ 0.00^{\rm f} \end{array}$	$\begin{array}{c} 0.10 \ \pm \\ 0.01^{f} \end{array}$	$\begin{array}{c} 0.03 \ \pm \\ 0.00^{\rm f} \end{array}$	$\begin{array}{c} 2.24 \pm \\ 0.24^{bc} \end{array}$	$\begin{array}{c} \textbf{2.10} \pm \\ \textbf{0.28}^{cd} \end{array}$	$\begin{array}{c} 0.11 \ \pm \\ 0.01^{\rm f} \end{array}$	$\begin{array}{c} 0.03 \ \pm \\ 0.00^{\rm f} \end{array}$	$\begin{array}{c} 0.03 \pm \\ 0.00^{f} \end{array}$	$\begin{array}{c} 2.86 \pm \\ 0.31^a \end{array}$	$\begin{array}{c} \textbf{2.21} \pm \\ \textbf{0.28}^{bc} \end{array}$	$\begin{array}{c} 0.09 \ \pm \\ 0.01^{\rm f} \end{array}$	$\begin{array}{c} 0.06 \ \pm \\ 0.01^{\rm f} \end{array}$	$\begin{array}{c} 0.06 \pm \\ 0.01^{\rm f} \end{array}$
Cysteine	$\begin{array}{c} 0.131 \\ \pm \ 0.02^{f} \end{array}$	$\begin{array}{c} 0.147 \ \pm \\ 0.027^{f} \end{array}$	$\begin{array}{c} 0.16 \pm \\ 0.018^{f} \end{array}$	0.246 ±	$0.732 \pm 0.091^{a}$	$0.135 \pm 0.011^{\rm f}$	$\begin{array}{c} 0.15 \pm \\ 0.01^{\rm f} \end{array}$	$0.31 \pm 0.029^{de}$	0.278 ±	$\begin{array}{c} 0.59 \ \pm \\ 0.034^c \end{array}$	$0.142 \pm 0.010^{\rm f}$	$\begin{array}{c} 0.143 \pm \\ 0.028^{f} \end{array}$	0.278 ±	$\begin{array}{l} 0.301 \pm \\ 0.041^{de} \end{array}$	$\begin{array}{c} 0.657 \pm \\ 0.08^b \end{array}$	$\begin{array}{c} 0.131 \ \pm \\ 0.014^{\rm f} \end{array}$	$\begin{array}{l} 0.248 \ \pm \\ 0.02^{e} \end{array}$	$\begin{array}{l} 0.353 \ \pm \\ 0.024^{d} \end{array}$	$\begin{array}{l} 0.301 \ \pm \\ 0.033^{de} \end{array}$	$\begin{array}{l} 0.749 \ \pm \\ 0.082^{a} \end{array}$
Glutamin(L)	84.11 ± 7.54 <sup>a</sup>	$\begin{array}{l} 64.04 \pm \\ 5.32^{bcdef} \end{array}$	$\begin{array}{c} 61.77 \pm \\ 4.65^{def} \end{array}$	0.017 70.76 ±	0.081 71.50 ±	69.11 ±	63.49 ±	60.30 ± 4.52 <sup>ef</sup>	$58.59 \pm 4.58^{\rm f}$	67.15 ± 5.76 <sup>bcde</sup>	0.019 67.73 ±	$\begin{array}{c} 66.62 \pm \\ 5.78^{bcdef} \end{array}$	$\frac{0.027}{71.70} \pm 5.64^{b}$	$\begin{array}{l} 61.16 \pm \\ 4.95^{def} \end{array}$	$\begin{array}{l} 66.94 \pm \\ 5.39^{bcde} \end{array}$	$\begin{array}{l} 65.22 \pm \\ 5.23^{bcdef} \end{array}$	$\begin{array}{l} 64.30 \pm \\ 5.43^{bcdef} \end{array}$	${\begin{array}{c} 62.53 \pm \\ 5.05^{def} \end{array}}$	${\begin{array}{c} 62.01 \pm \\ 4.63^{def} \end{array}}$	${\begin{array}{c} 62.13 \pm \\ 4.85^{def} \end{array}}$
Histidine(L)	$\begin{array}{c} 0.97 \pm \\ 0.07^{hi} \end{array}$	$\begin{array}{c} 1.29 \pm \\ 0.09^g \end{array}$	$\begin{array}{c} 1.02 \pm \\ 0.08^h \end{array}$	$2.08 \pm 0.18^{\rm bc}$	$2.43 \pm 0.21^{a}$	$0.91 \pm 0.09^{\text{hi}}$	$1.40 \pm 0.14^{ m fg}$	1.77 ± 0.14 <sup>de</sup>	$\begin{array}{c} 1.83 \pm \\ 0.12^{de} \end{array}$	2.112 ± 0.17 <sup>bc</sup>	$0.79 \pm 0.06^{i}$	$\begin{array}{c} 1.51 \pm \\ 0.10^{\rm f} \end{array}$	$\begin{array}{c} 1.71 \pm \\ 0.18^e \end{array}$	$\begin{array}{c} 1.82 \pm \\ 0.14^{de} \end{array}$	$\begin{array}{c} 2.39 \pm \\ 0.16^a \end{array}$	$\begin{array}{c} 1.08 \pm \\ 0.08^h \end{array}$	$\begin{array}{c} 1.40 \pm \\ 0.11^{fg} \end{array}$	$\begin{array}{c} 1.96 \pm \\ 0.13^{cd} \end{array}$	$\begin{array}{c} 2.12 \pm \\ 0.15^{bc} \end{array}$	$\begin{array}{c} 2.27 \pm \\ 0.15^{ab} \end{array}$
Serine(L)	$\begin{array}{c} 3.03 \pm \\ 0.23^{de} \end{array}$	$\begin{array}{l} 3.00 \pm \\ 0.22^{de} \end{array}$	${\begin{array}{c} 3.44 \ \pm \\ 0.23^{ab} \end{array}}$	$\begin{array}{c} 3.65 \pm \\ 0.28^a \end{array}$	$\begin{array}{c} 3.59 \pm \\ 0.24^a \end{array}$	$\begin{array}{c} 2.73 \pm \\ 0.18^{ef} \end{array}$	$\begin{array}{c} 3.10 \ \pm \\ 0.21^{cd} \end{array}$	$\begin{array}{c} 3.11 \pm \\ 0.20^{cd} \end{array}$	$\begin{array}{l} 3.21 \pm \\ 0.21^{bcd} \end{array}$	$3.24 \pm 0.21^{bcd}$	$\begin{array}{c} 2.47 \pm \\ 0.17^{f} \end{array}$	$\begin{array}{c} 3.06 \pm \\ 0.21^{cd} \end{array}$	$\begin{array}{l} 3.46 \pm \\ 0.23^{ab} \end{array}$	$\begin{array}{c} \textbf{3.58} \pm \\ \textbf{0.24}^{a} \end{array}$	$\begin{array}{c} 3.26 \pm \\ 0.24^{bcd} \end{array}$	$\begin{array}{c} 2.74 \pm \\ 0.18^{ef} \end{array}$	$\begin{array}{c} \textbf{2.75} \pm \\ \textbf{0.18}^{ef} \end{array}$	${ 3.23 \pm \atop 0.225^{cd} }$	$3.39 \pm 0.27^{ab}$	${\begin{array}{c} 3.01 \pm \\ 0.20^{de} \end{array}}$

#### Table 2 Metabolites composition of kimchi at different garlic concentrations (%) during the four-week fermentation period.

#### Table 2 (continued)

Metabolites (mM)	A0					A1					A2					A4				
	0W 1V	W	2W	3W	4W	ow	1W	2W	3W	4W	0W	1W	2W	3W	4W	0W	1W	2W	3W	4W
Threonine	$0.47 \pm 0.1$	.55 ±	$0.58~\pm$	$\textbf{0.78} \pm$	$0.75~\pm$	0.47 $\pm$	$0.63~\pm$	$0.67~\pm$	0.74 $\pm$	$0.72~\pm$	$0.42\ \pm$	$0.64 \ \pm$	0.75 $\pm$	0.76 $\pm$	0.76 $\pm$	$0.50~\pm$	$0.57~\pm$	$0.62 \ \pm$	0.71 $\pm$	$0.72~\pm$
	0.03 <sup>ij</sup> 0.0	.03 <sup>gh</sup>	0.04 <sup>efg</sup>	0.04 <sup>a</sup>	0.05 <sup>ab</sup>	0.04 <sup>ij</sup>	0.04 <sup>de</sup>	0.04 <sup>cd</sup>	0.04 <sup>ab</sup>	0.04 <sup>bc</sup>	0.03 <sup>j</sup>	0.04 <sup>de</sup>	0.04 <sup>ab</sup>	0.04 <sup>ab</sup>	0.04 <sup>ab</sup>	$0.03^{hi}$	$0.03^{efg}$	0.04 <sup>def</sup>	0.04 <sup>bc</sup>	0.04 <sup>bc</sup>
Valine(L)	$2.14 \pm 2.0$	.64 $\pm$	$2.68~\pm$	3.35 $\pm$	$3.26~\pm$	1.99 $\pm$	$2.68~\pm$	$3.27~\pm$	3.24 $\pm$	$3.27~\pm$	$\textbf{2.20}~\pm$	$2.96~\pm$	3.41 $\pm$	$3.29~\pm$	$\textbf{3.49} \pm$	$2.55~\pm$	$2.85~\pm$	$2.89~\pm$	$2.65~\pm$	$\textbf{2.82} \pm$
	0.17 <sup>e</sup> 0.1	.22 <sup>cd</sup>	0.21 <sup>cd</sup>	0.26 <sup>a</sup>	0.26 <sup>ab</sup>	$0.14^{e}$	$0.20^{cd}$	$0.25^{ab}$	$0.23^{ab}$	$0.27^{ab}$	$0.18^{e}$	$0.21^{bc}$	0.24 <sup>a</sup>	$0.23^{a}$	$0.27^{a}$	0.24 <sup>d</sup>	$0.22^{cd}$	0.25 <sup>c</sup>	0.21 <sup>cd</sup>	0.23 <sup>cd</sup>
Methionine(DL)	$0.12\pm~0.2$	$.21 \pm$	0.22 $\pm$	$0.32~\pm$	$0.36~\pm$	0.12 $\pm$	0.21 $\pm$	0.26 $\pm$	$0.30~\pm$	0.30 $\pm$	$0.12~\pm$	0.21 $\pm$	$0.32~\pm$	0.36 $\pm$	$0.38~\pm$	$0.14~\pm$	0.21 $\pm$	$0.25 \pm$	$0.29~\pm$	0.33 $\pm$
	0.01 <sup>h</sup> 0.0	.02 <sup>g</sup>	0.03 <sup>g</sup>	$0.04^{bcd}$	0.04 <sup>a</sup>	$0.01^{\rm h}$	0.02 <sup>g</sup>	$0.02^{\rm ef}$	$0.03^{cde}$	$0.03^{cde}$	$0.01^{h}$	$0.02^{g}$	0.03 <sup>bcd</sup>	$0.03^{ab}$	0.05 <sup>a</sup>	$0.01^{h}$	0.02 <sup>g</sup>	$0.02^{\mathrm{fg}}$	$0.03^{def}$	$0.03^{\rm abc}$
Isoleucine(L)	$2.08\pm~3.0$	$.06 \pm$	$3.06 \pm$	$\textbf{4.53} \pm$	$4.53~\pm$	1.97 $\pm$	$3.06~\pm$	$4.04~\pm$	4.54 $\pm$	$4.25~\pm$	$2.09~\pm$	$3.38 \pm$	$3.90~\pm$	$4.46~\pm$	4.76 $\pm$	$2.56~\pm$	$3.07~\pm$	$3.47 \pm$	3.84 $\pm$	4.19 $\pm$
	0.11 <sup>i</sup> 0.	.15 <sup>g</sup>	0.15 <sup>g</sup>	0.31 <sup>ab</sup>	0.25 <sup>ab</sup>	$0.11^{i}$	0.15 <sup>g</sup>	0.21 <sup>de</sup>	$0.24^{ab}$	0.24 <sup>cd</sup>	$0.11^{i}$	$0.16^{f}$	0.21 <sup>e</sup>	$0.22^{bc}$	0.24 <sup>a</sup>	$0.13^{h}$	0.18 <sup>g</sup>	$0.18^{f}$	0.18 <sup>e</sup>	0.21 <sup>d</sup>
Leucine(L)	$2.09 \pm 3.0$	$.06 \pm$	$3.05~\pm$	$\textbf{4.52} \pm$	$4.52~\pm$	1.97 $\pm$	$3.07~\pm$	$4.03~\pm$	$4.53~\pm$	4.24 $\pm$	$2.09~\pm$	3.38 $\pm$	$3.89~\pm$	4.45 $\pm$	4.74 $\pm$	$2.56~\pm$	$3.05 \pm$	3.46 $\pm$	$3.82~\pm$	4.18 $\pm$
	0.11 <sup>i</sup> 0.	.15 <sup>g</sup>	0.15 <sup>g</sup>	$0.31^{ab}$	$0.25^{ab}$	$0.11^{i}$	0.15 <sup>g</sup>	0.21 <sup>de</sup>	0.24 <sup>ab</sup>	0.24 <sup>cd</sup>	$0.11^{i}$	$0.16^{f}$	0.20 <sup>e</sup>	$0.22^{bc}$	0.24 <sup>a</sup>	0.13 <sup>h</sup>	0.17 <sup>g</sup>	$0.18^{\rm f}$	0.18 <sup>e</sup>	0.20 <sup>d</sup>
Tyrosine(L)	$3.21 \pm 5.0$	.61 $\pm$	5.96 $\pm$	8.11 $\pm$	$8.73~\pm$	3.38 $\pm$	5.21 $\pm$	$8.10~\pm$	$9.29~\pm$	8.45 $\pm$	$4.63~\pm$	$6.73~\pm$	7.33 $\pm$	9.91 $\pm$	$8.82~\pm$	$4.90\ \pm$	$6.27 \pm$	8.21 $\pm$	8.22 $\pm$	8.48 $\pm$
	0.15 <sup>k</sup> 0.3	.36 <sup>hi</sup>	0.37 <sup>gh</sup>	$0.45^{d}$	0.43 <sup>bcd</sup>	$0.21^{k}$	$0.27^{ij}$	$0.50^{d}$	$0.66^{b}$	0.45 <sup>cd</sup>	0.23 <sup>j</sup>	$0.32^{\rm f}$	0.79 <sup>e</sup>	0.46 <sup>a</sup>	$0.93^{bc}$	0.26 <sup>j</sup>	$0.29^{\mathrm{fg}}$	0.63 <sup>cd</sup>	0.39 <sup>cd</sup>	0.45 <sup>cd</sup>
Phenyl alanine(L)	$0.53 \pm 1.5$	$.54 \pm$	$1.04\ \pm$	$1.07~\pm$	$1.29~\pm$	1.18 $\pm$	$1.18~\pm$	$1.00~\pm$	$\textbf{2.42} \pm$	1.63 $\pm$	0.96 $\pm$	$1.29~\pm$	$2.52~\pm$	$2.98~\pm$	$2.40~\pm$	$1.03~\pm$	$1.53 \pm$	$2.02~\pm$	1.35 $\pm$	3.54 $\pm$
	0.03 <sup>j</sup> 0.0	.08 <sup>ef</sup>	$0.07^{hi}$	$0.06^{hi}$	0.13 <sup>g</sup>	$0.09^{gh}$	0.06 <sup>gh</sup>	$0.09^{hi}$	0.27 <sup>c</sup>	0.14 <sup>e</sup>	$0.09^{i}$	$0.12^{g}$	0.15 <sup>c</sup>	$0.26^{b}$	$0.17^{c}$	$0.07^{hi}$	0.09 <sup>ef</sup>	0.16 <sup>d</sup>	$0.08^{\mathrm{fg}}$	$0.21^{a}$
Tryptophan(L)	$0.22\pm~0.3$	$.31 \pm$	$0.34 \pm$	$0.43~\pm$	$0.45~\pm$	0.21 $\pm$	$0.29~\pm$	$0.42~\pm$	0.41 $\pm$	0.43 $\pm$	$0.22~\pm$	$0.34 \pm$	$0.45~\pm$	0.46 $\pm$	0.46 $\pm$	0.20 $\pm$	0.43 $\pm$	0.46 $\pm$	$0.36~\pm$	0.47 $\pm$
	0.02 <sup>f</sup> 0.	.03 <sup>de</sup>	0.03 <sup>d</sup>	0.04 <sup>ab</sup>	0.04 <sup>ab</sup>	$0.02^{\rm f}$	0.03 <sup>e</sup>	0.03 <sup>ab</sup>	0.04 <sup>bc</sup>	0.04 <sup>ab</sup>	$0.02^{\mathrm{f}}$	0.04 <sup>de</sup>	0.04 <sup>ab</sup>	0.04 <sup>a</sup>	0.04 <sup>a</sup>	$0.02^{\rm f}$	0.03 <sup>ab</sup>	0.05 <sup>a</sup>	0.03 <sup>cd</sup>	0.04 <sup>a</sup>
Secondary Metabolites																				
r-Aminobutyric	$0.24 \pm 0.2$	$.29 \pm$	$0.28~\pm$	$0.29~\pm$	$0.29~\pm$	0.22 $\pm$	0.31 $\pm$	0.28 $\pm$	0.28 $\pm$	$0.29~\pm$	$0.24\ \pm$	0.31 $\pm$	$0.30~\pm$	0.28 $\pm$	$0.29~\pm$	0.24 $\pm$	$0.29~\pm$	$0.28~\pm$	$0.33~\pm$	0.31 $\pm$
acid	0.02 <sup>ef</sup> 0.	.03 <sup>abcd</sup>	0.03 <sup>abcde</sup>	0.03 <sup>abc</sup>	$0.03^{ab}$	$0.02^{\rm f}$	$0.02^{ab}$	$0.02^{bcde}$	0.03 <sup>bcde</sup>	0.037 <sup>ab</sup>	$0.02^{def}$	$0.04^{ab}$	$0.03^{ab}$	0.03 <sup>abcde</sup>	e 0.04 <sup>abcd</sup>	0.02 <sup>cdef</sup>	$0.03^{ab}$	0.02 <sup>abcde</sup>	0.03 <sup>a</sup>	$0.03^{ab}$
2-Hydroxy isocaproic	$0.23\pm~0.2$	$.28 \pm$	0.48 $\pm$	$0.42~\pm$	$0.49~\pm$	N.D.	N.D.	0.44 $\pm$	0.47 $\pm$	0.47 $\pm$	N.D.	0.23 $\pm$	0.54 $\pm$	0.45 $\pm$	0.55 $\pm$	N.D.	0.41 $\pm$	0.51 $\pm$	0.49 $\pm$	0.56 $\pm$
acid	0.01 <sup>g</sup> 0.	$.02^{\rm f}$	$0.02^{bcd}$	$0.02^{\rm e}$	$0.02^{bc}$			$0.02^{de}$	$0.02^{bcd}$	0.03 <sup>bcd</sup>		0.01 <sup>g</sup>	$0.03^{a}$	0.04 <sup>cd</sup>	0.03 <sup>a</sup>		$0.02^{\rm e}$	$0.03^{b}$	$0.03^{bc}$	$0.05^{a}$
Phenyl lactic acid	N.D. N.	.D.	$0.07~\pm$	$0.07~\pm$	$0.09\ \pm$	N.D.	N.D.	$0.08~\pm$	$0.08~\pm$	$0.05~\pm$	N.D.	N.D.	$0.01~\pm$	$0.03~\pm$	$0.02 \ \pm$	N.D.	N.D.	$0.03~\pm$	$0.02~\pm$	0.01 $\pm$
			0.01 <sup>c</sup>	$0.00^{\mathrm{b}}$	0.01 <sup>a</sup>			$0.00^{\mathrm{b}}$	$0.01^{b}$	0.00 <sup>d</sup>			0.00 <sup>gh</sup>	0.00 <sup>e</sup>	$0.00^{\mathrm{fg}}$			0.00 <sup>ef</sup>	0.00 <sup>gh</sup>	0.00 <sup>h</sup>

Values (mM) represent the means  $\pm$  standard deviations of five measurements (n = 5). Superscripts (a–l) within a row indicate significant differences in garlic content and fermentation periods among the metabolites (p < 0.05). Sample names A0–A4 represent the garlic content (0 %, 1 %, 2 %, and 4 %, respectively) at different time points (week 0–4) during the fermentation period. N.D.: not detected.

9



**Fig. 4.** Correlation analysis of metabolites in kimchi depending on the garlic content. Changes in metabolites and garlic content during fermentation were analyzed using Principal Component Analysis (PCA). Red dots represent the PCA scores for garlic-containing kimchi and blue dots represent the loading plot for metabolites in the kimchi. The PCA biplot illustrates the correlation between garlic content in kimchi and metabolites at various time points: (A) initial time point, (B) 1 week, (C) 2 weeks, (D) 3 weeks, and (E) 4 weeks of fermentation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 5.** Heatmap correlation between lactic acid bacteria (LAB) and metabolites. A distance correlation test (Spearman's rank correlation coefficients) was conducted to analyze the correlation between LAB and metabolites. Significant correlations are depicted in the colored heatmap. Metabolites are listed on the upper side and LAB are shown on the left. Positive and negative correlations between LAB and metabolites are indicated by red and blue colors, respectively. Statistical significance is denoted as \* p < 0.05, \*\*p < 0.01, and \*\*\*p < 0.005. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

kimchi. It has a high acid tolerance and facilitates lactic acid production [28]. The bacterial community during kimchi fermentation is affected by diverse factors. In an anaerobic environment, as kimchi fermentation progresses, the growth of aerobic bacteria decreases and anaerobic bacteria become dominant [29]. Moreover, the lower pH of kimchi is a suitable environment for the growth of *Lactobacillus*, which exhibits homo-fermentation characteristics and acid resistance [7]. Furthermore, LAB from raw materials vary based on the region and season of ingredient procurement [7], and the lower acidity observed in the A2 and A4 groups in the present study could affect the bacterial community diversity. Thus, the addition of garlic to kimchi increased the abundance of LAB. This observation was consistent with the findings of a previous study [7].

Furthermore, we observed that changes in bacterial community composition led to noticeable differences in functional diversity among the bacterial communities. The A0 and A1 groups showed variations in only two metabolic pathways, whereas the A2 and A4 groups exhibited significant differences (p < 0.05) in eight key metabolic pathways, including carbohydrate, amino acid, and energy metabolism. These results suggested that an increase in garlic concentration in kimchi contributes to more complex metabolic activities within the bacterial community.

Subsequently, sugars, organic acids, amino acids, and secondary metabolites in kimchi were analyzed. A previous study showed that the addition of garlic increased the citric acid content and the reduction of glucose [30]. The findings of the present study were consistent with this report, and the increase in citric acid at the initial time point suggested that garlic is a source of citric acid. Moreover, we observed that the organic acid content was further reduced in the garlic-containing kimchi samples. The reduction in malic and citric acid contents corresponded to the increase in garlic content. Malic and citric acids are metabolized in the tricarboxylic acid cycle, key metabolic pathway involved in organic acid production, and converted to pyruvate via oxaloacetate, which is further metabolized to lactic acid. Additionally, some LAB, such as *L. sakei, L. plantarum, L. mesenteroides*, and *L. citreum*, which harbor malolactic enzymes [31], can metabolize malic acid to lactic acid through the action of a malolactic enzyme, and they may have contributed to the rapid reduction in malic acid content in the A2 and A4 groups.

The overall amino acid and secondary metabolite content increased slightly throughout the fermentation period and were not influenced by the garlic content. The content of arginine, the most abundant amino acid in garlic [32], increased linearly with the increase in garlic content at the initial time point (Table 2) and significantly decreased at two weeks of fermentation. Arginine is metabolized into citrulline and ornithine by the ADI activity of LAB [9,33]. We previously reported that among the LAB, *Weissella* species possess strong ADI activity [9,33].

Moreover, our results showed that the LAB in garlic kimchi contributed to the fermentation process by rapidly metabolizing sugars and producing organic acids at the early stage of fermentation, resulting in metabolic diversity. However, it should be noted that garlic is abundant in aroma components [12,13] and possesses anti-bacterial properties that prevent the emergence and growth of spoilage yeast [25]. Therefore, several diverse factors must be considered to control the garlic content in kimchi fermentation to achieve the desired flavor, microbial safety, and fermentation speed.

One limitation of this study is its small sample size. In addition, the bacterial composition of kimchi may depend on the geological and seasonal differences in the raw ingredients, including garlic. Therefore, further research is needed to determine the correlation

between bacterial community with garlic from geographically different regions and different storage conditions, which might contribute to the improvement of specific bacterial regulation in kimchi fermentation.

#### 5. Conclusions

In this study, we demonstrated that garlic serves as a source of LAB in kimchi fermentation. Compared with kimchi with a low garlic content, that with a high garlic content ( $\geq 2$  %) exhibited higher LAB abundance and diversity that led to differences in functional diversity of the bacterial community. Metabolite analysis revealed that the metabolism of sugars and organic acids in kimchi was significantly affected by the addition of garlic, indicating that garlic enhances the metabolic processes during kimchi fermentation. Furthermore, the increased bacterial diversity and metabolite production, resulting from the addition of garlic, potentially contributed to a distinct flavor profile, which might affect the taste and overall quality of kimchi.

#### Data availability statement

All data supporting the findings of this study are available from the corresponding author [J.-H. Lee] upon reasonable request.

# CRediT authorship contribution statement

Ha-Young Jang: Writing – original draft, Methodology, Investigation. Min Ji Kim: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. Ji Young Jeong: Investigation. In Min Hwang: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. Jong-Hee Lee: Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Data curation, Conceptualization.

# Declaration of competing interest

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

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24919.

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