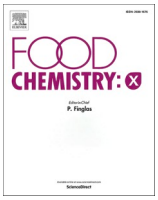
Food Chemistry: X 22 (2024) 101348

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Impact of essential and optional ingredients on microbial and metabolic profiles of kimchi

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ARTICLE INFO

Keywords:
Kimchi
Fermented food
Ingredient
Microbiota
Metabolite
Mix-omics

ABSTRACT

This study aimed to examine the impacts of essential and optional ingredients on the microbial and metabolic profiles of kimchi during 100 days of fermentation, using a mix-omics approach. Kimchi manufactured without essential ingredients (e.g., red pepper, garlic, ginger, green onion, and radish) had lower lactic acid content. The absence of garlic was associated with a higher proportion of *Latilactobacillus* and *Lactococcus*, while the absence of red pepper was associated with a greater proportion of *Leuconostoc* than the control group. In addition, red pepper and garlic served as primary determinants of the levels of organic acids and biogenic amines. Sugar was positively correlated with the levels of melibiose, and anchovy sauce was positively correlated with the levels of amino acids such as methionine, leucine, and glycine. These findings contribute to a fundamental understanding of how ingredients influence kimchi fermentation, offering valuable insights for optimizing kimchi production to meet various preferences.

1. Introduction

Food ingredients are a major factor in improving the nutritional value and defining the overall quality of food products. In the case of spontaneously fermented foods, which are typically not sterilized and do not require the inoculation of starter cultures, raw ingredients can be considered sources of microorganisms for fermentation (Song et al., 2020). Various microorganisms derived from the surfaces of ingredients participate in fermentation, enabling metabolism in the food matrix (Tamang, Watanabe, & Holzapfel, 2016). Namely, the presence or absence of specific ingredients in fermented food can significantly influence its quality, thereby affecting its organoleptic and overall characteristics.

Kimchi is a traditional Korean fermented food manufactured by the spontaneous fermentation of microorganisms derived from various ingredients (Patra, Das, Paramithiotis, & Shin, 2016). Numerous types of kimchi are available, ranging from main vegetables to diverse combinations of seasoning ingredients. Accordingly, unlike most fermented foods with consistent raw ingredients, kimchi is characterized by the use of various ingredients depending on the manufacturer, making it difficult to standardize. Therefore, some official institutions (Codex Alimentarius Commission, Ministry of Food and Drug Safety, Ministry of Agriculture and Forestry, and Korean Standards Association) have slightly different definitions for kimchi ingredients. The ingredients of kimchi range from commonly used ingredients, such as kimchi cabbage, red pepper, garlic, ginger, green onion, and radish, to uncommon ingredients, such as seaweed and water dropwort. Therefore, to understand the characteristics of kimchi, it is necessary to comprehensively identify the effects of each ingredient on its quality.

Kimchi ingredients such as red pepper, garlic, ginger, green onion, and radish are mainly used as spices and seasonings. As additional ingredients, salted fish sauce, sugar, leek, and glutinous rice paste can be used differently depending on personal preferences, the production region, and tradition (Lee et al., 2023). The taste of kimchi is determined by its constituent ingredients, wherein these ingredients not only serve as sources of fermentative lactic acid bacteria (LAB) but also establish a unique fermentation environment. Accordingly, many studies have investigated the influence of each ingredient on kimchi microbiota and

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https://doi.org/10.1016/j.fochx.2024.101348
Received 1 February 2024; Received in revised form 20 March 2024; Accepted 1 April 2024
Available online 2 April 2024

metabolites during fermentation (Baek, Kim, Han, Lee, & Jeon, 2023; Jeong et al., 2013; Jung et al., 2018). However, as these studies have primarily focused on individual ingredients and have considered them as either qualitative or quantitative variables, it is essential to conduct comprehensive comparisons to gain a better understanding of the collective impact of each ingredient on kimchi, a complex fermented vegetable food.

Therefore, this study aimed to investigate the changes in multiple profiles of kimchi, including physicochemical characteristics, microbial communities, and metabolites, according to various combinations of ingredients in kimchi by dividing it into essential and optional ingredients during 100 days of fermentation. Metabolomics, metataxonomics, and mix-omics approaches were used to elucidate the role and effect of each ingredient throughout the fermentation period.

2. Materials and methods

2.1. Preparation of kimchi samples

Kimchi was prepared using salted kimchi cabbage as the primary vegetable. The standard for selecting ingredients was set by referring to the kimchi CODEX (CODEX STAN 223–2001), Ministry of Agriculture and Forestry, Korean Standards Association, and related previous studies (Cho, Lee, Rhee, & Park, 1998; Lee et al., 2023). Specifically, kimchi ingredients were prepared by dividing them into essential ingredients such as red pepper (RP), garlic (GA), ginger (GI), green onion (GO), and radish (RA), and optional ingredients such as anchovy sauce (AS), sugar (SU), leek (LE), and glutinous rice paste (GR). To investigate the impact of individual ingredients on fermentation, a total of eleven distinct groups were devised as follows: either an essential or optional ingredient was either excluded (Ex) or included (In) (**Table S1**). The control group was prepared including all essential ingredients. To compare the overall effects of the optional ingredients, a group that included all ingredients (AL) used (both essential and optional ingredients) was also prepared (Fig. 1a). All groups were prepared in five replicates. The prepared kimchi was stored at 20 °C for five hours in order to activate fermentation (Kim et al., 2020), and then stored at 4 °C for 100 days. Sampling was conducted on 0, 1, 2, 5, 10, 30, 50, and 100 days of fermentation. After centrifuging the kimchi broth (13,572 × g for 5 min at 4 °C), supernatants and pellets were stored at −80 °C for subsequent metabolite and microbial community analyses, respectively.

2.2. Physicochemical analysis

The pH was measured using a pH meter (pH -250 L, ISTEK, Seoul, Republic of Korea). The titratable acidity (TA) was measured as lactic acid by titrating to pH 8.3 with a 0.1 N NaOH volume and then calculated using the titration equation. The physicochemical measurements were recorded as the mean of five replicates.

2.3. Microbial community profiling

DNA was extracted from the pellets using the AccuFAST automated system (AccuGene, Incheon, Republic of Korea) in accordance with the manufacturer's instructions. This protocol was similar to those described in a previous metataxonomics study (Kim et al., 2022). For Illumina MiSeq sequencing, microbial DNA was amplified using KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Wilmington, MA, USA) and the primer pair 341F/805R containing Nextera adapter sequences (Klindworth et al., 2013). The V3–V4 hypervariable regions of 16S rRNA genes were amplified by 25 cycles of polymerase chain reaction (PCR). The resulting PCR products (~428 bp) were purified using HiAccuBeads (AccuGene, Incheon, Republic of Korea). Libraries were pooled by 600 cycles of MiSeq Reagent Kit v3 (Illumina, San Diego, CA, USA) at an equimolar ratio. The pooled libraries were sequenced using an Illumina MiSeq system.

The raw sequence datasets were denoised by correcting amplicon errors, and exact amplicon sequence variants (ASVs) were inferred using the DADA2 v. 1.16 pipeline (Callahan et al., 2016). To classify the ASVs obtained from DADA2, a Naïve Bayes classifier was created based on the SILVA release rRNA reference database v. 138.1 (Glöckner et al., 2017). Downstream analyses of quality and chimera filtering were performed using the QIIME2–2022.2 software package (Bolyen et al., 2019). The ASVs associated with the chloroplasts and mitochondria were eliminated. The ASVs datasets derived from the DADA2 were assigned to bacterial taxa using QIIME2 workflow scripts and the SILVA database classifier (threshold of 99% pairwise identity). The alpha (within groups) and beta diversity (between groups) of the microbial communities were analyzed using the plugin q2-diversity. Multivariate statistical analysis was applied to process the final data file using principal coordinate analysis (PCoA).

2.4. Metabolic profiling

The derivatization protocol and GC–MS conditions were similar to those described in a previous metabolomics study (Lee et al., 2023). For ten-fold dilution, 10 μL of the sample supernatant and 90 μL of distilled water were mixed. The diluted sample and 20 μL of ribitol solution (internal standard, 0.5 mg/mL in water) were mixed and lyophilized. To inspect the stability and reliability of the analysis, the quality control (QC) samples were prepared by blending 10 μL of each sample. Subsequently, 100 μL of methoxyamine hydrochloride (20 mg/mL in pyridine) was added, and the samples were ultra-sonicated (Powersonic 520, Hwashin, Seoul, Republic of Korea) for 20 min at 4 °C. After vortexing the sample mixture for 30 s, mixture was shaking-incubated (75 rpm for 90 min at 30 °C) in the dark. Silylation was performed by vortex-mixing 50 μL of *N*-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and shaking-incubation once more (75 rpm for 30 min at 37 °C). Finally, the derivatized sample was centrifuged (13,572 ×*g* for 5 min at 4 °C), and then 80 μL of supernatant was put in each vial for metabolite analysis. The derivatized samples were analyzed using a GC–MS-QP2020 instrument (Shimadzu, Kyoto, Japan) equipped with an RTX-5MS capillary column (Restek, Bellefonte, PA, USA). The conditions of GC–MS analysis were as follows: injector, 230 °C; transfer line, 250 °C; flow rate of helium gas, 1 mL/min; detector, 280 °C. The initial temperature of the GC oven was set at 80 °C, held for 2 min, increased at a rate of 15 °C/min, and the final temperature was maintained at 330 °C for 6 min. One microliter of the sample was analyzed in split mode (1:30), and the *m/z* range was set to 85–500. The ionization was set to electron impact (70 eV). To inspect the stability, performance, and reproducibility of the analysis conditions, QC samples were analyzed with samples during the run. The retention index (RI) values of the compounds were calculated from the retention times (RT) of standard alkane mixtures consisting of C7–C40 alkanes (Sigma-Aldrich, St. Louis, MO, USA) under the same GC–MS conditions.

The raw peak datasets were converted into CDF format files using Shimadzu GC–MS Postrun Analysis software (Shimadzu, Kyoto, Japan). Subsequently, datasets of peaks were converted to ABF format files with the converter software and processed with MS-DIAL v. 4.9 for detection, noise removal, and calibration of baseline, alignment, deconvolution, identification, and integration of height of peak (Tsugawa et al., 2015). To detect the peaks, the mass range was set between 0 and 1000 Da, and a minimum peak height of 1000 amplitudes was applied. Deconvolution was performed using a sigma window value of 0.5 and an EI spectra cut-off of 10 amplitudes. For identification, a retention index tolerance of 20, an EI similarity cut-off of 90%, and an identification score cut-off of 90% was utilized. Alignment of the peaks was performed with a retention index tolerance of 20, a retention time tolerance of 0.075 min, and a retention time factor of 0.5. To identify the compounds based on their mass spectral patterns, the publicly available spectral library of EI-MS and Kovats RI, was used for data annotation. The mass spectra of the identified metabolites were further compared to the NIST v. 20.0, library

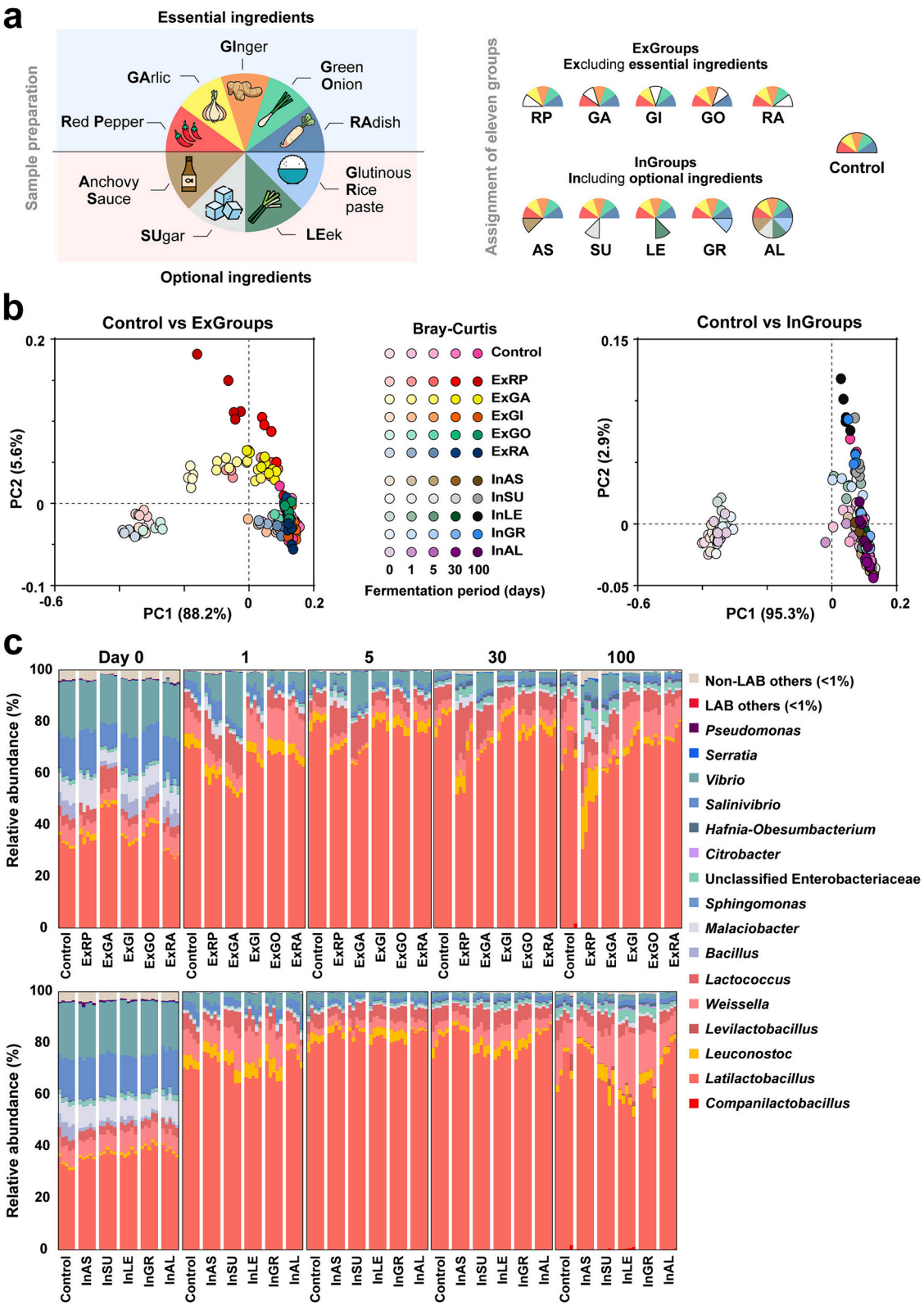

Fig. 1. Microbial profiling of kimchi prepared with various ingredient compositions. (a) Overview of the kimchi preparation process and the classification of eleven groups. (b) Beta diversity of microbial communities of kimchi samples during 100 days of fermentation (0, 1, 5, 30, and 100 days). Beta diversity was visualized using principal coordinates analysis (PCoA) score plot based on Bray-Curtis matrix of 16S rRNA gene sequence dataset. (c) Relative abundance profiles of microbial communities at the genus level. The legend colors represent lactic acid bacteria (LAB) taxa in shades of red and non-LAB taxa in shades of blue. The control group was compared to both groups with excluded ingredients (ExGroups) and groups with included ingredients (InGroups). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and standard reagents. The information regarding the retention time, RI, and quantitative mass of all identified metabolites are presented in **Table S2**. Peak intensities of the metabolites were normalized to those of the internal standard (ribitol). Multivariate statistical analysis was applied to process the final data file using SIMCA v. 16.0 (Umetrics, Umea, Sweden). Principal component analysis (PCA) was performed, and the quality of the model was estimated using R^2 (variance in the data) and Q^2 (prediction of the model) values.

2.5. Statistical analysis

Statistical analyses of the metabolome and microbiota datasets were performed using GraphPad Prism v. 9.0 software (GraphPad Software Inc., San Diego, CA, USA) and MicrobiomeAnalyst (https://www.microbiomeanalyst.ca/) or MetaboAnalyst (https://www.metaboanalyst.ca/). After the normality of the dataset was confirmed using the Kolmogorov-Smirnov test, significant differences between the groups were analyzed. The comparison of six groups was performed using the analysis of variance (ANOVA) test of Brown-Forsythe and the Kruskal-Wallis test for parametric and nonparametric data analysis. Multiple comparisons were conducted using the two-stage step-up method of the Benjamini, Krieger, and Yekutieli or Dunn's test. To compare the differences between the control and experimental groups, Wilcoxon tests or ANOVA tests were utilized in MicrobiomeAnalyst and MetaboAnalyst, respectively. False discovery rate-adjusted p-values <0.05 were considered statistically significant. The dataset was expressed as mean $\pm$ standard deviation.

2.6. Multivariate integration analysis

Multivariate integration analysis was performed using the metabolome, microbiota, and ingredient datasets in R software v. 4.3.0. The mixOmics package was used to perform a data integration analysis for biomarker discovery using latent components (DIABLO) (Singh et al., 2019). The resulting plots were generated by visualizing the DIABLO outputs.

3. Results

3.1. Physicochemical characteristics during kimchi fermentation

Eleven experimental groups were prepared by various ingredient compositions, by either excluding essential ingredients (ExGroups: ExRP, ExGA, ExRI, ExGO, and ExRA) or including optional ingredients (InGroups: InAS, InSU, InLE, InGR, and InAL) (Fig. 1a). The last two uppercase abbreviations in the group name represent the following: RP, red pepper; RA, garlic; GO, green onion; RA, radish; AS, anchovy sauce; SU, sugar; LE, leek; AL, all ingredients. The changes in pH and titratable acidity (TA) in kimchi during 100 days of fermentation are shown in **Fig. S1**. Throughout the fermentation period, all groups exhibited decreasing pH and increasing TA, regardless of the ingredient composition. Within such trends, at day 30, the ExGA group had lower pH and higher TA compared to other groups. Interestingly, on the last day of fermentation, the TA values were lower in the ExGroups than in the control group, whereas the InGroups had higher TA values than the control group. These results show that the production of organic acids, especially lactic acid, is higher in a fermentation environment where all the essential ingredients of kimchi are present.

3.2. Microbial community profiling during kimchi fermentation

The alpha diversity, as estimated by the Shannon index, is shown in **Fig. S2a**. The alpha diversity values of all groups exhibited a rapid decline in the early stages of fermentation, followed by a slight increase as fermentation progressed. On day 100 of fermentation, the alpha diversity values of the ExRP and ExGA groups were higher than those of the control group, whereas the alpha diversity values of the InAS and InAL groups which both include anchovy sauce were slightly lower. Fig. 1b depicts the beta diversity (between groups) visualized using PCoA based on the Bray-Curtis matrix. Samples from day 0 and day 1 were clearly separated from the other samples in the PCoA score plot, indicating rapid microbial succession during the initial stages of fermentation. Interestingly, the microbial community profiles of the ExGroups showed more distinct differences than those of the InGroups. The ExGA group displayed distinct profiles from day 0 of fermentation, whereas the ExRP group exhibited different profiles as the fermentation progressed. This trend was also observed in the beta diversity plots for each fermentation day (**Fig. S2b**). The changes in the microbial community profiles of kimchi at the genus level are presented in Fig. 1c. Throughout the fermentation period, the dominant genus was *Latilactobaillus*, regardless of the ingredient composition. From the beginning of the fermentation, noticeable differences were observed in the ExGA group, and as the fermentation period progressed, the ExRP group began to show different patterns. On day 0 of fermentation, the proportion of *Latilactobacillus* was significantly higher in the ExGA group, suggesting that garlic may have inhibited the growth of *Latilactobacillus*. In the ExRP group, *Bacillus* was not detected on day 0 of fermentation, suggesting that *Bacillus* originated from the red pepper. In the early stages of fermentation, the InGroups showed microbial community profiles similar to those of the control group. However, as the fermentation period progressed, the LAB profiles, including genera *Companilactobacillus*, *Latilactobacillus*, *Leuconostoc*, *Levilactobacillus*, *Weissella*, and *Lactococcus* in the InSU, InLE, and InGR groups were different from those of the control group. Taken together, the differences in the ingredients used in kimchi can alter the succession of the microbial community during fermentation.

3.3. Taxonomic relative abundance affected by kimchi ingredients

To compare the relative abundance of the major LAB between kimchi groups with various ingredient compositions, the difference in the relative abundance of the four LAB genera in each group was calculated based on the control group on days 0 and 100 of fermentation (Fig. 2a). On day 0 of fermentation, the relative abundances of *Latilactobacillus* and *Weissella* in the InGroups were generally higher than those in the control group, and the relative abundances of *Leuconostoc* and *Lactococcus* in the InGroups were lower than those in the control group. However, this trend was not observed at day 100 of fermentation. In ExGroups, the ExRP and ExGA groups showed distict trends than the other groups throughout oveall fermentation preiod. On day 0 of fermentation, the ExRP and ExGA groups showed higher relative abundances of the three LAB genera, with the exception of *Weissella*, than the control group. In the ExRP and ExGA groups, *Latilactobacillus* showed the opposite tendency, showing a lower relative abundance than those in the control group at day 100 of fermentation, whereas *Leuconostoc, Weissella,* and *Lactococcus* showed a similar pattern from day 0 to day 100 of fermentation. Additionally, heat tree analysis revealed significant differences in the microbial community profiles of the ExRP and ExGA groups compared to those of the other groups and the control group (**Fig. S3**).

To further elucidate the microbes originating from the ingredients or those influenced by the complex fermentation environment, we investigated the microbiota throughout the fermentation period. It was specifically focused on the microbes that disappeared upon the removal of specific ingredients or emerged when specific ingredients were added (Fig. 2b). In the control group, 25 genera of microbiota were detected. However, compared to the control group, six genera were not detected in the ExGroups, whereas seven genera were additionally detected in the InGroups. Some microbes could potentially be derived from red pepper (*Bacillus*), garlic (*Methylophilus*, *Pseudarcobacter*, *Shewanella*, and *Sphingomonas*), and green onion (*Serratia*). Additionally, some microbes could also originate from optional ingredients in anchovy sauce

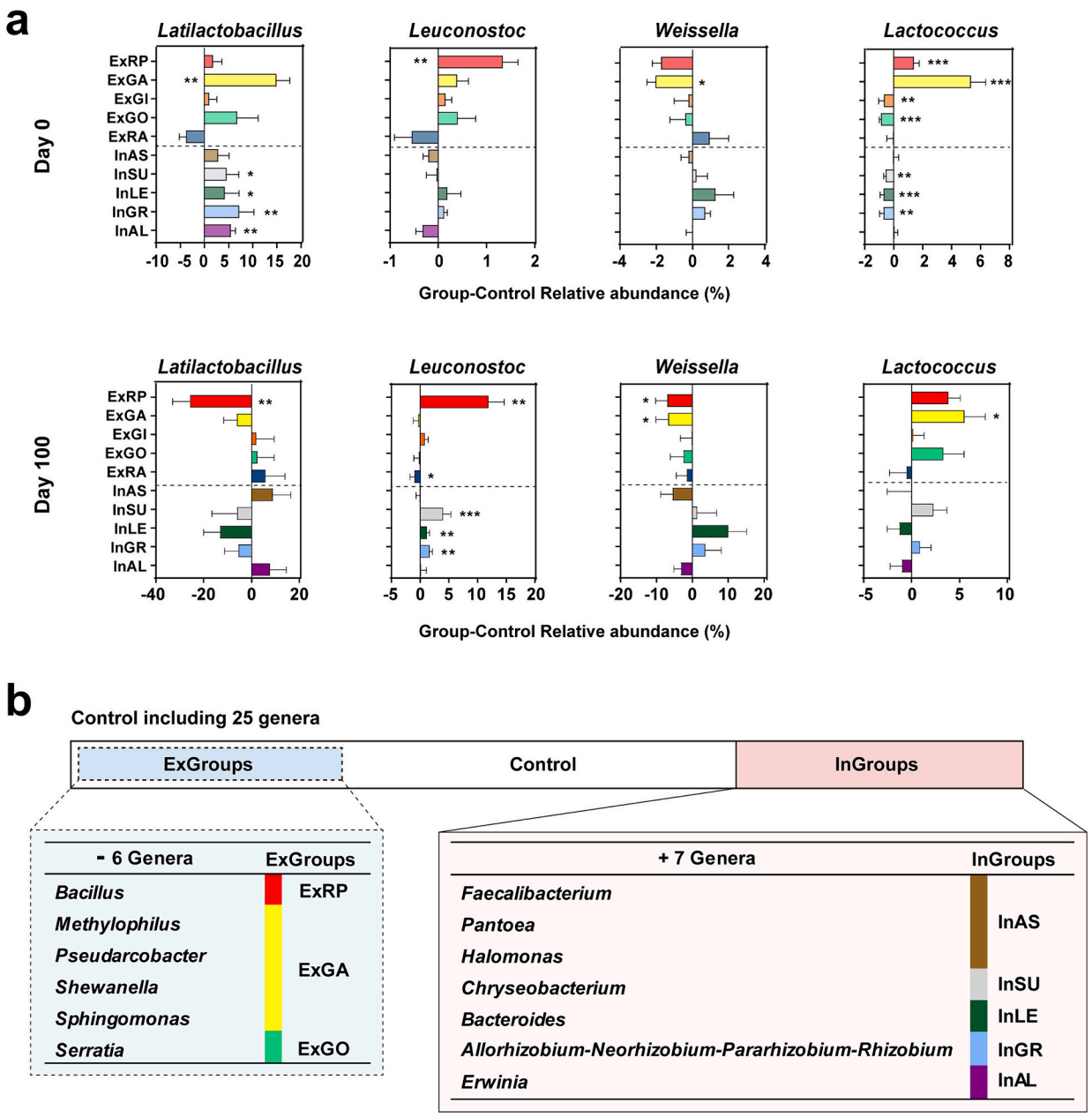

Fig. 2. Comparative analysis of the microbial relative abundance profiles of kimchi prepared with various ingredient compositions. (a) Comparison of the relative abundance of LAB genera based on the control group on day 0 and day 100 of fermentation (cut-off >1%). (b) Vent diagram showing microbes that disappear when essential ingredients are removed (ExGroups) and microbes that appear when optional ingredients are added (InGroups) at the geneus level. Microbes were considered present if detected in three or more of the five replicates each day (cut-off >0.1%) and at least once from day 0 to day 100. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

(*Faecalibacteriium*, *Pantoea*, and *Halomonas*), sugar (*Chryseobacterium*), leek (*Bacteroides*), glutinous rice paste (*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*), and a combination of all these optional ingredients (*Erwinia*). Although a previous study has investigated the origin of fermentative microbiota in particular ingredients, such as red pepper, garlic, and ginger (Song et al., 2020), microbial communities in same type of ingredients could vary depending on factors such as the production area, harvest time, and storage conditions. Hence, future research will be necessary to analyze the microbial communities within each ingredient in order to identify the specific microbial species originating from particular ingredients in kimchi. In addition, these results indicate that the proportion of these non-fermentative bacteria is relatively minor compared to that of LAB, the influence of ingredients on the growth of major LAB could be more significant. While it is unclear whether specific ingredients define the characteristics of kimchi, this finding suggests that the ingredients used in kimchi can induce inoculation of specific microorganisms.

3.4. Metabolic profiling during kimchi fermentation

PCA was conducted to examine alterations in the metabolic profiles during 100 days of fermentation (Fig. 3a). As fermentation progressed, there was a noticeable trend of samples being plotted from left to right based on their PC1 values, with more significant differences observed in the ExRP group, indicating that the red pepper in kimchi may play the most significant role in determining the metabolic profile of kimchi. To distinguish the overlapping samples, additional PCA score plots were generated to visualize the data for each fermentation day (Fig. 3b).

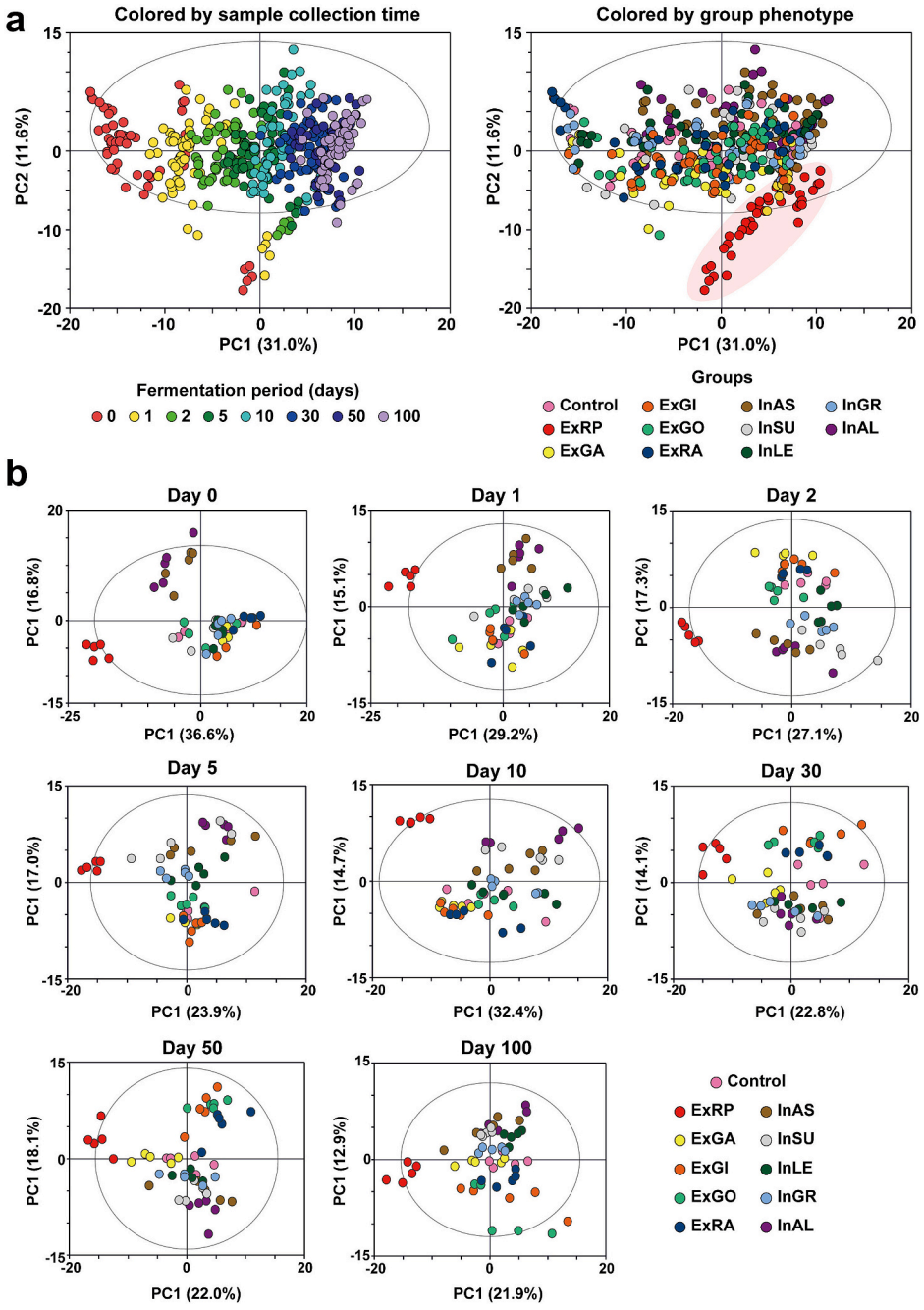

Fig. 3. Metabolic profiling of kimchi prepared with various ingredient compositions. Principal component analysis (PCA) score plot derived from the GC–MS dataset of kimchi samples during 100 days of fermentation: (a) Overall fermentation period highlighting sample collection time (left) and group phenotype (right), (b) Each day of fermentation (day 0, 1, 2, 5, 10, 30, 50, and 100).

Throughout the fermentation period, the ExRP group as well as the InAS and the InAL groups (both of which include anchovy sauce) clustered separately from the other groups. On day 100 of fermentation, each group slightly differed from the other groups. Subsequently, a heat map was generated to investigate the overall patterns of differences in the metabolites depending on the composition of the ingredients during 100 days of fermentation (**Fig. S4**). The study detected 55 metabolites, among which those exhibiting the highest significance of the ANOVA test were visualized. The main trends could be summarized as follows: The ExRP group had low levels of organic acids, whereas the InAS and InAL groups exhibited elevated levels of amino acids, which can be attributed to the addition of fish sauce. In addition, the InSU group had high sugar contents.

The metabolites that displayed differences on day 100, as indicated by each comparative group, are shown in Fig. 4. During fermentation, distinct changes in metabolites were observed in the kimchi environment. The levels of 4-aminobutyric acid (GABA) and quinic acid were the lowest in the ExRA and ExRP groups, respectively. The ExRP group also exhibited differences in threonic acid levels (with the ExGO group), as well as aspartic acid and glucuronic acid levels. The levels of amino acids, such as 5-aminovaleric acid, glycine, and leucine, were higher in the InAS and InAL groups, while the levels of glucuronic acid were lower compared to the other groups, excluding the EXRP group. In the InSU group, mannitol and tyrosine (with the InGR group) exhibited higher levels, while sucrose showed higher levels only initially, decreasing thereafter (with the InAL group). Among the metabolites exhibiting

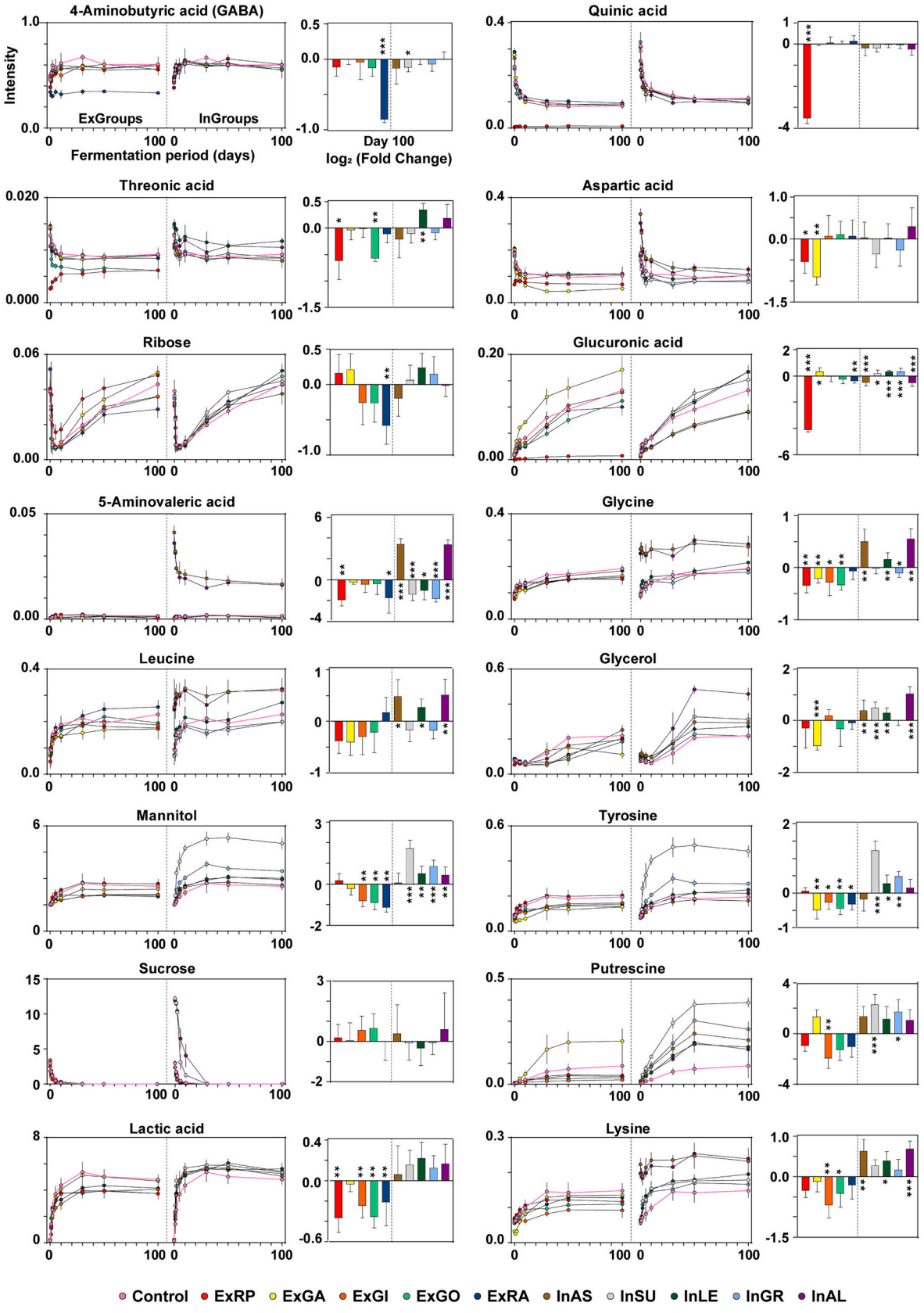

Fig. 4. Comparative analysis of the metabolic change of kimchi prepared with various ingredient compositions. Changes in metabolites in each group during 100 days of fermentation were expressed as groups that excluded ingredients (ExGroups) and groups that included ingredients (InGroups), respectively. A bar graph was used to show the difference in the peak intensity of kimchi metabolites on day 100. The x-axis of the bar graph shows the fold change (FC) values on the $\log_2$ scale relative to the control group. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

significant differences in intensities, putrescine, a putrefactive indicator of kimchi, was observed at higher levels in both the ExGA and all the InGroups. This finding suggests that excluding garlic or incorporating optional ingredients along with essential ingredients may potentially contribute to the deterioration of kimchi. Interestingly, based on the control group, the levels of lactic acid and leucine were lower in the ExGroup and higher in InGroups. These distinct changes seem to be influenced by the selective exclusion or inclusion of the ingredients. Several metabolites could be produced or metabolized through microbial metabolism during fermentation; however, specific metabolites, such as quinic acid, 5-aminovaleric acid, and glycine, suggest that they originate from specific ingredient (red pepper and anchovy sauce) rather than from fermentation.

3.5. *Comprehensive exploration through mix-omics approach*

To attain a more comprehensive understanding of the influence of each essential or optional ingredient on kimchi fermentation, an

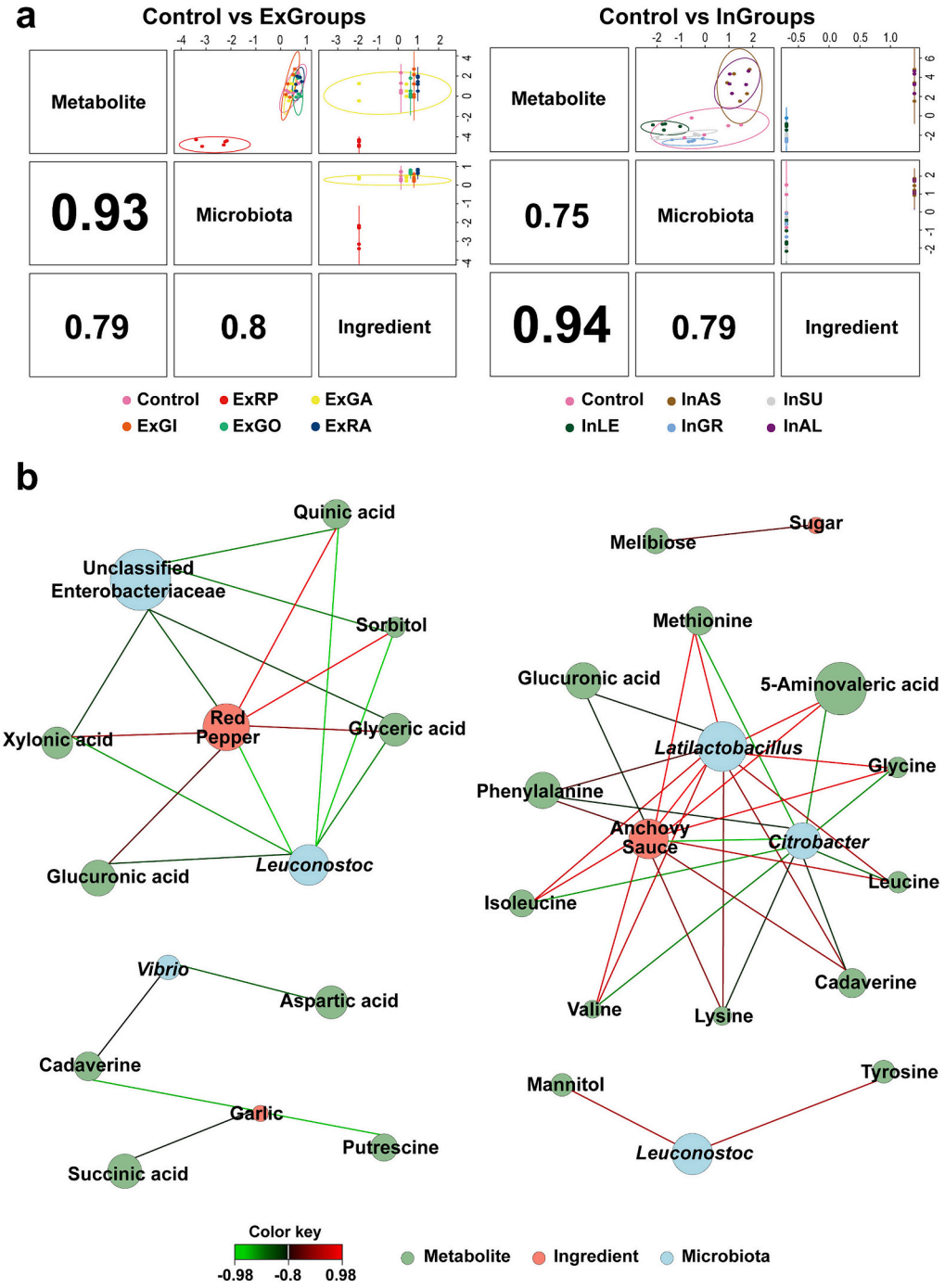

Fig. 5. Schematization of a comprehensive exploration mix-omics analysis with data integration analysis for biomarker discovery using latent components (DIABLO). The input dataset represents the kimchi profile on day 100. (a) Inter-omics correlations presented by sample scatterplots displaying the first components of each dataset (microbiota, metabolite, and ingredient). Correlations were calculated using Pearson correlation between each dataset (lower diagonal plot). (b) Relevance network plot showing positive (red lines) and negative (green lines) correlations between features from each dataset. The control group was analyzed in conjunction with the groups excluded ingredients (ExGroups) (left) and the groups included ingredients (InGroups) (right), respectively ($|r| > 0.8$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

exploration into the interrelationships among the ingredients, microbiota, and metabolites of kimchi was conducted via DIABLO (Fig. 5). A strong correlation between the microbiota and metabolites was observed within the ExGroups, whereas a strong correlation between the ingredients and metabolites was noted within the InGroups (Fig. 5a). These results indicate that changes in metabolic profiles could be attributed to shifts in the microbial profile resulting from the exclusion of essential ingredients or the metabolic profile could be modified by the compounds derived from the inclusion of optional ingredients. Fig. 5b shows the relevance network plots derived from the correlations between the features of each dataset. Among the essential ingredients, red pepper was positively correlated with the levels of organic acids such as quinic acid, xylonic acid, glucuronic acid, and glyceric acid, whereas it was negatively correlated with the relative abundance of *Leuconostoc* and unclassified Enterobacteriaceae. Additionally, garlic was negatively correlated with the levels of biogenic amines, including cadaverine and putrescine. Meanwhile, anchovy sauce and sugar, optional ingredients, was correlated with the levels of some metabolites. The sugar ratio was positively correlated with the levels of melibiose, and anchovy sauce was positively correlated with the levels of amino acids such as methionine, leucine, and glycine. Furthermore, anchovy sauce was positively and negatively correlated with the relative abundances of *Latilactobacillus* and *Citrobacter*, respectively.

4. Discussion

The presence or absence of particular ingredients establishes an environment that significantly affects the growth and succession of the fermentative microbiota in kimchi, which is considered a closed ecosystem during fermentation (Lee, Song, Jung, Lee, & Chang, 2017). The essential ingredients of kimchi, vegetables such as red pepper, garlic, ginger, green onion, and radish, are predominantly cultivated in the soil, thereby possessing the potential to incorporate diverse environmental microorganisms into the final product, kimchi. Among these ingredients, some studies have reported garlic may serve as a source of LAB during the initial stages of kimchi fermentation (Lim et al., 2015; Song et al., 2020). Our investigation revealed distinct LAB profiles within the groups. Notably, the absence of garlic was associated with a higher proportion of *Latilactobacillus* and *Lactococcus* compared to the control group, while the absence of red pepper was associated with a greater proportion of *Leuconostoc* than the control group. Additionally, our study provides evidence that red pepper could serve as an inoculum for *Bacillus*. Jeong et al. (2013) reported that radish kimchi without red pepper showed an increased relative abundance of *Leuconostoc* and maintained this trend throughout the fermentation period, which is consistent with the results of the present study. The two aforementioned ingredients (red pepper and garlic), predominantly responsible for differences in microbial profiles, are not only utilized as spicy seasonings in Korean foods, but also as natural antimicrobial agents that contribute to the selective suppression or enhancement of the proliferation of microorganisms during kimchi fermentation (Lee et al., 2019). In the present study, a strong correlation between microbiota and metabolites was observed within the ExGroups, suggesting that differences in metabolic profiles could be attributed to changes in the microbial profile resulting from the exclusion of essential ingredients. The group excluding garlic exhibited increased levels of biogenic amines, such as putrescine and cadaverine. Biogenic amines are primarily synthesized by specific microorganisms found in traditional fermented foods, which typically undergo open fermentation processes. Among them, putrescine, characterized by its unpleasant odor, may pose risks to human health when consumed excessively (Gao et al., 2023; Gao et al., 2023). Additionally, the levels of some organic acids, including lactic acid and quinic acid, were found to be lower in kimchi prepared without red pepper. These findings suggest that manufacturing kimchi without garlic or red pepper, which are essential ingredients, may have a negative impact on quality of kimchi (Cheigh, Park, & Lee, 1994; Jung et al., 2022; Kim, Dang, & Ha, 2022). Furthermore, the lowest levels of GABA were observed in the group that excluded radish, with no significant difference in the proportion of LAB compared to the control group, suggesting that radish could potentially acquire a pivotal component for GABA production within the kimchi environment. Overall, the absence of specific essential kimchi ingredients may lead to adverse effects on metabolites related to flavor and functionality, potentially negatively impacting kimchi quality.

Some ingredients can be used as additives to enhance flavor profile of kimchi. They also provide opportunities for fermentative microorganisms to metabolize their constituents during fermentation (Anagnostopoulos & Tsaltas, 2019). Our results revealed that the groups that included optional ingredients did not show primarily difference in the microbial profile compared with the control group, except for a slightly higher ratio of LAB. Especially, until the last day of fermentation, the addition of anchovy fish sauce also influenced the maintenance of a high proportion of *Latilactobacillus*. However, a strong correlation ($r = 0.94$) was observed between the optional ingredients and the metabolic profiles. Among these ingredients, the addition of anchovy sauce significantly contributed to the increase in levels of most amino acids, leading to distinct amino acid profiles in the groups containing anchovy sauce. Anchovy sauce, the aqueous fraction resulting from the fermentation of salted anchovy, is characterized by an abundance of amino acids originating from the enzymatic degradation of fish proteins (Jung et al., 2018). Due to its distinct umami flavor, this ingredient is frequently used for kimchi production in Korean households (Lee et al., 2023). Additionally, the group that included sugar exhibited the highest levels of melibiose and mannitol. Among the groups included in the optional ingredients, those that included sugar or glutinous rice paste exhibited similar patterns for several metabolites, including mannitol, tyrosine, and putrescine. Despite the distinct chemical structures of sugar (sucrose) and glutinous rice paste (starch), both were reported to promote kimchi fermentation by increasing the free sugar content within the kimchi (Jeong, Lee, & Chung, 2018). Park et al. (2023) reported that the level of mannitol, a flavor-enhancing factor produced by *Leuconostoc*, increased in starch paste-supplemented kimchi, which is consistent with the results of the current study. Meanwhile, lactic acid is acknowledged as a prominent kimchi metabolite produced during lacto-fermentation (Lee, Jeon, Yoo, & Kim, 2021). Interestingly, the results of this study revealed that the levels of lactic acid were higher in the samples including optional ingredients, indicating that the presence of all essential ingredients is necessary to enhance lactic acid production, ultimately facilitating LAB growth. Additionally, these findings suggest that optional ingredients could accelerate fermentation. Consequently, the desired level of maturation might be adjusted by selecting the ingredients and controlling its ratio.

This study focused on evaluating the effect of ingredients on the microbial and metabolic profiles of kimchi using a mix-omics approach, which has the potential to extract reliable biomarkers, thereby enriching our understanding (Singh et al., 2019). To conduct a comprehensive examination, we considered the complex effects of various compositions rather than solely focusing on the qualitative or quantitative variables of a single ingredient. Our results exhibited that the essential ingredients of kimchi were mainly related to the microbial community composition, and some optional ingredients of kimchi were related to the production of metabolites, especially flavor-related compounds. To the best of our knowledge, this is the first study to report the effects of classified kimchi ingredients, which are difficult to standardize according to specific standards. While our study focused on nine ingredients, future research may require the investigation of a broader range of ingredient compositions and validation of the sources of microbes and metabolites from these ingredients. Further research is also needed to determine the effects of essential and optional ingredients on the sensory characteristics of kimchi, including both taste and aroma profiles, specifically the volatile organic compound profiles. Additionally, future studies could focus on developing tools capable of objectively predicting the ripening

progression of kimchi. This could potentially lead to the establishment of a method for standardizing the ripening stages of kimchi based on its constituent ingredients, thereby facilitating the production of customized kimchi to consumer preferences.

5. Conclusions

The ingredients of spontaneously fermented kimchi, which contribute to both the metabolic profiles and sources of microorganisms, play important roles in the fermentation process. This study revealed that the presence or absence of specific ingredients resulted in significant variations in the kimchi profiles. Lactic acid level was higher in the group with all the essential ingredients. Spicy seasonings, such as red pepper and garlic, served as primary determinants of organic acids and biogenic amines levels. Among optional ingredients, the sugar was positively correlated with the levels of melibiose, and anchovy sauce was positively correlated with the levels of amino acids such as methionine, leucine, and glycine. Through conducting mix-omics analysis across multiple profiles, this study demonstrates that ingredients in kimchi not only influence the microbial and metabolic profiles within the environment, but also contribute to the presence of several microbes or compounds, likely originating from the ingredients. This study may enhance the fundamental understanding of the effects of ingredients on kimchi fermentation and provide valuable insights for manufacturing kimchi of various qualities.

CRediT authorship contribution statement

Do-Yeon Lee: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **Eun-Ju Kim:** Funding acquisition, Writing – review & editing. **Seong-Eun Park:** Software, Visualization. **Kwang-Moon Cho:** Investigation, Writing – review & editing. **Sun Jae Kwon:** Writing – review & editing. **Seong Woon Roh:** Writing – review & editing. **Suryang Kwak:** Writing – review & editing. **Tae Woong Whon:** Funding acquisition, Writing – review & editing. **Hong-Seok Son:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

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.

Data availability

The data supporting the findings of this study are available from the corresponding authors upon reasonable request because of the large volume of raw data collected in this study.

Acknowledgments

This research was supported by the Korea Innovation Foundation (INNOPOLIS) grant funded by the Korean Government (Ministry of Science and ICT) through a Science and Technology Project that opens the future of the region (2021-DD-UP-0380), the World Institute of Kimchi (KE2401-1-1), the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (NRF-2021R1A6A3A01086359), a Korea University Grant, and the Institute of Biomedical Science & Food Safety, CJ Food Safety Hall at Korea University, Republic of Korea.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101348.

References

Anagnostopoulos, D. A., & Tsaltas, D. (2019). Fermented foods and beverages. In *Innovations in traditional foods* (pp. 257–291). Elsevier.

Baek, J. H., Kim, K. H., Han, D. M., Lee, S. H., & Jeon, C. O. (2023). Effects of glutinous rice paste and fish sauce on kimchi fermentation. *LWT, 173*, Article 114253. https://doi.org/10.1016/j.lwt.2022.114253

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology, 37*, 852–857. https://doi.org/10.1038/s41587-019-0209-9

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods, 13*, 581–583. https://doi.org/10.1038/nmeth.3869

Cheigh, H.-S., Park, K.-Y., & Lee, C. Y. (1994). Biochemical, microbiological, and nutritional aspects of kimchi (Korean fermented vegetable products). *Critical Reviews in Food Science and Nutrition, 34*, 175–203. https://doi.org/10.1080/10408399409527656

Cho, E.-J., Lee, S.-M., Rhee, S.-H., & Park, K.-Y. (1998). Studies on the standardization of Chinese cabbage kimchi. *Korean Journal of Food Science and Technology, 30*, 324–332.

Gao, X., Li, C., He, R., Zhang, Y., Wang, B., Zhang, Z.-H., & Ho, C.-T. (2023). Research advances on biogenic amines in traditional fermented foods: Emphasis on formation mechanism, detection and control methods. *Food Chemistry, 405*, Article 134911. https://doi.org/10.1016/j.foodchem.2022.134911

Gao, X., Zhao, X., Hu, F., Fu, J., Zhang, Z., Liu, Z., … Ho, C.-T. (2023). The latest advances on soy sauce research in the past decade: Emphasis on the advances in China. *Food Research International, 113407*. https://doi.org/10.1016/j.foodres.2023.113407

Glöckner, F. O., Yilmaz, P., Quast, C., Gerken, J., Beccati, A., Ciuprina, A., … Ludwig, W. (2017). 25 years of serving the community with ribosomal RNA gene reference databases and tools. *Journal of Biotechnology, 261*, 169–176. https://doi.org/10.1016/j.jbiotec.2017.06.1198

Jeong, D., Lee, J.-H., & Chung, H.-J. (2018). Analysis of targeted metabolites and molecular structure of starch to understand the effect of glutinous rice paste on kimchi fermentation. *Molecules, 23*, 3324. https://doi.org/10.3390/molecules23123324

Jeong, S. H., Lee, H. J., Jung, J. Y., Lee, S. H., Seo, H.-Y., Park, W.-S., & Jeon, C. O. (2013). Effects of red pepper powder on microbial communities and metabolites during kimchi fermentation. *International Journal of Food Microbiology, 160*, 252–259. https://doi.org/10.1016/j.ijfoodmicro.2012.10.015

Jung, M.-J., Kim, J., Lee, S. H., Whon, T. W., Sung, H., Bae, J.-W., … Roh, S. W. (2022). Role of combinated lactic acid bacteria in bacterial, viral, and metabolite dynamics during fermentation of vegetable food, kimchi. *Food Research International, 157*, Article 111261. https://doi.org/10.1016/j.foodres.2022.111261

Jung, M. Y., Kim, T.-W., Lee, C., Kim, J. Y., Song, H. S., Kim, Y. B., … Lee, S. H. (2018). Role of jeotgal, a Korean traditional fermented fish sauce, in microbial dynamics and metabolite profiles during kimchi fermentation. *Food Chemistry, 265*, 135–143. https://doi.org/10.1016/j.foodchem.2018.05.093

Kim, E.-J., Seo, S.-H., Park, S.-E., Lim, Y.-W., Roh, S. W., & Son, H.-S. (2020). Initial storage of kimchi at room temperature alters its microbial and metabolite profiles. *LWT, 134*, Article 110160. https://doi.org/10.1016/j.lwt.2020.110160

Kim, J. Y., Park, S.-E., Kim, E.-J., Seo, S.-H., Whon, T. W., Cho, K.-M., … Son, H.-S. (2022). Long-term population dynamics of viable microbes in a closed ecosystem of fermented vegetables. *Food Research International, 154*, Article 111044. https://doi.org/10.1016/j.foodres.2022.111044

Kim, S.-Y., Dang, Y.-M., & Ha, J.-H. (2022). Effect of various seasoning ingredients on the accumulation of biogenic amines in kimchi during fermentation. *Food Chemistry, 380*, Article 132214. https://doi.org/10.1016/j.foodchem.2022.132214

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research, 41*. https://doi.org/10.1093/nar/gks808. e1-e1.

Lee, D.-Y., Park, S. H., Park, S.-E., Kim, E.-J., Kim, H.-W., Seo, S.-H., … Son, H.-S. (2023). Comprehensive elucidation of the terroir of Korean kimchi through the study of recipes, metabolites, microbiota, and sensory characteristics. *Food Research International, 166*, Article 112614. https://doi.org/10.1016/j.foodres.2023.112614

Lee, M., Song, J. H., Jung, M. Y., Lee, S. H., & Chang, J. Y. (2017). Large-scale targeted metagenomics analysis of bacterial ecological changes in 88 kimchi samples during fermentation. *Food Microbiology, 66*, 173–183. https://doi.org/10.1016/j.fm.2017.05.002

Lee, M.-A., Kim, T.-K., Hwang, K.-E., Choi, Y.-J., Park, S.-H., Kim, C.-J., & Choi, Y.-S. (2019). Kimchi extracts as inhibitors of colour deterioration and lipid oxidation in raw ground pork meat during refrigerated storage. *Journal of the Science of Food and Agriculture, 99*, 2735–2742. https://doi.org/10.1002/jsfa.9441

Lee, S.-J., Jeon, H.-S., Yoo, J.-Y., & Kim, J.-H. (2021). Some important metabolites produced by lactic acid bacteria originated from kimchi. *Foods, 10*, 2148. https://doi.org/10.3390/foods10092148

Lim, S. B., Shin, S.-Y., Moon, J. S., Otgonbayar, G.-E., Joo, W., Lee, S. J., … Han, N. S. (2015). Garlic is a source of major lactic acid bacteria for early-stage fermentation of cabbage-kimchi. *Food Science and Biotechnology, 24*, 1437–1441. https://doi.org/10.1007/s10068-015-0184-y

Park, S.-E., Cho, K.-M., Kwon, S. J., Kim, E.-J., Seo, S.-H., Jeong, D., … Son, H.-S. (2023). Effects of the addition of starches with different amylose contents on kimchi microbiota and metabolites. *LWT, 175*, Article 114475. https://doi.org/10.1016/j.lwt.2023.114475

Patra, J. K., Das, G., Paramithiotis, S., & Shin, H.-S. (2016). Kimchi and other widely consumed traditional fermented foods of Korea: A review. *Frontiers in Microbiology, 7*, 1493. https://doi.org/10.3389/fmicb.2016.01493

Singh, A., Shannon, C. P., Gautier, B., Rohart, F., Vacher, M., Tebbutt, S. J., & Lê Cao, K.-A. (2019). DIABLO: An integrative approach for identifying key molecular drivers from multi-omics assays. *Bioinformatics, 35*, 3055–3062. https://doi.org/10.1093/bioinformatics/bty1054

Song, H. S., Whon, T. W., Kim, J., Lee, S. H., Kim, J. Y., Kim, Y. B., … Roh, S. W. (2020). Microbial niches in raw ingredients determine microbial community assembly during kimchi fermentation. *Food Chemistry, 318*, Article 126481. https://doi.org/10.1016/j.foodchem.2020.126481

Tamang, J. P., Watanabe, K., & Holzapfel, W. H. (2016). Diversity of microorganisms in global fermented foods and beverages. *Frontiers in Microbiology, 7*, 377. https://doi.org/10.3389/fmicb.2016.00377

Tsugawa, H., Cajka, T., Kind, T., Ma, Y., Higgins, B., Ikeda, K., … Arita, M. (2015). MS-DIAL: Data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nature Methods, 12*, 523–526. https://doi.org/10.1038/nmeth.3393