



Effects of ingredient size on microbial communities and metabolites of radish kimchi

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ABSTRACT

In this study, changes in physicochemical characteristics, microbial communities, and metabolites were investigated to identify the fermentation characteristics of radish kimchi according to the size of radish cubes used. The small-sized radish kimchi group showed the highest hardness value and glucose content in the early stages of fermentation. The relative abundance of major lactic acid bacteria, including *Leuconostoc*, *Weissella*, and *Lactobacillus*, was the highest in the small-sized radish kimchi group on day 5 of fermentation, which resulted in rapid production of lactic acid, thereby causing a decrease in pH and an increase in titratable acidity. The size of the radish in kimchi plays a pivotal role in determining various factors, most notably during the first 5 days of fermentation, leading to marked metabolic changes. A total of 17 metabolites, including glucose, sucrose, lactic acid, malic acid, citric acid, and GABA, exhibited significant differences in the small-size radish kimchi group. Interestingly, the sucrose content was higher in the large-sized radish group at the beginning of fermentation. This study revealed that ingredient size can selectively affect the growth of specific microorganisms in an environment where several microorganisms coexist, which could change the quality of radish kimchi.

1. Introduction

Control of ingredient size during food processing contributes to the quality of the final product (Sharma et al., 2020). In addition, several studies have reported that the particle size of an ingredient is a major part in deciding its practical and qualitative characteristics (such as water absorption, molecular traits of the components, and bio-availability of bioactive compounds) (Khan et al., 2020; Lazaridou et al., 2018; Ma et al., 2020). Therefore, controlling the size of ingredients, which changes the surface to volume ratio, has been widely used to enhance the physicochemical and functional properties of food materials (Chen et al., 2018; Du et al., 2020).

The differences in the surface area of the ingredients in solid-state fermentation may lead to differences in microbial activity (Kumar et al., 2021; Singhania et al., 2010). Physicochemical and microbiological differences according to ingredient size have been reported in some fermented foods, such as *meju* (a Korean fermented soybean brick) (Lee et al., 2012) and cabbage kimchi (Moon et al., 2019), which have

different sizes and shapes. The exposed surface area of food is known to be involved in the growth of microorganisms through supplying nutrients (Hamad, 2012). In particular, because the size of the ingredient affects the sugar availability of microorganisms (Sanguansri & Augustin, 2006), the smaller the size of the ingredient, the faster the fermentation rate is. As the taste and quality of fermented foods are greatly influenced by the activity of microorganisms during fermentation (Hu et al., 2022; Sharma et al., 2020), it is important to understand the effect of ingredient size on the quality of fermented foods. In addition, the size of the ingredients can selectively affect the growth of specific microorganisms in environments where numerous microorganisms coexist. As several traditional fermented foods are manufactured without starter inoculation and many microorganisms coexist, it is necessary to investigate the effect of the size of the ingredients on the growth of specific microorganisms and quality of fermented foods.

Kimchi, a traditional Korean fermented food consumed worldwide, is manufactured in many forms using various raw ingredients (Park et al., 1996). Korean radish kimchi (*kkakdugi*) is a type of kimchi that uses

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radish as its main ingredient in an evenly diced shape. Thus, radish kimchi can be considered a suitable sample to determine the effect of ingredient size during fermentation. Based on previous studies (Lee et al., 2012; Moon et al., 2019; Nagaraju & Manohar, 2000), it is expected that even if the same raw ingredients and amounts are used, the difference in the sizes of the ingredients will affect the fermentation of radish kimchi. However, the kinetics of microbial communities and metabolite alterations according to ingredient size in fermented foods have not yet been reported. Therefore, in this study, microbial communities, metabolites, physicochemical and texture properties were investigated to identify the fermentation characteristics according to the raw ingredients of radish kimchi. This study could help to elucidate the effect of ingredient size of fermented foods on quality, or to determine the size of the ingredients suitable for the characteristics of fermented foods.

2. Materials and methods

2.1. Preparation of samples

Radish kimchi was prepared according to the modified manufacturing method described in a study (Park et al., 2017). Briefly, cubed radish was prepared using the central part of radish cutting into $1 \times 1 \times 1 \text{ cm}^3$, $2 \times 2 \times 2 \text{ cm}^3$, and $3 \times 3 \times 3 \text{ cm}^3$ cubes. The experimental groups ($n = 5$ per group) were as follows: small-sized radish kimchi group (SK, $1 \times 1 \times 1 \text{ cm}^3$), medium-sized radish kimchi group (MK, $2 \times 2 \times 2 \text{ cm}^3$), and large-sized radish kimchi group (LK, $3 \times 3 \times 3 \text{ cm}^3$). The seasoning mixture was prepared as follows: red pepper powder (5.0 %), garlic (2.5 %), ginger (1.0 %), green onions (3.5 %), salt (1.6 %), sugar (2.0 %), and water (4.4 %). This seasoning was then mixed with the cubed radish at a ratio of 8:2 (radish:seasoning mixture). This radish kimchi mixture was stored at 20°C for 6 h to allow active fermentation, then stored at 4°C for 50 days. Samples were taken at 0, 5, 15, 30, and 50 days of fermentation for analysis. To analyze the microbial community and metabolites, the supernatants were obtained from radish kimchi samples by centrifugation (4°C , 10 min, 12,000 rpm), and subsequently stored at -80°C .

2.2. Measurement of pH and titratable acidity

After centrifuging the radish kimchi samples, pH was determined using a pH meter (pH-250L, ISTEK, Seoul, Korea). The titratable acidity (TA) was determined by titrating 0.1 N NaOH to a pH of 8.3 and expressed as a percentage of lactic acid (%). The outcomes are presented as mean values of five measurements.

2.3. Texture analysis

Texture profile analysis was performed using a texture analyzer (TA-XT plus C, Stable Micro Systems, Surrey, UK). Prior to analysis, all the samples were prepared in the form of cubes of the same size ($1 \times 1 \times 1 \text{ cm}^3$). The samples were compressed twice at 50 % strain using a flat-surfaced aluminum cylinder probe (20 mm diameter) at a speed of 1.0 mm/s. Texture parameters (hardness, adhesiveness, cohesiveness, and springiness) were measured five times, and the averages and standard deviations were reported.

2.4. Sensory evaluation

Electronic tongue analysis was conducted using an Astree taste sensor electronic tongue (Alpha MOS, Toulouse, France). For each radish kimchi sample, 1 mL was filtered through Whatman No. 1 filter paper (GE Healthcare, Chicago, IL, USA), and the supernatant was collected. This supernatant was then diluted 100-fold using distilled water and subsequently filtered through a $0.45 \mu\text{m}$ polyvinylidene difluoride (PVDF) syringe filter prior to analysis with the electronic

tongue. Each determination was repeated five times, and statistical analysis was carried out using Alpha MOS software (AlphaSoft version 14.2). The representative taste measurements employed sensors for AHS (sourness), PKS (sweetness), CTS (saltiness), NMS (umami), and ANS (bitterness) to gauge the signal intensity of each sensor.

2.5. Qualitative and quantitative analyses of ingredients released from the raw radish

Cubed raw radish was prepared using the central part of the radish, cut into $1 \times 1 \times 1 \text{ cm}^3$, $2 \times 2 \times 2 \text{ cm}^3$, and $3 \times 3 \times 3 \text{ cm}^3$ cubes. The experimental groups ($n = 3$ per group) were as follows: small-sized radish group (SR, $1 \times 1 \times 1 \text{ cm}^3$), medium-sized radish group (MR, $2 \times 2 \times 2 \text{ cm}^3$), and large-sized radish group (LR, $3 \times 3 \times 3 \text{ cm}^3$). For qualitative and quantitative analysis, the raw materials were prepared as follows: 40 g of radish and 4 g of salt. These materials were then mixed in a zipper bag ($18 \times 20 \text{ cm}$). The radish mixture was stored at 4°C for 15 days. Samples were taken at 0, 5, and 15 days of fermentation for analysis. The weight of the radish, excluding the eluate, was measured for each sample. To analyze the metabolites using GC-MS, supernatants were obtained from each radish sample by centrifugation (4°C , 10 min, 12,000 rpm) and subsequently stored at -80°C .

2.6. 16S rRNA gene sequencing and data processing

An AccuFAST automation system (AccuGene Inc., Incheon, Korea) was used to extract DNA according to the manufacturer's instructions. To perform bacterial genomic DNA amplification, MiSeq sequencing was performed using primers containing 341 fb and 805 rb with Nextera adaptor sequences to target the V3-V4 hypervariable region of the 16S rRNA genes (King et al., 2019). Using the KAPA HiFi HotStart ReadyMix (Roche sequencing, Pleasanton, CA, USA), 16S rRNA genes were amplified through 25 polymerase chain reaction (PCR) cycles. HiAccuBeads for NGS library purification (AccuGene Inc.) was used to purify the PCR products ($\sim 428 \text{ bp}$). The amplicon libraries were pooled at an equimolar ratio and the pooled libraries were sequenced on an Illumina MiSeq system using the MiSeq Reagent Kit v3 for 600 cycles (Illumina, San Diego, CA, USA).

All raw datasets were deionized by correcting amplicon errors and were used to infer exact amplicon sequence variants (ASVs) using DADA2 v1.16 (Callahan et al., 2016). The SILVA release 138 rRNA reference database (Glöckner et al., 2017) was used to create a Naïve Bayes classifier to classify the ASVs obtained from DADA2. Downstream analyses of quality-filtered and chimera-filtered reads were performed using the QIIME2-2022.2 software package (Bolyen et al., 2019). Each sequence obtained from the DADA2 datasets was assigned to taxonomy with a threshold of 99 % pairwise identity using QIIME2 workflow scripts and the SILVA release 138 rRNA reference database classifier. Alpha diversity was estimated using Chao1, Shannon, and Simpson indices. To explore dissimilarities in species composition among samples, principal coordinate analysis (PCoA) was conducted based on the Bray-Curtis distance of beta-diversity.

2.7. GC-MS analysis and data processing

A GC-MS-based metabolomic approach was used to monitor metabolic changes during fermentation. GC-MS, in combination with derivatization, is suitable for analyzing small polar metabolites that cover many primary metabolisms (Doerfler et al., 2013). Following centrifugation (13,000 rpm, 4°C , 15 min), 100 μL of diluted solution mixed with 10 μL of supernatant and 90 μL of distilled water was gathered in a 1.5 mL Eppendorf tube. Then, 20 μL of the internal standard (ribitol in water, 0.5 mg/mL) was added. After lyophilization, 100 μL of methoxyamine hydrochloride dissolved in pyridine (20 mg/mL) was added to the dried sample, followed by incubation in dark (30°C , 90 min, 75 rpm). For silylation, 50 μL of *N*-methyl-*N*-(trimethylsilyl)

trifluoroacetamide (MSTFA) was added to the sample, and the mixtures were incubated with shaking at 75 rpm for 30 min in 37 °C (HB-201SF, Hanbaek Scientific Co., Bucheon, Korea). Following incubation, the sample was centrifuged at 13,000 rpm for 10 min, and 100 μ L of the resulting supernatant was transferred into GC-MS vials. Derivatized samples were analyzed using a QP 2020 GC-MS (Shimadzu, Kyoto, Japan). The GC oven temperature was initially held at 80 °C for 2 min and increased to the final temperature of 330 °C, at a rate of 15 °C/min, and held for 6 min. The m/z range was set to 85–500 with electron impact ionization (70 eV). The sample was injected in the split mode (1:30).

GC-MS raw data were transformed into a netCDF format and processed using MetAlign (<https://www.metAlign.nl>) for peak detection, noise reduction, normalization, and alignment (Lommen, 2009). The resulting data were incorporated into AIoutput software (https://prime.psc.riken.jp/Metabolomics_Software/AIoutput/index.html) for peak identification (Tsgawa et al., 2011). GC-MS data were processed using principal component analysis (PCA) and partial least squares projection to latent structures and discriminant analysis (PLS-DA) to visualize the variance of metabolites using SIMCA-P 16.0 (Umetrics, Umea, Sweden). The quality of the model score plot was represented by R^2 (the variance in the data) and Q^2 values (the prediction of the model). Analysis of metabolic pathways, including enrichment analysis and pathway topological analysis, was performed using MetaboAnalyst 5.0 (<https://www.metaboanalyst.ca>).

2.8. Statistical analysis

For all the statistical data analyses, IBM SPSS statistics (version 26.0, SPSS, Inc., Chicago, IL, USA) were performed with analysis of variance

(ANOVA) and Tukey's post hoc test ($p < 0.05$). All data are presented as the mean \pm standard deviation.

3. Results and discussions

3.1. Changes in physicochemical, texture, and sensory properties

Fig. 1A shows the size and surface area to volume ratio of the radish cubes used for making radish kimchi in each experimental group. As the radish size increased, the surface area to volume ratio decreased. Changes in the pH and TA of radish kimchi of different sizes are shown in Fig. 1B. The initial pH of the SK group decreased rapidly during fermentation, indicating that fermentation occurred rapidly in the group with the smallest radish size. Meanwhile, the TA of SK was relatively higher during the fermentation period than that of the other groups. The most rapid decrease in pH and increase in TA in the SK group indicated that lactic acid bacteria (LAB) proliferated most rapidly. It is well known that differences in the surface to volume ratio affect the growth rate of microorganisms (Harris & Theriot, 2018). Several studies have reported that smaller ingredient sizes increase surface area and thus increase nutrient availability to bacteria (Mshandete et al., 2006; Singhania et al., 2010). When the diameter of the radish cube was doubled, the surface area and volume increased by four and eight times, respectively. Eventually, the greater the diameter of the radish kimchi, the greater is the decrease in the surface area to volume ratio. The larger the surface area to volume ratio of ingredients, the more nutrients, such as sugar, can be eluted from the ingredient, so LAB can quickly obtain nutrients for growth from the raw ingredients.

To better understand how the raw material influences fermentation outcomes, we analyzed the weight changes in radishes and the eluted

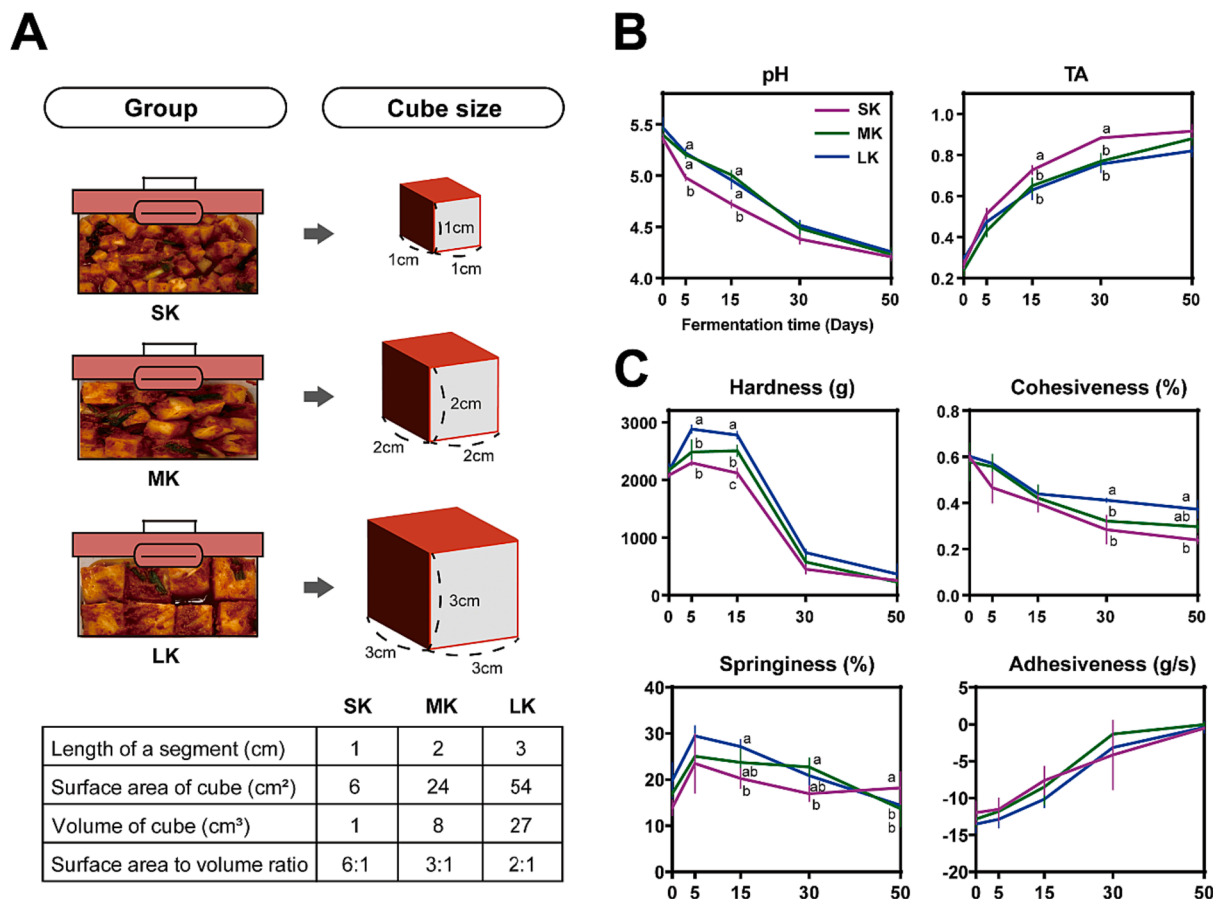


Fig. 1. (A) Differences in the surface area to volume ratio of radishes of different sizes. Three groups of radish kimchi were prepared using three different sizes of cube-shaped radish. (B) Changes in pH and titratable acidity (TA) and (C) texture properties of radish kimchi during fermentation.

metabolites (Fig. S1). The weight of all radish samples decreased during storage, with the smallest (SR) sample showing a significant reduction in weight on days 5 and 15 (Fig. S1A). This suggests that, despite having the same initial material and weight, radish samples with a higher surface area-to-volume ratio may experience a faster rate of weight loss, potentially indicating greater nutrient effusion and fuel for fermentative microbes. Additionally, Fig. S1B displays the major sugars among the identified metabolites. Notably, there was no significant difference in glucose levels, although the SR sample showed a relatively higher pattern. Sucrose levels were highest in the LR sample on day 5 and showed a significant difference. Overall, fructose and arabinose levels did not differ significantly among the experimental groups and exhibited similar patterns. A high amount of eluted ingredients in the SR group might result in fast growth of fermentative microbes, as evidenced by the lower pH and higher TA in SK group shown in Fig. 1B.

The textural properties of various foods are important factors that are widely used to compare their rheological properties (Friedman et al., 1963). To measure the textural properties, hardness, cohesiveness, springiness, and adhesiveness were measured (Fig. 1C). The hardness values were significantly different among the groups after 5 and 15 days of fermentation ($p < 0.05$). In particular, the LK group, with the largest ingredient size, exhibited the highest hardness value. Meanwhile, the SK group, which had the smallest ingredient size, reported more shrinkage than the other groups during fermentation. These results suggest that the

degree of water elution from the radish cube during fermentation differs depending on the size of the radish cube. The texture of the products can be significantly affected by their water content (Figiel & Tajner-Czopek, 2006). Kimchi exhibits a decrease in hardness, known as tissue softening, as the fermentation period elapses (Van Buren, 1991), which may be closely related to the water content of the ingredients. The difference in the hardness values of radish kimchi suggests that the water content in radish differs depending on the size of the radish cube, which can affect the texture of radish kimchi. However, as fermentation progressed, there was no significant difference in the hardness values of all groups. Although the cohesiveness and springiness values were not significant at the initial stages, there was a significant difference in some groups after day 30.

Next, we evaluated the impact of different radish cube sizes on sensory properties. To minimize variations between samples within each group, we pooled five replicates to create a single representative sample for sensory evaluation using an electronic tongue. The electronic tongue's sensors successfully categorized the taste of radish kimchi into five metrics: sourness, sweetness, saltiness, umami, and bitterness (Fig. S2A). A longitudinal sensory evaluation revealed that the LK group had considerable differences in all taste metrics compared to the other two groups after 30 days of fermentation. However, by the 50-day mark, all three groups had converged to similar levels across all taste indicators (Fig. S2B). These results suggest that the size of the radish cube can

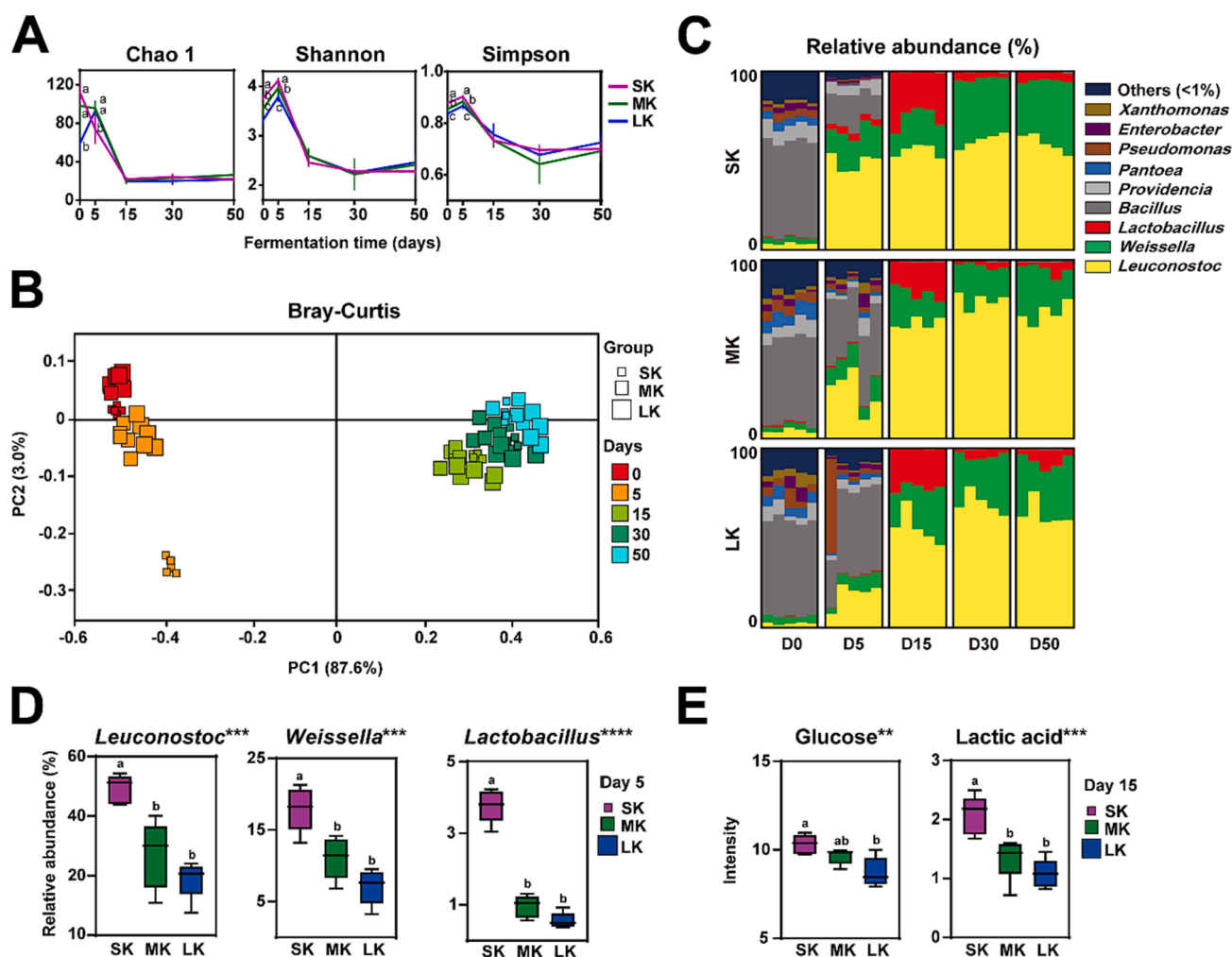


Fig. 2. (A) Alpha diversity as estimated using Chao 1, Shannon, and Simpson indices. (B) Beta-diversity is measured using principal coordinate analysis (PCoA) plot based on the Bray-Curtis of the bacterial 16S rRNA gene amplicon sequence data. (C) Taxa changes at the genus level during 50 days of radish kimchi fermentation. (D) Box plots of relative abundance of the identified major LAB (*Leuconostoc*, *Weissella*, and *Lactobacillus*) on day 5 of fermentation. (E) Box plots of glucose and lactic acid levels measured using GC-MS on day 15 of fermentation. Symbols indicate significant difference (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).

influence sensory properties during a specific period within the fermentation process.

3.2. Changes in microbial community

By the 5th day of fermentation, the values of alpha diversity indices (Chao1, Shannon, and Simpson) were higher in the SK group, indicating that the smaller the size of the ingredient was, the more diverse microorganisms were present at the beginning of fermentation (Fig. 2A). Changes in the bacterial communities were compared to investigate the bacterial succession in radish kimchi during fermentation. When Bray-Curtis was used for the PCoA of beta-diversity, differences in bacterial composition between groups were observed during fermentation (Fig. 2B). The microbial profiles of radish kimchi on the 5th day of fermentation showed a great difference between the SK group and the other groups, indicating that the microbial community structure had changed differently. Meanwhile, until the 50th day of fermentation, the MK and LK groups showed relatively closer distances in beta-diversity, indicating that the changes in the microbial community were relatively similar compared to the SK group. Although several studies have reported that the surface area to volume ratio affects microbial growth (Kumar et al., 2021), there are few reports that demonstrate that it

promotes the growth of specific microorganisms differently in an environment where several microorganisms coexist. Changes in the microbial community structure of each group are shown in Fig. 2C. Overall, the genera *Leuconostoc*, *Weissella*, and *Lactobacillus* were the dominant microorganisms after 5 days of fermentation, and as fermentation progressed, the proportion of *Leuconostoc* increased in all groups. Fig. 2D shows the relative abundances of major LAB (*Leuconostoc*, *Weissella*, and *Lactobacillus*), which are essential microorganisms for kimchi production (Jeong, Lee, Jung, Choi, et al., 2013), on day 5 of fermentation. The relative abundances of *Leuconostoc* (49.2 %), *Weissella* (17.9 %), and *Lactobacillus* (3.0 %) were higher in the SK group than in the other groups, suggesting that these bacteria proliferated rapidly in the SK group with smaller radishes. Similar results have been reported for *meju* (a Korean fermented soybean brick) (Lee et al., 2012) and cabbage kimchi (whole cabbage and sliced cabbage) (Moon et al., 2019) the smaller the size of the ingredients, the higher was the number of LAB. Radish is rich in nutrients, such as glucose and fructose (Seo et al., 2018), which are the main energy sources for LAB. In this study, the glucose content on the 15th day of fermentation was significantly higher in the SK group than in the LK group (Fig. 2E), suggesting that more nutrients, including glucose, were eluted from small-sized radish. The increased availability of nutrients can lead to the rapid growth of LAB in

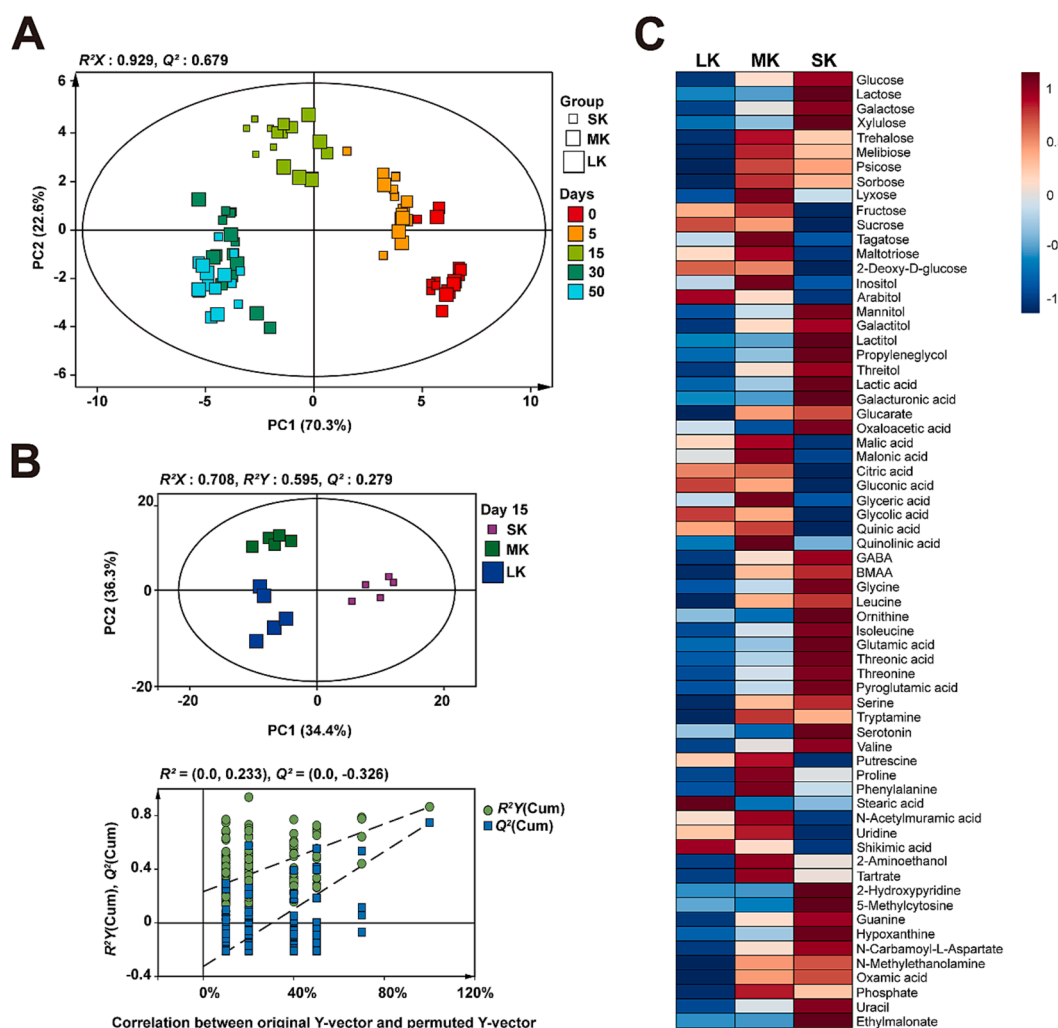


Fig 3. (A) Principal component analysis (PCA) score plot derived from GC-MS data of radish kimchi samples during 50 days of fermentation. (B) Projection to latent structures and discriminant analysis (PLS-DA) score plot and 200 permutations test obtained from GC-MS data of radish kimchi samples at the 15th day of fermentation. (C) Heatmap showing the 66 identified metabolite variations between the groups (mean value) at the 15th day based on analysis of variance (ANOVA) using Pearson distance. The concentration of each metabolite is colored based on a normalized scale of minimum - 1 (dark blue) to a maximum of 1 (dark red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the SK group; as a result, the lactic acid content was the highest in the SK group on day 15 of fermentation (Fig. 2E).

3.3. Changes in metabolic profiles

To investigate metabolic changes in radish kimchi during 50 days of fermentation, PCA was applied using the features obtained from the GC–MS data (Fig. 3A). The PCA score plot showed that the samples moved from right to left as fermentation progressed, indicating a continuous change in the metabolites during fermentation ($R^2X = 0.929$ and $Q^2 = 0.679$). The SK group on the 15th day of fermentation in the PCA score plot was slightly different from the other groups, which suggests that the metabolite profile of this group was different from that of the other groups at this time point. To investigate differences in metabolite profiles between the groups, PLS-DA, a supervised pattern recognition method, was performed using radish kimchi samples on the 15th day of fermentation (Fig. 3B). The PLS-DA score plot showed an evident separation between groups ($R^2X = 0.708$, $R^2Y = 0.595$, and $Q^2 = 0.279$), indicating that the metabolites of radish kimchi samples differed depending on the ingredient size used. To understand the overall difference in metabolites on day 15 of fermentation between

groups, a heatmap was generated using the levels of 66 metabolites (Fig. 3C). The heatmap showed different patterns of metabolic profiles between groups, indicating that metabolites of radish kimchi samples differed according to the size of the ingredients used. These results are likely owing to the differences in microbial profiles according to the size of the ingredient.

A total of 17 metabolites showed significant differences ($p < 0.05$) among the groups at specific sampling points during the fermentation period. Changes in the levels of the 17 metabolites during fermentation are shown in Fig. 4. Generally, the main sugars detected during kimchi fermentation include fructose, glucose, galactose, and sucrose (Jung et al., 2012); these sugars were also detected in this study (Fig. 4A). The increase in sugar content in the early stages of kimchi fermentation is caused by the extraction of ingredients, such as vegetables (Jeong, Lee, Jung, Lee, et al., 2013; Jeong, Lee, Jung, Choi, et al., 2013). The levels of glucose and galactose were the highest in the SK group on the 15th day of fermentation, indicating that more of these sugars were extracted from the smaller-sized radish. In contrast, sucrose and fructose levels were significantly lower in the SK group on day 1 of fermentation and showed a decreasing pattern during the fermentation period. Sucrose is decomposed into glucose and fructose by LAB, such as *Leuconostoc* and

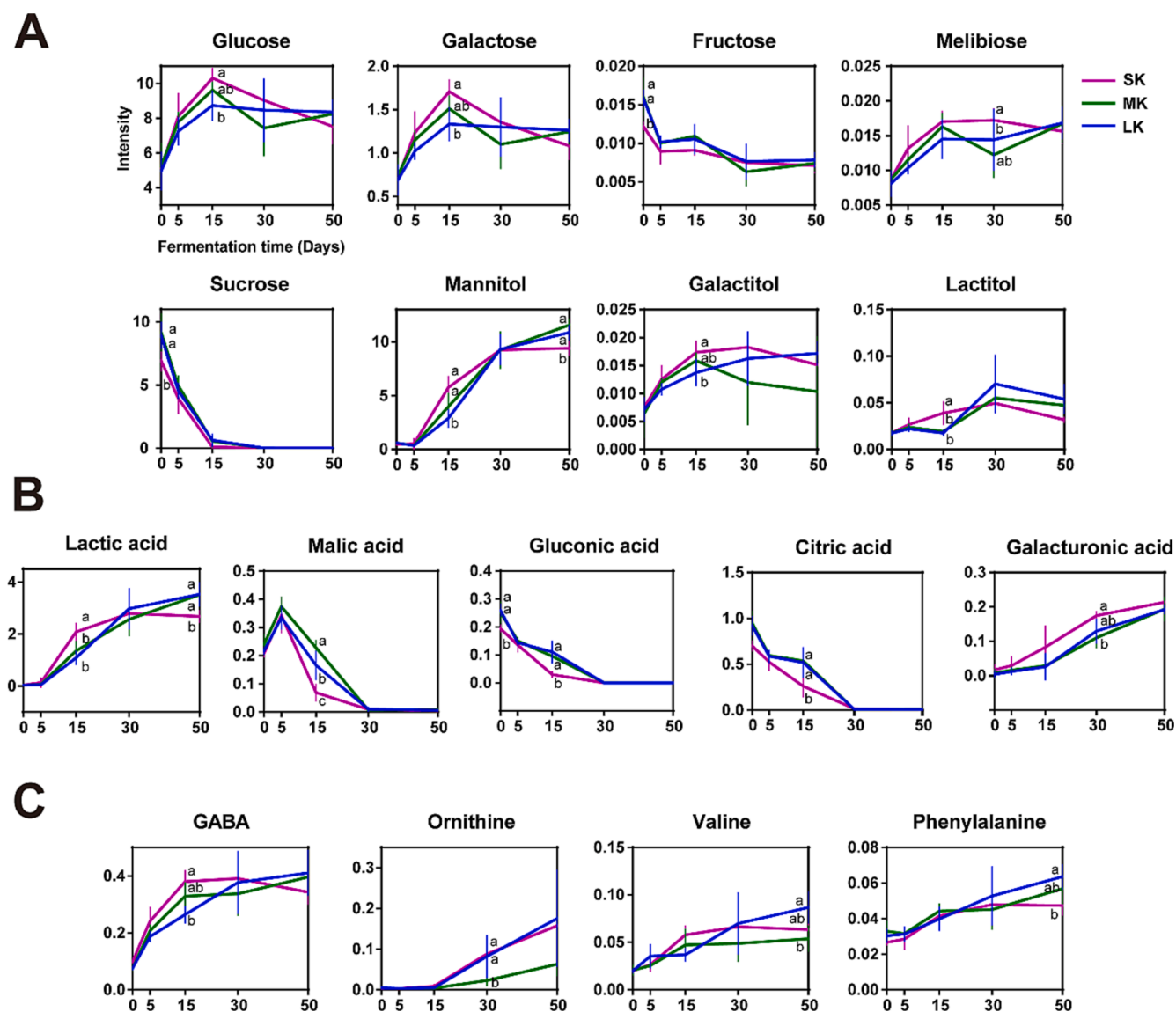


Fig. 4. Changes of (A) sugars and sugar alcohols, (B) organic acids, and (C) amino acids levels in radish kimchi samples during fermentation. Metabolites that showed significant differences ($p < 0.05$) between groups at specific sampling points during the fermentation period were selected.

Weissella (Choi et al., 2019). Therefore, the rapid decrease in sucrose in the SK group was probably related to the rapid growth of LAB. On the 15th day of fermentation, mannitol levels were higher in the SK group than in the LK group. Mannitol is a sugar alcohol produced by heterofermentative LAB such as *Leuconostoc* and *Lactobacillus* during kimchi fermentation (Wisselink et al., 2002). However, on the 50th day of fermentation, the mannitol level was the lowest in the SK group, suggesting that mannitol levels may vary between groups depending on the fermentation time. Some organic acid levels showed a significant difference between the SK group and the other groups on the 15th or 30th day of fermentation (Fig. 4B). Although the SK group had relatively higher levels of lactic acid than MK and LK on the 15th day of fermentation, the levels of lactic acid were lower in the SK group on the 50th day of fermentation. The higher lactic acid levels in the SK group on day 15 of fermentation can be explained by rapid LAB proliferation and glucose consumption. In contrast, in the SK group, the levels of malic acid and citric acid rapidly decreased, showing significantly lower contents on the 15th day of fermentation. The levels of some amino acids in radish kimchi increased during fermentation; however, there was no specific trend between the groups (Fig. 4C). The levels of GABA and ornithine were significantly different after 15th and 30th day of fermentation, respectively, and the levels of valine and phenylalanine were significantly different after 50 days of fermentation. Taken together, the levels of some metabolites, such as sugars, organic acids, and GABA, were significantly different in the SK group from other groups within 30 days of fermentation but did not reveal a visible trend

at 50 days of fermentation. These results suggest that the levels of some metabolites in fermented foods may vary in the early stages of fermentation, depending on the size of the raw ingredients used. This is because LAB proliferate quickly in radish kimchi with small raw ingredients, and thus, some metabolites are quickly consumed or produced by LAB. To identify which pathways were affected by the identified metabolites from radish kimchi on the 15th day of fermentation, two distinct groups (SK and LK) were used for the metabolic pathway and enrichment analysis module in MetaboAnalyst (Fig. 5). Majority of the metabolic pathways affected by the different sizes of ingredients were related to sugar or amino acid metabolism. A link is evident between the sugars leached from radishes and the metabolic pathways where microorganisms consume these sugars. Notably, pyruvate metabolism indicates a potential relationship between these sugars and microorganisms, influenced by the size of the radish ingredients; pyruvate being a product of aerobic and anaerobic glucose metabolism (Vander Heiden et al., 2009). Nevertheless, further research is imperative to fully understand the relationships between various metabolic pathways.

The smaller the size of the ingredient, the higher the surface area to volume ratio is and the faster will be the removal of the compounds from the ingredient. In this study, the SK group, which had the smallest ingredient size, showed the lowest hardness value and the highest glucose content in the early stage of fermentation, indicating that water and nutrients were quickly eluted from the small-sized radish cubes. Therefore, the fermentation environment of the SK group must have changed to a relatively favorable environment (increase in water

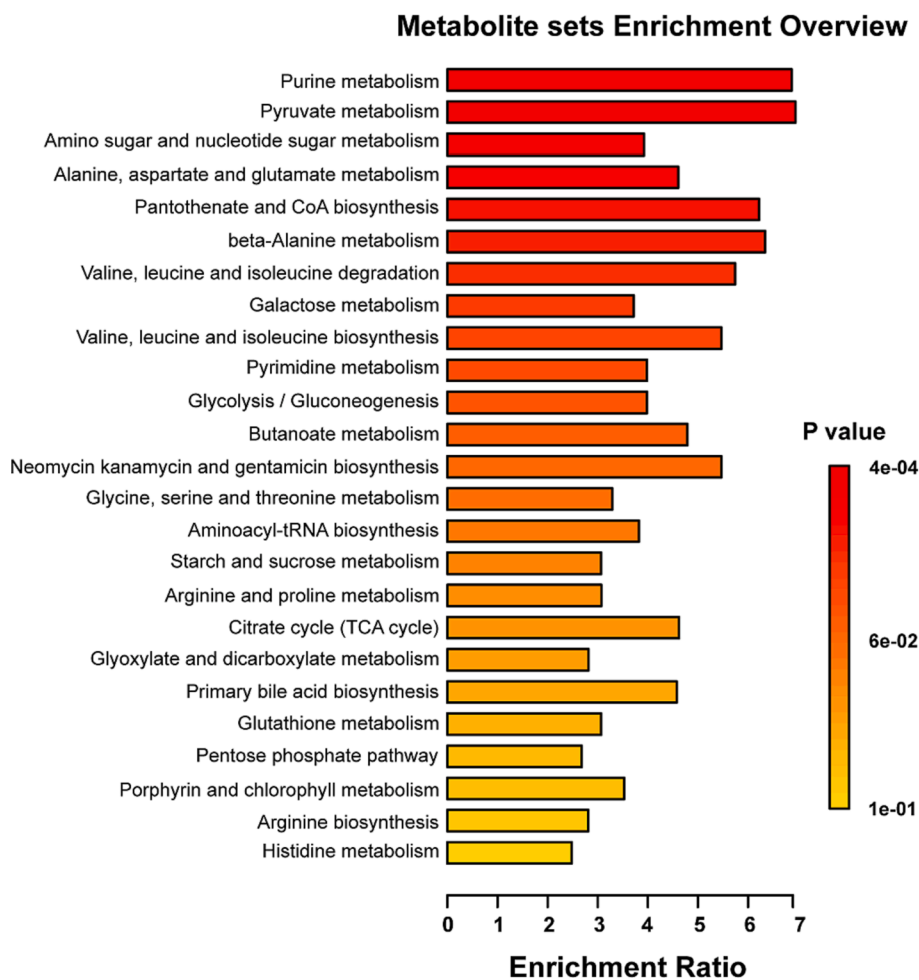


Fig 5. Enrichment analysis performed in MetaboAnalyst using a set of 66 metabolites found to be significantly different between small-sized radish kimchi group (SK) and large size radish kimchi group (LK) at 15th day of fermentation. The enrichment ratio is calculated as the number of hits within a particular metabolic pathway divided by the expected number of hits.

activity and supply of nutrients) for LAB growth. The relative abundance of major LAB (*Leuconostoc*, *Weissella*, and *Lactobacillus*) was the highest in the SK group on day 5 of fermentation. The rapid proliferation of LAB in the SK group resulted in the rapid production of lactic acid, which in turn decreased the pH and increased TA. In addition, it is estimated that the rapid proliferation of LAB also affects the production rate of metabolites, such as mannitol and GABA, and the consumption rate of metabolites such as malic acid and citric acid. Overall, after 15 days of fermentation, the SK group revealed different values of texture, total acidity, pH, and metabolites than the other groups, suggesting that the size of the ingredient affects the quality of the fermented food. Indeed, the results of the sensory evaluation showed that the SK group exhibits the highest level of sourness and the lowest levels of saltiness, sweetness, and bitterness compared to the other two groups during the early period of fermentation. Intriguingly, the sensory evaluation also revealed considerable differences in all taste metrics for the LK group compared to the other two groups at 30 days post kimchi generation. These results might be attributed to the slow fermentation rate, which could be due to the small surface area resulting from the large size of the radish cubes. However, after 50 days of fermentation, they tended to be similar among the groups, indicating that the size of the ingredient particularly affected the fermentation rate. A study by Koo *et al.* (Koo *et al.*, 2016) reported that several amino acids (e.g., glutamic acid, alanine, and glycine) contribute to the umami taste, while leucine, isoleucine, and arginine contribute to the bitter taste in kimchi. Accordingly, we attempted to integrate the metabolic profiles that contribute to the taste of radish kimchi with the results of sensory evaluation. The LK group showed a significantly higher level of glutamic acid after 50 days of fermentation compared to other groups (Fig. S2C). This group also exhibited a slightly elevated level of umami taste after 50 days of fermentation (Fig. S2B). At this sampling time, the level of glycine was also slightly higher in the LK group than in the other groups. In terms of bitterness, we could not find a meaningful relationship with the levels of leucine or isoleucine. Variations in specific amino acids and organic acids can influence flavor nuances, suggesting that taste modulation might be contingent on the size of the ingredient. Taken collectively, this study showed that microbial and metabolic profiles can vary depending on the size of the raw ingredients in radish kimchi. However, further studies are required to determine how the size of raw ingredients affects the sensory properties of fermented foods.

4. Conclusion

In this study, we observed that the size of the radish cube used for the fermentation of radish kimchi affected the microbial and metabolic profiles during fermentation. The smaller the size of the radish, the faster the LAB growth, leading to differences in the consumption or production of metabolites, such as sugars, organic acids, and amino acids, during fermentation. This study is significant as it is the first to identify that microbial and metabolic profiles can vary depending on the size of the raw ingredients in radish kimchi. This study highlights the importance of understanding the impact of ingredient size on the fermentation rate. Ingredient size selectively affected the growth of specific microorganisms in an environment where numerous microorganisms coexist, which could change the quality of fermented foods, particularly at the beginning of fermentation.

CRediT authorship contribution statement

Hyun-Woong Choi: Conceptualization, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Seong-Eun Park:** Formal analysis, Software, Visualization. **Eun-Ju Kim:** Writing – review & editing. **Seung-Ho Seo:** Formal analysis, Investigation. **Tae Woong Whon:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft. **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

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2023.100950>.

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