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Effects of glutinous rice paste and fish sauce on kimchi fermentation

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ABSTRACT

To investigate the effects of glutinous rice paste (GRP) and fish sauce (FS) on kimchi's the fermentation characteristics, kimchi, kimchi with GRP, and kimchi with FS were prepared and the bacterial communities, metabolites, and volatile compounds were analyzed over 30 days of fermentation. *Leuconostoc* was most dominant, and *Lactobacillus* was next in all kimchi, and *Lactobacillus* was slightly more abundant in kimchi with GRP or FS. Bacterial abundances were clearly lower in kimchi with FS than other two kimchi. Compared to the other two kimchi, glucose (only initial period) and lactic acid, acetic acid, and ethanol concentrations were higher in kimchi with GRP, whereas mannitol (30 days) and amino acid concentrations were higher in kimchi with FS. The metabolite profiles of kimchi and kimchi with GRP were relatively similar, but slightly different from that of kimchi with FS. Volatile compounds were slightly higher in kimchi with GRP or FS compared to kimchi and their profiles in the three kimchi were a little different at the initial period (5 days), but they became relatively similar during the late periods (20–30 days). This enhanced understanding of GRF's or FS′ effects on kimchi's fermentation will contribute to the production of high-quality kimchi.

1. Introduction

Kimchi is the most representative Korean traditional fermented food, emblematic of Korean culture, served together with almost every meal in Korea. Kimchi is made by the fermentation of vegetables such as cabbage, radish, and green onion with other major seasoning components, including red pepper powder, garlic, leek, and ginger (Lee, Whon, Roh, & Jeon, 2020; Surh, Kim, & Kwon, 2008). Spontaneous fermentation of salted vegetables leads to the growth of various lactic acid bacteria (LAB) producing diverse metabolic compounds such as organic acids, amino acids, vitamins, bacteriocins, prebiotic factors, and mannitol that contribute to the organoleptic, health-promoting, and nutritional properties of kimchi (Choi et al., 2019; Jung, Lee, & Jeon, 2014; Kim et al., 2020; Kim, Lee, Chun, Jeong, & Jeon, 2019; Lee et al., 2020; Park, Jeong, Lee, & Daily III, 2014).

Despite these health-promoting and nutritional properties, the consumption of kimchi in Korea is declining, especially among young people. The Korean kimchi market share is also decreasing due to the imports of kimchi from abroad (Kang & Lee, 2020). Therefore, the development of kimchi with new taste or high quality is necessary to increase kimchi consumption and protect the Korean kimchi market share from imported kimchi. In Korea, depending on the region or household, various sub-ingredients such as glutinous rice paste (GRP), fish sauce (FS), pine nuts, and certain fruits have been often supplemented to enhance or modify the taste, texture, and flavor of kimchi. The supplementation of these sub-ingredients to kimchi could represent an important approach to producing high-quality kimchi; thus, studies examining the effects of these additives on the quality and taste of kimchi are necessary.

Based on mainly major raw materials, kimchi is classified into hundreds of varieties. The fermentation features of kimchi such as microbial community, metabolites, and volatile compounds affecting the taste, texture, and flavor of kimchi can be very different depending on major raw materials, the region, household, and fermentation conditions (Lee, Jung, & Jeon, 2015; Lee, Song, Park, & Chang, 2019; Lee et al., 2020; Lim et al., 2015; Park et al., 2012). However, not only major raw materials and fermentation conditions, but also the sub-ingredients of kimchi can also have a great influence on the fermentation of kimchi, so many studies on their effects on kimchi fermentation have been conducted (Jeong, Lee, et al., 2013; Jeong, Jung, Lee, Jin, & Jeon, 2013; Song, Shi, Gninguue, Wei, & Luo, 2017). Among the sub-ingredients, GRP and FS in particular have been commonly added as sub-ingredients during the preparation of kimchi to enhance taste or quality. Recently, some studies on microbial community and metabolite

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changes according to the addition of GRP or FS during kimchi fermentation have been partially conducted (Cha, Kim, & Cadwallader, 1998; Jeong, Lee, & Chung, 2018; Jung et al., 2018), but the fermentation characteristics of kimchi, including bacterial cell counts, bacterial community, metabolites, and volatile compounds, have not been comprehensively explored.

The main objective of this study was to more comprehensively investigate the effects of GRP and FS on the fermentation characteristics of kimchi. For the study, we prepared kimchi supplemented with GRP and FS, analyzed the absolute bacterial community, metabolites, and volatile compounds through Illumina MiSeq 16S rRNA gene sequencing of spike-in kimchi samples of external bacterial cells, ^{1}H NMR spectrometry, and gas chromatography/mass spectrometry (GC/MS), respectively, during fermentation, and compared their profiles statistically. This study will contribute to the production of high-quality kimchi with new flavors by providing a better understanding of the effects of GRP and FS on kimchi's fermentation characteristics.

2. Materials and methods

2.1. Kimchi preparation

Three types of kimchi, kimchi, kimchi with glutinous rice paste (GRP), and kimchi with fish sauce (FS), were prepared in triplicate according to the procedure described previously with some modifications (Jung et al., 2018). Briefly, baechu-cabbages (also known as Chinese cabbage) were soaked for 16 h in 13% (w/v) solar salt solution, and the salted baechu-cabbages were briefly rinsed with tap water and drained of excessive water. Three types of seasoning mixtures for kimchi, kimchi with GRP, and kimchi with FS were prepared by mixing ground garlic, ground ginger, red pepper powder, water, GRP, solar salt solution, and FS at the ratios of 16.7:8:20:28.7:0:26.6:0, 16.7:8:20:8.7:20:26.0:0, and 16.7:8:20:28.7:0:0:26.6 by weight, respectively. Here, glutinous rice paste was prepared by adding glutinous rice powder into water (10%, w/v), boiling for 10 min, and cooling. Solar salt solution was prepared by dissolving solar salts (Shinan, Korea) in water (29%, w/v). Myeolchi-aekjeot, a commercial anchovy sauce (CJ, Korea), was used as a fish sauce. The salted baechu-cabbages were dispensed into 9 plastic boxes containing 5.0 kg of baechu-cabbages and mixed with three different seasoning mixtures at the ratios of 85:15 (w/w). The kimchi plastic boxes were incubated at 4 °C for 30 days.

2.2. Sampling and pH measurement

Kimchi soups (the liquid fractions of kimchi) were sampled during fermentation and their pH values were measured. Kimchi soups were filtered through four layers of sterile coarse gauze (Daehan, Korea) to remove large particles. One milliliter of the filtered kimchi soups was centrifuged to harvest microorganisms at 16,100×*g* and 4 °C for 5 min. Cell pellets from the triplicate samples were combined and stored at −80 °C for bacterial community analysis. However, the supernatants of the triplicate samples were separately stored for the analysis of metabolites and volatile compounds at −80 °C.

2.3. Bacterial community analysis

To estimate bacterial cell numbers and to obtain absolute bacterial communities in kimchi samples during fermentation, the same numbers of *Methylobacterium* sp. SyP6R (GenBank acc. no., JAAQRC000000000) (approximately 10^{9} cells) were added into respective cell pellets, and total genomic DNA was extracted using FastDNA Spin Kit (MP Biomedicals, USA). Bacterial communities of the kimchi samples were analyzed according to the procedure described previously (Han, Chun, Feng, Kim, & Jeon, 2020). Briefly, the V3–V4 regions of bacterial 16S rRNA genes were PCR-amplified using the universal primer set, 341F (5′-adaptor-CCT ACG GGN GGC WGC AG-3′)/805R (5′-adaptor-GAC TAC HVG GGT ATC TAA TCC-3′) and sequenced using an Illumina MiSeq paired-end platform (× 300 bp; Illumina, USA) at Macrogen (Korea). The sequencing reads were sorted into respective kimchi samples based on their barcode sequences, and barcode and adapter sequences were removed using Scythe (https://github.com/vsbuffalo/scythe). Low-quality nucleotides less than 20 quality thresholds (0.01 error rate) were trimmed from both sides of nucleotide sequences using Sickle (https://github.com/najoshi/sickle). The sorted and high-quality sequencing reads were processed and taxonomically analyzed using the Qiime2 plugin software package (https://qiime2.org/). Briefly, the paired-end sequencing reads were assembled using VSEARCH (Rognes, Flouri, Nichols, Quince, & Mahé, 2016), and sequencing reads less than 450 nucleotides and chimeric sequences were removed using Deblur (ver. 1.0.0; Amir et al., 2017). The high-quality sequencing reads were classified at the genus level based on the Silva database (silva-119-99-nb-classifier) (Quast et al., 2012), and sequencing reads classified as chloroplast 16S rRNA gene sequences were removed. Based on the read counts from spiked-in *Methylobacterium* sp. SyP6R cells and relative bacterial communities in each kimchi sample, absolute bacterial communities were calculated.

2.4. Analysis of metabolic compounds

Metabolic compounds, including free sugars, amino acids, organic acids, ethanol, and mannitol, in each kimchi sample during fermentation were analyzed using ^{1}H NMR spectroscopy, according to a previously described protocol (Han et al., 2020). Briefly, 350 μL of kimchi supernatants were mixed with the same volume of 99.9% D_2O (Sigma-Aldrich, USA) containing 5 mM sodium 2, 2-dimethyl-2-silapentane-5-sulfonate (DSS; Sigma-Aldrich) and their ^{1}H NMR spectra were measured on a Varian Inova 600-MHz NMR spectrometer (Varian, USA). Metabolic compounds were identified and quantified using the Chenomx NMR Suite program (ver. 8.4; Chenomx, Canada) based on the DSS peak as an internal standard, and their concentrations in each kimchi sample were expressed as μmol/mL-kimchi supernatant.

To compare the metabolite profiles of the three types of kimchi throughout the fermentation period, a principal component analysis (PCA) was performed using all peaks obtained by ^{1}H NMR spectroscopy as described previously (Jung et al., 2012). Briefly, all ^{1}H NMR spectra were first baseline corrected, after which the spectral data of 0.5–10 ppm were reduced into 0.001 ppm spectral buckets and the NMR spectra of 4.6–4.8 ppm corresponding to water were removed using the Mestrenova software (Mestrelab Research SL, Spain). The NMR spectra were normalized to the total spectral area and converted to ASCII format using the Mestrenova software. All NMR spectra were aligned using MATLAB (Mathworks, USA), and the PCA was performed based on the covariance matrix using R (http://cran.r-project.org/).

2.5. Analysis of volatile compounds

Volatile compounds in each kimchi sample were analyzed using headspace solid-phase microextraction (HS-SPME) coupled to GC/MS (7820A/5977E MSD; Agilent, USA), according to the procedure described previously with some modifications (Chun, Kim, Jeong, & Jeon, 2020). Briefly, 0.5 mL of kimchi supernatants were adjusted to approximately pH 6.0, diluted to 5 mL with distilled water, and transferred into 20 mL vials with a silicon/Teflon septum (Supelco, USA). Methyl cinnamate (1000 μg/mL in methanol) as an internal standard was added into each vial to be 10 μg/mL. HS-SPME was performed using a 2-cm long divinylbenzene/carboxen/polydimethylsiloxane (DVB-CARPDMS) SPME fiber (Supelco, USA) for 30 min in the automatic-injector PAL RSI 85 (CTC analytics AG, Switzerland) with shaking 250 rpm at 50 °C. Volatile compounds were thermally desorbed in the injection port at 250 °C for 2 min and analyzed with split mode (1:10) by GC/MS equipped with a DB-Wax column (50 m × 200 μm ×

0.2 μm; Agilent). The oven temperature was set to 40 °C (held for 5 min), increased to 150 °C at a rate of 5 °C/min, increased to 200 °C at a rate of 7 °C/min, and then finally held at 200 °C for 10 min. Helium (1.5 mL/min) was used as a carrier gas. The scan range of mass was m/z 30 to 300 at the fragment voltage of 70 eV. The identification of GC/MS peaks was accomplished through a library search of their mass spectra against the NIST database (ver. 11).

All volatile compounds were quantified using an external standard method described previously with some modifications (Gao et al., 2020; Lasekan, Khatib, Juhari, Patiram, & Lasekan, 2013; Nuzzi, Lo Scalzo, Testoni, & Rizzolo, 2008). Briefly, an external standard curve was first prepared through the GC/MS analysis of seven levels of benzaldehyde containing methyl cinnamate (10 μg/mL) as an internal standard. The concentrations of volatile compounds were then calculated using the molecular weights of benzaldehyde and volatile compounds based on the benzaldehyde standard curve. The PCA of volatile compounds identified by GC/MS during fermentation in the three kimchi was performed based on their concentrations. The z-scores of respective volatile compounds were calculated based on their relative concentrations during fermentation and visualized as heatmaps using Morpheus tool (https://software.broadinstitute.org/morpheus/), and their PCA was also conducted. Volatile compounds showing significant differences between three types of kimchi or fermentation time were indicated using bar graphs.

The odor activity values (OAVs) of volatile compounds were calculated by dividing the concentrations of volatile compounds by their odor thresholds, which were taken from literatures (An et al., 2019; Gao et al., 2020; Genovese, Lamorte, Gambuti, & Moio, 2013; Marcinkowska, Frank, Steinhaus, & Jelen, 2021; Qian & Wang, 2005; Zhang et al., 2022a, 2022b; Zhou et al., 2019; Zhu, Wang, Xiao, & Niu, 2018).

3. Results

3.1. General fermentation features of three types of kimchi

Initial pH values of kimchi, kimchi with GRP, and kimchi with FS were approximately 4.43–4.60 (Fig. 1A). The pH values quickly increased in all kimchi samples during the early fermentation period (0–5 days), which were supposedly due to alkaline vegetable saps containing free sugars and minerals released from baechu-cabbage (Jeong, Lee, et al., 2013). The increase of the pH values in kimchi with GRP or FS occurred more quickly than in kimchi without GRP and FS, which was supposed because the release of vegetable saps from baechu-cabbage was promoted by the osmotic effects of GRP or FS. The pH values decreased rapidly to less than 4 after their maximum pH values and then became relatively constant after 20 days. Interestingly, the decrease of the pH values was greatest in kimchi without GRP and FS and smallest in kimchi with FS.

3.2. Changes of bacterial communities in the three types of kimchi during fermentation

Bacterial community analysis at the genus level during kimchi fermentation revealed that very diverse bacterial genera were identified in the initial kimchi samples (0 days), but lactic acid bacteria were not identified, suggesting that a very small number of LAB were present in the raw materials (baechu-cabbages and seasonings) used for the kimchi preparation (Fig. S1). However, within only three days of fermentation, the bacterial communities were predominated by two LAB genera, *Leuconostoc* and *Lactobacillus*. Regardless of kimchi types and fermentation time, *Leuconostoc* was most abundant, and *Lactobacillus* was next. The relative abundance of *Lactobacillus* gradually increased as the fermentation progressed in all three types of kimchi, and it was generally higher in kimchi with GRP or FS than in kimchi without GRP and FS.

Bacterial abundances and communities in three types of kimchi during fermentation were calculated based on the relative abundances

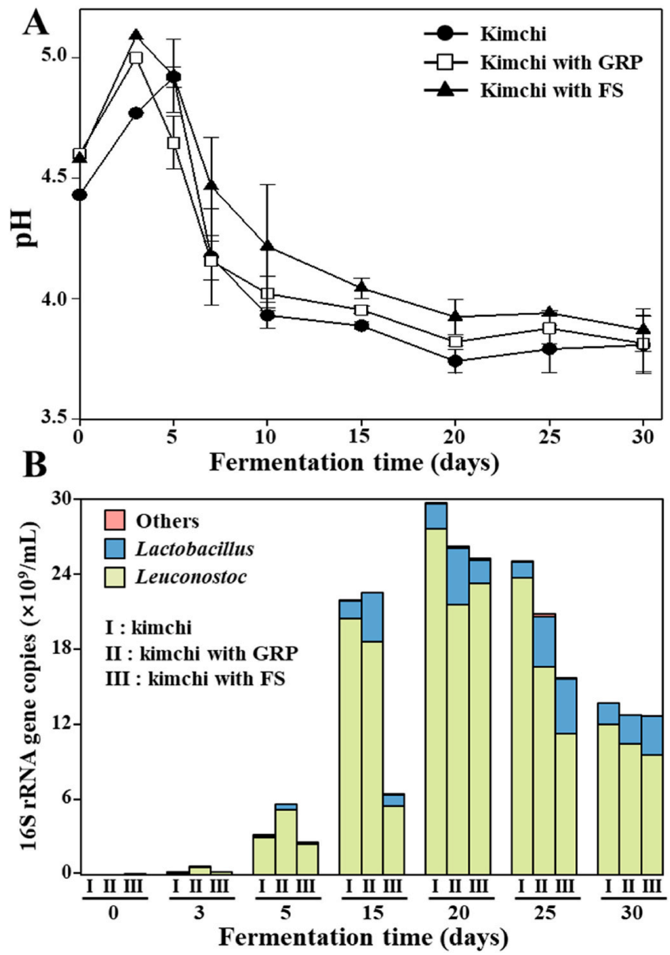

Fig. 1. Changes of pH (A) and bacterial abundance (B) during fermentation in kimchi, kimchi with glutinous rice paste (GRP), and kimchi with fish sauce (FS). The pH values and error bars represent the means and standard errors of triplicate samples, respectively. The 16S rRNA gene copies were estimated based on the sequencing read numbers of *Methylobacterium* sp. SyP6R spiked-in and the bacterial communities were analyzed using the 16S rRNA gene copy numbers and the relative abundance of Fig. S1. "Others" represents the sum of all other bacterial groups besides *Leuconostoc* and *Lactobacillus*.

and 16S rRNA gene copy numbers of *Methylobacterium* sp. SyP6R spiked-in before genomic DNA extraction. The abundance analysis showed that bacterial abundances in the initial kimchi samples (0 days) were very low (less than 1.76×10^7 16S rRNA gene copies/mL) (Fig. 1B). Regardless of the kimchi types, bacterial abundances rapidly increased to approximately $2.53–2.96 \times 10^{10}$ 16S rRNA gene copies/mL at 20 days of fermentation, and thereafter the bacterial abundances gradually decreased. Interestingly, the bacterial abundance in kimchi with FS were clearly lower than those in other two kimchi during fermentation, especially at 15 days, suggesting that FS may not promote the bacterial growth during kimchi fermentation. The bacterial abundances in kimchi without GRP and FS were higher than those in kimchi with GRP or FS after 20 days of fermentation, which was in agreement with the results of Fig. 1A that the pH decrease during fermentation was greatest in kimchi without GRP and FS and smallest in kimchi with FS.

3.3. Changes of free sugars, fermentation products, and amino acids in the three types of kimchi during fermentation

Because kimchi metabolites such as free sugars, amino acids, organic acids, ethanol, and mannitol are very closely associated with kimchi tastes, they were analyzed using ^{1}H NMR spectroscopy during fermentation. Glucose, fructose, and sucrose were detected as major free sugars providing sweetness in kimchi, and among them fructose was most abundant, followed by glucose and sucrose (Fig. 2A), which was similar to the results in previous reports (Jung et al., 2011). In the initial kimchi (0 days), fructose and sucrose concentrations were similar regardless of kimchi types, but glucose concentration in kimchi with GRP was significantly greater than those in the other two kimchi, probably due to the supplementation of GRP. However, as the fermentation progressed, the free sugar concentrations decreased rapidly, and they became relatively similar in all three kimchi. On the other hand, it was shown that the free sugar concentrations were slightly higher in kimchi with FS than those in kimchi and kimchi with GRP, suggesting that the fermentation of kimchi with FS might progress a little more slowly compared to the other two kimchi.

Lactate, acetate, ethanol, and mannitol were identified as major fermentation products during the kimchi fermentation (Fig. 2B). The concentrations of lactate, acetate, and ethanol were generally higher in kimchi with GRP than in the other two kimchi, probably due to the supplementation of GRP. On the other hand, the concentrations of lactate, acetate, and ethanol were significantly lower in kimchi with FS than in the other two kimchi. These results also support that kimchi fermentation in kimchi with FS might slowly progress compared to the other two kimchi. These results also may explain well the results that pH values in kimchi with FS were higher than those in the other two kimchi during fermentation. However, interestingly the concentration of mannitol, a six-carbon sugar alcohol contributing to a refreshing taste, was significantly higher in kimchi with FS compared to the other two kimchi at the end of fermentation (30 days), which suggests that kimchi with FS may have a higher refreshing taste compared to the other two kimchi.

The analysis of amino acids showed that the concentrations of amino acids in kimchi with FS were generally higher than those in the other two kimchi, as predicted, but most amino acids were almost constantly retained regardless of kimchi types during the kimchi fermentation (Fig. 3), suggesting that only very small amounts of amino acids were used for the growth of kimchi LAB.

To compare the profiles of all metabolites in the three types of kimchi during fermentation, a PCA was performed using all ^{1}H NMR peaks (Fig. S2). The PCA plot showed that all three types of kimchi had very similar metabolite profiles during the early fermentation periods (0 and

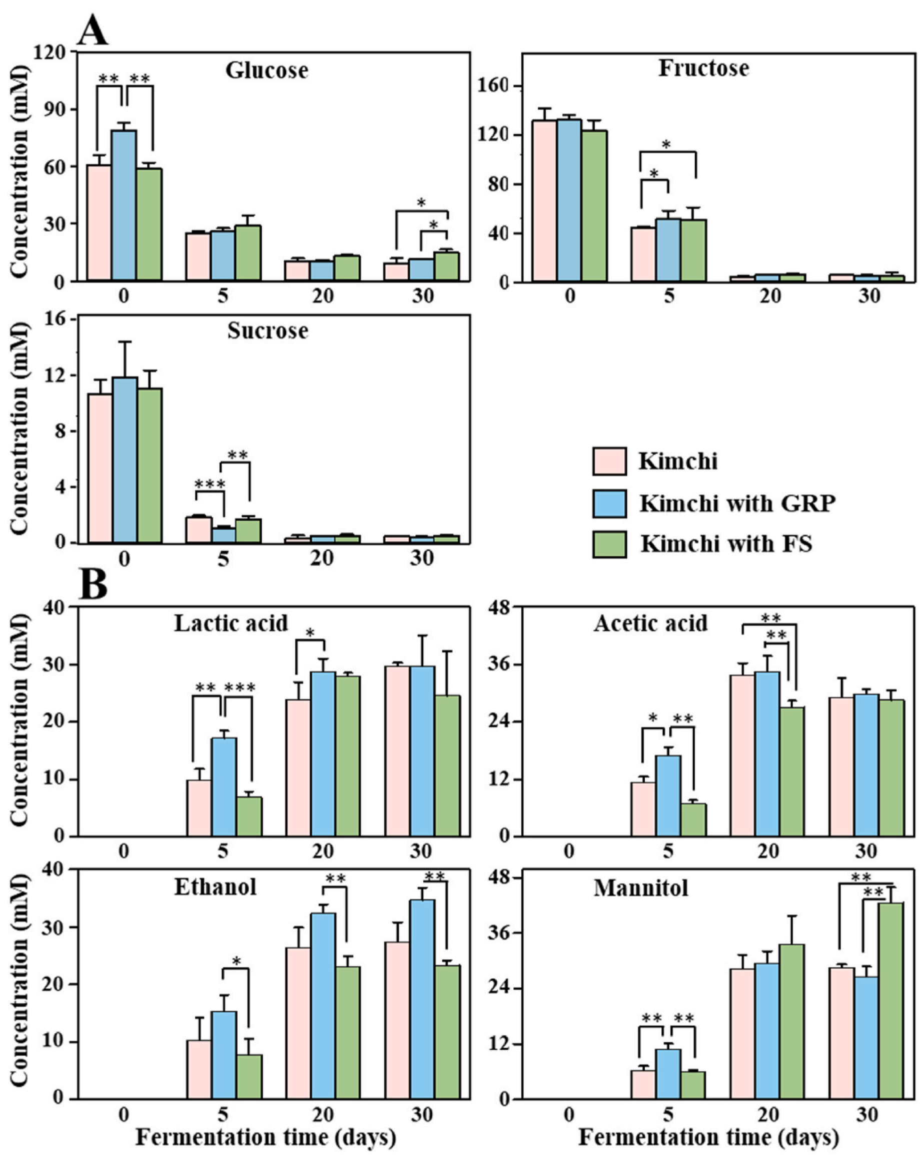

Fig. 2. Profiles of major free sugars (A) and fermentation products (B) identified in kimchi, kimchi with glutinous rice paste (GRP), and kimchi with fish sauce (FS) during fermentation. Data measured in triplicate are given as mean ± standard errors, and significant differences between samples are indicated with p values < 0.05 (*), <0.01 (**), or < 0.001 (***).

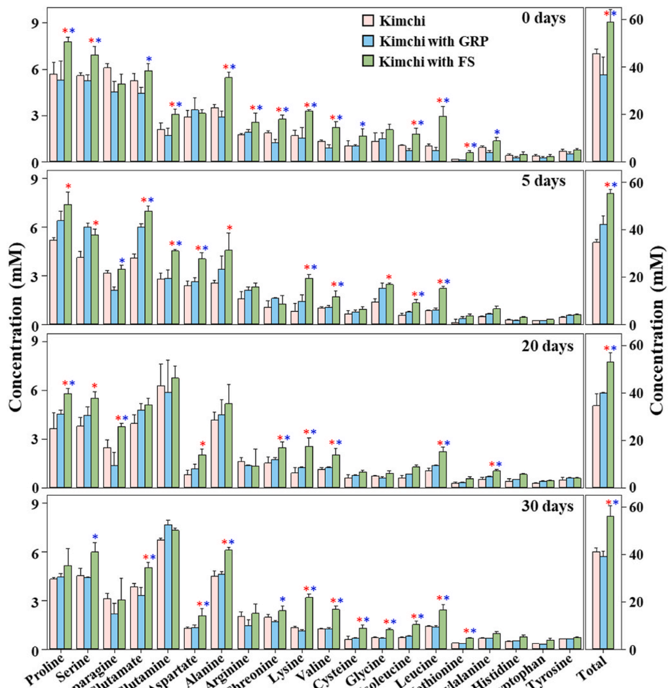

Fig. 3. Profiles of amino acids identified in kimchi, kimchi with glutinous rice paste (GRP), and kimchi with fish sauce (FS) during fermentation. Data measured in triplicate are given as mean ± standard errors. Amino acids with significantly higher concentrations (p value < 0.001) in kimchi with FS compared to kimchi and kimchi with GRP are indicated with red and blue asterisks, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

5 days), suggesting that the metabolite profiles of kimchi are not significantly affected by only the addition of GRP or FS. However, the metabolite profiles greatly changed after 5 days, and they became clearly different depending on the kimchi types at 20 and 30 days. In particular, the metabolite profiles in kimchi with FS were shown to be quite different from those in the other two kimchi at 20 and 30 days.

3.4. Changes of volatile compounds in the three types of kimchi during fermentation

The analysis of volatile compounds in the three types of kimchi during fermentation revealed that sulfur compounds, terpenes, and benzenes were identified as major volatile compounds in all three kimchi, and acids, nitrogen compounds, alcohols, and ketones were also identified as minor volatile compounds (Table S1, Fig. 4A). The overall concentrations of volatile compounds were slightly higher in kimchi with GRP or FS compared to kimchi without GRP and FS. The total concentrations of the volatile compounds increased at the early fermentation period (5 days), but thereafter gradually decreased as the fermentation progressed (Fig. 4A). The profiles of volatile compounds during fermentation were relatively similar in the three types of kimchi. The PCA also showed that the profiles of volatile compounds were somewhat different at 5 days, but eventually they became relatively similar during the late fermentation periods (20 and 30 days) (Fig. 4B). Diallyl sulfide, 1,3-dithiane, diallyl disulfide, and methyl 2-propenyl trisulfide belonging to sulfur-containing compounds, α-curcumene and β-phellandrene belonging to terpenes, and 4-propylbenzaldehyde belonging to benzenes were identified as major volatile compounds in all three types of kimchi during all fermentation periods (Fig. 4A), which suggests that these compounds may be common volatile compounds in kimchi.

To investigate the changes of respective volatile compounds during fermentation in the three types of kimchi, their profiles were visualized using z-scores based on the fermentation periods, which showed that the profiles of volatile compounds during the kimchi fermentation generally had three different patterns (Fig. 4C). Volatile compounds such as benzaldehyde, diallyl sulfide, 3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate, 3-chloro-2-methylcyclopent-2-enol, diallyl disulfide, di-2-propenyl trisulfide, and 3-vinyl-1,2-dithiacyclohex-4-ene were highly detected in the initial kimchi samples (0 days), but they decreased as the fermentation progressed (Fig. 4CII and D). It is thought that these volatile compounds may be derived from raw materials such as cabbage and garlic and metabolized during fermentation. Volatile compounds such as ethyl cinnamate, α-phellandrene, camphene, m-cymene, β-phellandrene, and α-curcumene increased at 5 days, and thereafter decreased during the late fermentation periods (Fig. 4CI). Volatile compounds such as benzenepropanenitrile, 2,3-butanediol, 1,3-bis(1,1-dimethylethyl)benzene, linalool, 5-hexenenitrile, 2-isothiocyanato-ethylbenzene, and 1-isothiocyanato-heptane were detected at very low concentrations in the initial kimchi samples, but they gradually increased as the fermentation progressed (20 and 30 days) (Fig. 4CIII and D), suggesting that these volatile compounds may be produced through microbial metabolisms during kimchi fermentation and contribute to the flavoring characteristics of kimchi products. Volatile compounds showing significant differences between the three types of kimchi were also investigated (Fig. 4D). Although some volatile compounds such as 3-vinyl-1,2-dithiacyclohex-5-ene and 5-hexenenitrile showed slight differences in the three types of kimchi, it was shown that most of volatile compounds were relatively similar, especially during late fermentation kimchi, which was in good agreement with the PCA results showing similar volatile compound profiles in the three types of kimchi (Fig. 4B).

To identify the volatile compounds that are major contributors to kimchi's overall odor activity, and to compare odor activities among three types of kimchi, the OAVs of volatile compounds identified in kimchi were calculated based on their odor thresholds, which were obtained from previous studies (Table S2, Fig. 5). Sulfur compounds, including dimethyl disulfide, dimethyl trisulfide, diallyl sulfide, and diallyl disulfide, classified as pungent odor compounds, showed very high OAVs (32–28,650), suggesting that these volatile compounds may contribute most to kimchi's odor activity. Benzaldehyde, 2-undecanone, and linalool, classified as fruit odor compounds, 2-isothiocyanato-ethylbenzene and benzenepropanenitrile, classified as vegetable-like odor compounds, and β-phellandrene, m-cymene, and α-phellandrene, classified as wood odor compounds, all showed OAVs of more than 10, suggesting that they are likely also major contributors to kimchi's odor activity. Because the volatile compounds showing high OAVs in kimchi were abundantly identified, even in the initial kimchi samples (0 days), these compounds might derive from raw materials such as baechu cabbages. The OAVs increased in all three types of kimchi during the early fermentation period (5 days), and thereafter gradually decreased as fermentation progressed (Fig. 5). This trend was consistent with the concentration profiles of volatile compounds (Fig. 4A). Although there were some OAV differences among the three types of kimchi during fermentation, their OAVs were generally similar (Fig. 5). In particular, the OAVs during the end of fermentation (20–30 days) were shown to be relatively similar, lacking significant differences between the three types of kimchi.

4. Discussion

Kimchi fermentation is greatly affected by the main raw materials used for the preparation of kimchi as well as external factors such as fermentation temperature and salinity (Jeong, Lee, et al., 2013; Jeong, Jung, et al., 2013; Lee, &; Lee et al., 2021; Song et al., 2020; Song, Park, & Chang, 2019). In addition, in Korea, various sub-ingredients such as GRP, FS, pine nuts, and fruits are often added to kimchi in small amounts to improve or modulate the quality, taste, and flavor of kimchi and these

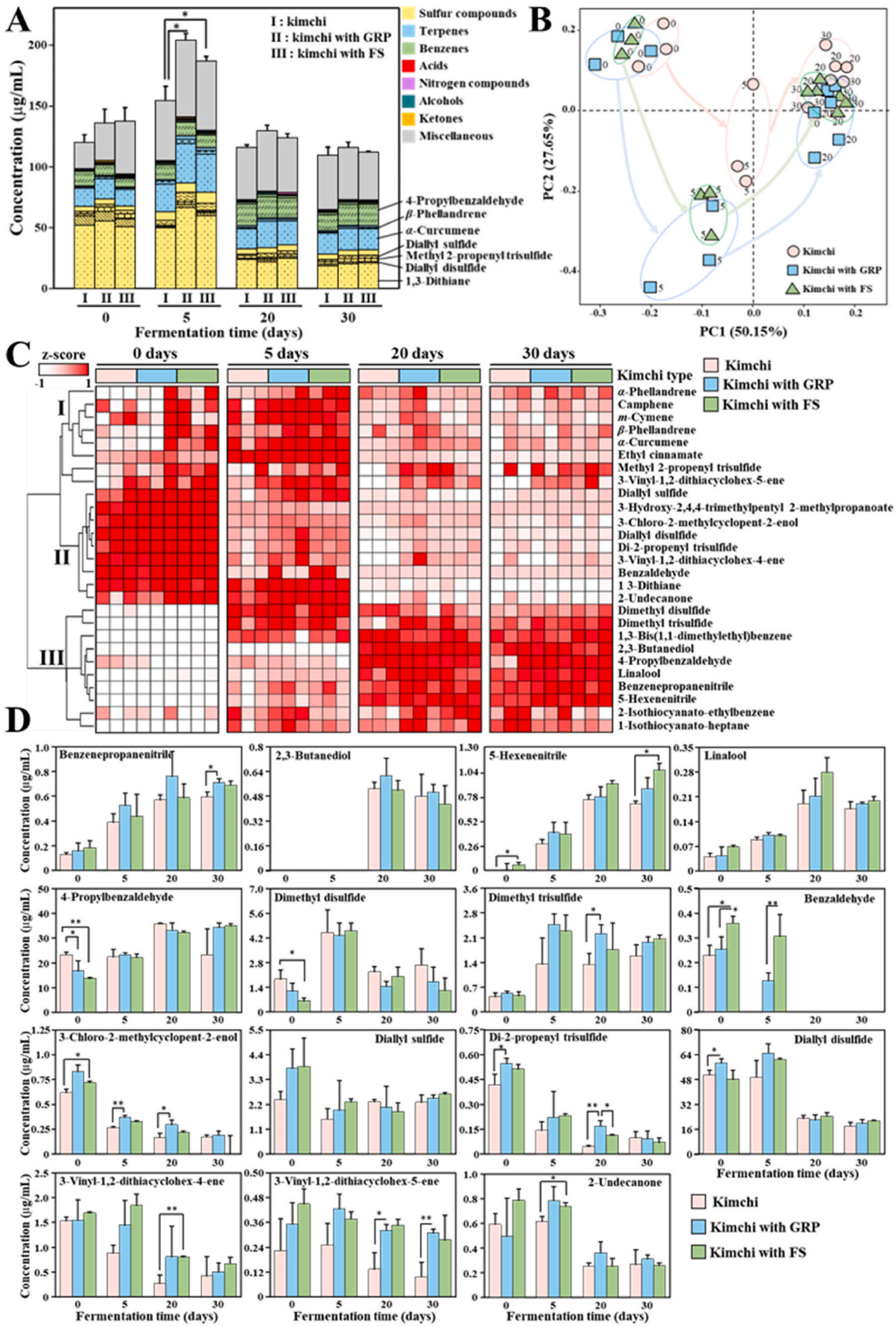

Fig. 4. Profiles (A) and principal component analysis (B) of volatile compounds identified in kimchi, kimchi with glutinous rice paste (GRP), and kimchi with fish sauce (FS) during fermentation. The volatile compounds were also indicated using heatmaps of the z-scores representing the relative concentrations of respective volatile compounds (C), and volatile compounds showing significant differences between kimchi samples were compared (D). Data were measured in triplicate samples and error bars represent standard errors. Significant differences between kimchi types are indicated with p values < 0.05 (*), <0.01 (**), or < 0.001 (***).

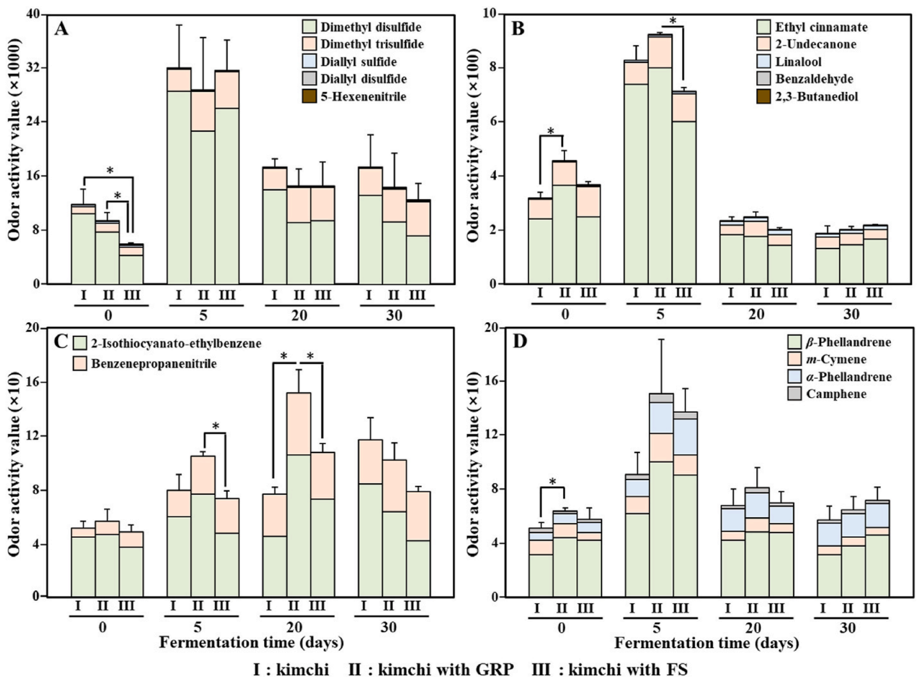

Fig. 5. Profiles of odor activity values (OAVs) of pungent (A), fruity (B), vegetable-like (C), and woody (D) flavor compounds identified in kimchi, kimchi with glutinous rice paste (GRP), and kimchi with fish sauce (FS) during fermentation. Data represent mean OAVs for each volatile compound measured in triplicate samples, and error bars indicate standard errors for total OAVs. Significant differences of total OAVs between kimchi types are indicated at $p < 0.05$ (*), $p < 0.01$ (**), or $p < 0.001$ (***).

sub-ingredients may also affect the fermentation features of kimchi (Choi et al., 2018; Jung et al., 2018; Lim et al., 2015). Because the effects of sub-ingredients on the microbial community, metabolites, and flavor components during kimchi fermentation will ultimately affect the taste, flavoring characteristics, and functionality of kimchi, studies on their effects on kimchi fermentation are necessary.

GRP and FS are the most common sub-ingredients used for the preparation of kimchi in Korea, although their use or not varies by region and household. It has been generally considered that these GRP and FS can be used as carbon and nitrogen sources, respectively, able to promote the growth of kimchi LAB during fermentation (Choi et al., 2018; Jung et al., 2018). However, because it has been known that LAB generally have a low amylolytic activity for starch (Xu et al., 2020), it is questionable how much LAB can use GRP as a carbon source for their growth during kimchi fermentation. In addition, it is not well known how much FS can promote the growth of kimchi LAB through using it as a nitrogen source. Some studies on the effects of GRP or FS on the microbial community and metabolites during kimchi fermentation have been conducted (Choi et al., 2018; Jung et al., 2018), but the effects of GRP or FS on the LAB growth, metabolites, and volatile compounds have not been comprehensively explored in detail. Therefore, in this study, kimchi, kimchi with GRP, and kimchi with FS were prepared, and their absolute microbial community, metabolites, and volatile compounds were analyzed and statistically compared.

The pH profiles representing the metabolic features of microbes during kimchi fermentation showed that kimchi fermentation might not be promoted by the addition of GRP or FS (Fig. 1A). The decrease of pH levels during fermentation was less in kimchi with FS than in just kimchi, suggesting that kimchi fermentation might be inhibited by the addition of FS, not the growth promotion of LAB. A previous study also showed that the decrease of pH levels during fermentation was less in kimchi with FS than in kimchi without FS (Jung et al., 2018). However, in the study, it was shown that bacterial cell numbers also representing bacterial activity were higher in kimchi with FS than in just kimchi, representing a probably contradictory result with the pH results (Jung et al., 2018). These results might be caused by the limitation or bias of the plate counting method used in the study. In our study, to analyze more accurately bacterial cell numbers and communities without the limitation or bias of the plate counting method, absolute bacterial communities were analyzed by adding equal amounts of bacterial genus cells that were not present in the kimchi samples (Fig. 1B). The results clearly showed that the cell numbers of LAB were smaller in kimchi with FS, and it was also not observed that the LAB growth was promoted by the addition of GRP, which were in good accordance with the pH profiles during kimchi fermentation (Fig. 1A). These results suggest that the hypothesis that GRP and FS may promote the growth of LAB during kimchi fermentation because they can be used as carbon and nitrogen sources may be not correct. In kimchi with FS, the growth of LAB was inhibited during kimchi fermentation, which might be caused by antimicrobial peptides present in FS (Hartmann & Meisel, 2007; Song et al., 2017). The pH levels of kimchi with GRP or FS were higher than those of kimchi without GRP and FS, suggesting that kimchi with GRP or FS may have less sour taste compared to just kimchi, which may be one of the reasons for adding GRP or FS to kimchi.

The metabolite analysis showed that the concentration of glucose was higher in kimchi with GRP during the early fermentation period, but there was no significant difference thereafter (Fig. 2A). This suggests that glucose might be produced not by LAB, but by microbes or indigenous amylolytic enzymes present in raw materials used for kimchi preparation. A little more organic acids were produced probably due to the initially high glucose in kimchi with GRP (Fig. 2B) and kimchi with FS had higher amounts of amino acids (Fig. 3), which may result in different tastes of kimchi. Interestingly, mannitol concentration was higher in kimchi with FS at the late fermentation period (Fig. 2B), which also may have a significant effect on the taste of kimchi. The metabolite profiles of kimchi with FS were more differentiated from just kimchi compared to kimchi with GRP (Fig. S2), suggesting that kimchi fermentation may be more greatly affected by the addition of FS compared to the addition of GRP.

The analysis of volatile compounds showed that the volatile compound profiles of kimchi with GRP or FS were slightly different from that of kimchi without GRP and FS at the early fermentation period (5 days), but they became relatively similar at the end of fermentation (20–30 days), the typical intake time of kimchi in Korea (Figs. 4 and 5). These results suggest that flavoring characteristics conferred by volatile compounds in the final kimchi products that we eat may be relatively similar regardless of the addition of GRP or FS. However, because the OAVs of 1,3-dithiane, α-curcumene, and 4-propylbenzaldehyde, which were abundantly identified in kimchi (Table S1), were not available, their contribution to the odor activity of kimchi could not be evaluated. Therefore, the exact contribution of GRP or FS to kimchi flavor should be reevaluated by calculating the OAVs of all volatile compounds identified in kimchi.

5. Conclusion

In this study, we analyzed and statistically compared bacterial communities, metabolites, and volatile compounds produced in kimchi, kimchi with GRP, and kimchi with FS during fermentation to investigate the effects of GRP and FS on kimchi's fermentation characteristics. The results showed that the metabolites and volatile compounds affecting the taste, texture, and flavor of kimchi were influenced by the presence of GRP or FS, which suggests that the addition of GRP or FS to kimchi can improve or modify its quality and taste. However, the analysis of metabolites and volatile compounds cannot fully capture the taste and flavor experienced when consuming kimchi. Therefore, further study of how kimchi's taste and flavor are conferred by component metabolites and volatile compounds is necessary.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

CRediT authorship contribution statement

Ju Hye Baek: Methodology, Investigation, Writing – original draft. **Kyung Hyun Kim:** Investigation, Formal analysis. **Dong Min Han:** Investigation, Software. **Se Hee Lee:** Methodology, Investigation. **Che Ok Jeon:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors have no conflicts of interest.

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.lwt.2022.114253.

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