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Effects of the main ingredients of the fermented food, kimchi, on bacterial composition and metabolite profile

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ABSTRACT

Kimchi is a fermented food prepared via spontaneous fermentation by lactic acid bacteria originating from raw ingredients. To investigate the effect of these ingredients on food fermentation, four types of food that differed only in their main raw ingredients (kimchi cabbage, green onion, leaf mustard, and young radish) were evaluated. The major microorganisms were *Leuconostoc gelidum*, *Weissella kandleri*, and *Lactobacillus sakei* groups. The distribution of these species depended on the sample type. All three species were primarily distributed in the food prepared from kimchi cabbage and young radish; however, the *Lac. sakei* group was hardly found in the food prepared using green onion and leaf mustard. Metabolite analysis results showed that the free sugar, organic acid, ethanol, and amino acid profiles differed with the sample type. This study indicates that the main ingredients could be an important factor in determining the composition of the microbial community and the metabolite composition.

1. Introduction

Kimchi is a traditional fermented food in Korea, and there are several types depending on the available seasonal ingredients and the manufacturing methods used (Lee, Whon, Roh & Jeon, 2020). Kimchi cabbage [*Brassica rapa* L. var. *pekinensis* (Lour.)], radish (*Raphanus sativus* L.), green onion (Allium wakegi Araki), leaf mustard [*Brassica juncea* (L.) Czern], and young radish (*Raphanus sativus* L.) are typically used as the main ingredients for the various preparations of this food, and it is fermented after seasoning with other ingredients, such as garlic, ginger, salt, red pepper, and salt-fermented fish.

Kimchi fermentation is influenced by the ingredients, fermentation temperature, salt concentration, oxygen availability, and pH, which determine the taste and quality of the final fermented product (Jung, Lee & Jeon, 2014). Due to the activities of various lactic acid bacteria (LAB), predominantly *Leuconostoc*, *Lactobacillus*, *Weissella*, *Pediococcus*, and *Lactococcus* (Kim, Bang, Beuchat, Kim & Ryu, 2012), during the fermentation process, kimchi may be unevenly fermented each time, and fermentation characteristics may vary. LAB produce various compounds, such as lactate, mannitol, ethanol, vitamins, carbon dioxide, and antimicrobial compounds, through fermentation (Lee et al., 2020). Therefore, the sensory characteristics of kimchi vary depending on the dominant LAB type during fermentation. To manufacture high-quality fermented food with superior taste, LAB with excellent fermentation ability and functionality are used as starter.

LAB are typically found in the main ingredients of kimchi, such as kimchi cabbage and garlic, and play an important role in determining kimchi fermentation (Lee, Jung & Jeon, 2015; Song et al., 2020). To date, several investigations have been carried out to determine the effects of temperature, seasoning, salt concentration, presence of salt-fermented fish, and LAB starter type on kimchi fermentation. Food components and external factors are the main determinants of the microbial community. There are currently more than 100 types of kimchi (Ji et al., 2013). The previous studies primarily focused on the representative kimchi type, prepared using kimchi cabbage, and there are few studies on kimchi prepared using other ingredients. Therefore, this study determined the effects of the intrinsic microorganisms present in the different main ingredients of this food on its fermentation. To investigate the food fermentation characteristics, microbial community structure, and metabolite production in food samples prepared with different

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main ingredients, but with the same seasonings, the samples were evaluated using high-throughput sequencing and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy.

2. Materials and methods

2.1. Preparation of kimchi samples

Four types of kimchi were prepared using four different main ingredients: kimchi cabbage, green onion, leaf mustard, and young radish (Fig. 1). The kimchi was prepared by mixing ingredients in the following proportions: 85% main ingredient, 2.5% garlic, 3% red pepper, 1.8% salt, and 7.7% tap water. The four samples (sample C prepared using kimchi cabbage; sample G prepared using green onion; sample L prepared using leaf mustard; sample Y prepared using young radish) were stored at 4 °C for 30 days. During the fermentation period, pH and LAB counts were estimated, and microbial community diversity and metabolite changes were analyzed, as described below.

2.2. Sampling and measurement of lactic acid bacterial viability and pH

The soup of the food sample was periodically collected from the samples at 0, 5, 15, and 30 days. pH values were measured using a pH meter (Thermo Fisher Scientific, Waltham, MA, USA) in triplicates. One mL of the soup was used for the enumerations of LAB. Large particles in the soup were removed using a sterile stomacher filter bag (Nasco Whirl-Pak, Fort Atkinson, WI, USA). The filtered soups were serially diluted with sterilized 0.85% saline solution and inoculated onto 3M™ Petrifilm LAB count plates (3M™ Microbiology, Saint Paul, MN, USA) in triplicates. The plates were incubated at 30 °C for 2 days, and the microbial counts were expressed as CFU/mL of the soup.

2.3. DNA extraction and 16S rRNA gene-amplicon sequencing

Total DNA was extracted from the pellets obtained through the centrifugation (18,000*g*, 10 min) of 1 mL of each sample collected at different times using a FastDNA SPIN Kit for soil (MP Biomedicals, Santa Ana, CA, USA), according to the manufacturer's instructions. The concentrations of the extracted DNA were determined using a spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA), and the quantity and purity were estimated via 1% agarose gel electrophoresis. Microbial succession in each sample was monitored by amplifying the V3–V4 region of the 16S rRNA gene sequence using the bacterial primers 341F and 805R containing adapter sequences, as previously described (Fadrosh et al., 2014; Song et al., 2020). Three amplified PCR products were visualized using 1% agarose gel electrophoresis and purified using the QIAquick PCR purification kit (Qiagen, Hilden, NRW, Germany). Secondary PCR amplification was performed using i5 and i7 index primers to attach the Illumina Nextera barcodes to both amplicon ends. The indexed PCR products were quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). Three replicates per sample were pooled at equimolar concentrations. Amplicons were paired-end sequenced using a MiSeq sequencing platform (Illumina Inc., Albany, NY, USA) with V2 chemistry following the manufacturer's instruction.

2.4. 16S rRNA gene sequencing data processing

Paired-end sequencing data were filtered using Trimomatic ver. 0.32 to check the read quality, and low-quality reads with Phred quality score below 25 were removed (Bolger, Lohse & Usadel, 2014). The filtered paired-end reads were merged using PANDAseq (Masella, Bartram, Truszkowski, Brown & Neufeld, 2012), and the primer sequences were removed. Sequences were denoised using DUDE-Seq. Taxonomic assignments were prepared using the USEARCH program with EzBioCloud database, and the similarity was calculated through pairwise alignment. Potential chimeric sequences were identified and removed using UCHIME (Edgar, Haas, Clemente, Quince & Knight, 2011). The remaining sequences with <97% similarity were clustered into operational taxonomic units (OTUs) using CD-HIT and UCLUST (Edgar,

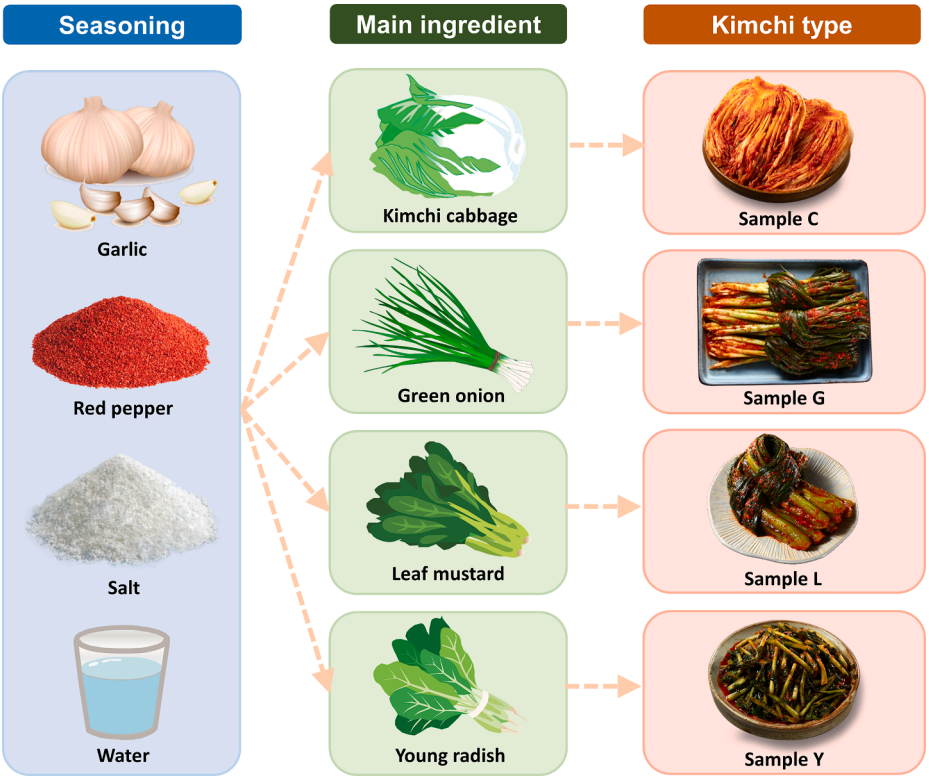

Fig. 1. Summary of each type of kimchi.

2010). Microbial diversity in each sample was estimated based on Chao1, abundance-based coverage estimators (ACE), Shannon index, and Simpson's index (Chao, 1987; Chazdon, Colwell, Denslow & Guariguata, 1998; Hill, 1973; Shannon, 1997).

2.5. Metabolome analysis

Metabolites were analyzed in three replicates using ^{1}H NMR spectroscopy. Supernatants obtained through the centrifugation (18,000*g*, 10 min) of 500 μL of each sample were mixed with 500 μL of deuterium oxide supplemented with 10 mM 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt (DSS; final concentration of 5 mM) as an internal standard. The ^{1}H NMR spectral analysis and identification of metabolites from the samples were performed, as described previously (Song et al., 2020). Briefly, ^{1}H-NMR measurements were performed using a Varian Inova 600-MHz NMR spectrometer (Varian Inc., Palo Alto, CA, USA). The ^{1}H NMR spectra were corrected using Chenomx NMR Suite version 8.3 (Chenomx, Edmonton, Alberta, Canada), using the internal standard DSS. Automatic baseline correction and automatic phase correction were performed according to the chemical form indicator. The quantification of the metabolites was performed using the built-in database of the Chenomx NMR Suite 8.3.

2.6. Availability of data and materials

The 16S rRNA gene amplicon datasets presented in this study were deposited in the National Center for Biotechnology Information (NCBI), the European Bioinformatics Institute (EBI), and the DNA Data Bank of Japan (DDBJ) under the accession number PRJNA674725.

2.7. Statistical analysis

Principal component analysis (PCA) was carried out using the prcomp, devtools, and ggbiplot packages of the R program. To compare the difference in metabolite production between the samples during the fermentation period, statistical analysis was performed using MetaboAnalyst 4.0. The metabolites that differed significantly among the samples were analyzed using one-way ANOVA and Tukey's HSD post-hoc test ($p \leq 0.001$). Additionally, to determine the association between the microbial community and metabolites of each sample, a non-similarity test of nonmetric multidimensional scaling (NMDS) was performed using the Bray–Curtis pseudo-distance matrix in R with the metaMDS and envfit (permutations = 999, $p < 0.5$) functions. Prism software version 8.0 (GraphPad, La Jolla, CA, USA) was used to verify the Spearman correlation between major bacterial taxa and metabolites. The analysis of similarities (ANOSIM) was used to determine the differences among the sample types.

3. Results

3.1. Changes in fermentation properties based on the specific sample

The changes in the pH and LAB counts of the four types of kimchi (samples C, G, L, and Y) during food fermentation were determined and are presented in Fig. 2A and B. At the beginning of fermentation, the average pH of all samples ranged from 4.44 to 4.68, which increased to 5.12–5.50 after 5 days. As fermentation progressed, the pH dropped in all samples, ranging from 3.9 to 4.36 after 30 days. Among the four samples, the pH of sample C decreased most quickly; however, the overall pH change followed the typical kimchi fermentation pattern, regardless of the sample type. Average LAB counts for the samples initially ranged from 4.63 to 4.87 log CFU/mL. This increased to 5.46–5.75 log CFU/mL by day 5 of fermentation in samples C, G, and L, whereas it rapidly increased to 6.99 log CFU/mL in sample Y. On day 15, the average LAB count in samples C, L, and Y ranged from 8.73 to 8.91 log CFU/mL, and in sample G, the count was 7.47 log CFU/mL, which was less than that in other samples.

3.2. Alpha diversities of the bacterial community during food fermentation

To define the microbial community of the food samples prepared using different main ingredients, bacterial composition in the samples was characterized based on 16S rRNA gene sequences. A total of 576,019 reads were obtained through the Illumina Miseq platform from all samples, and the Good's coverage values for each sample were 97.9–99.9%, based on clustering into OTUs at a 97% similarity level. This suggested that the reads obtained from each sample were sufficient for bacterial diversity analysis. Phylotype diversity of the bacterial community in each sample was expressed via Chao1, ACE, Shannon, and Simpson indices (Table S1). The Chao1, ACE, and Shannon indices of the bacterial community composition in all samples decreased as the fermentation started and stabilized after 15 days. In particular, sample Y exhibited the highest Chao1, ACE, and Shannon indices of all food types at day 0 of fermentation, and this decreased as fermentation progressed.

3.3. Bacterial community composition of the four sample types

To compare the bacterial profile of the samples based on their main ingredients during the fermentation period, the V3–V4 region of the 16S rRNA gene was targeted in the DNA extracts from the samples. The taxonomic compositions of the four types of food are shown in Fig. 3A, B,

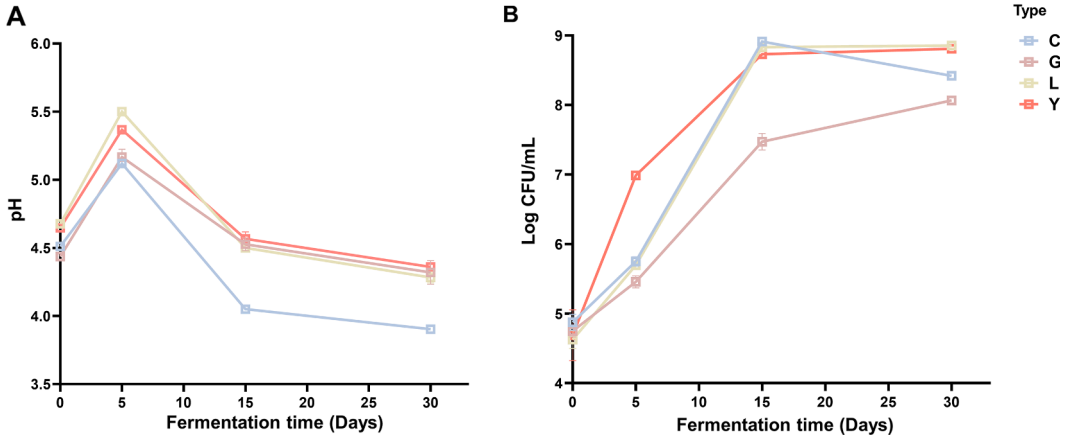

Fig. 2. Variation in pH (A) and lactic acid bacteria counts (B) in each food type during the fermentation period. C, Food prepared using kimchi cabbage; G, green onion; L, leaf mustard; and Y, young radish. The LAB count is expressed in log CFU/mL.

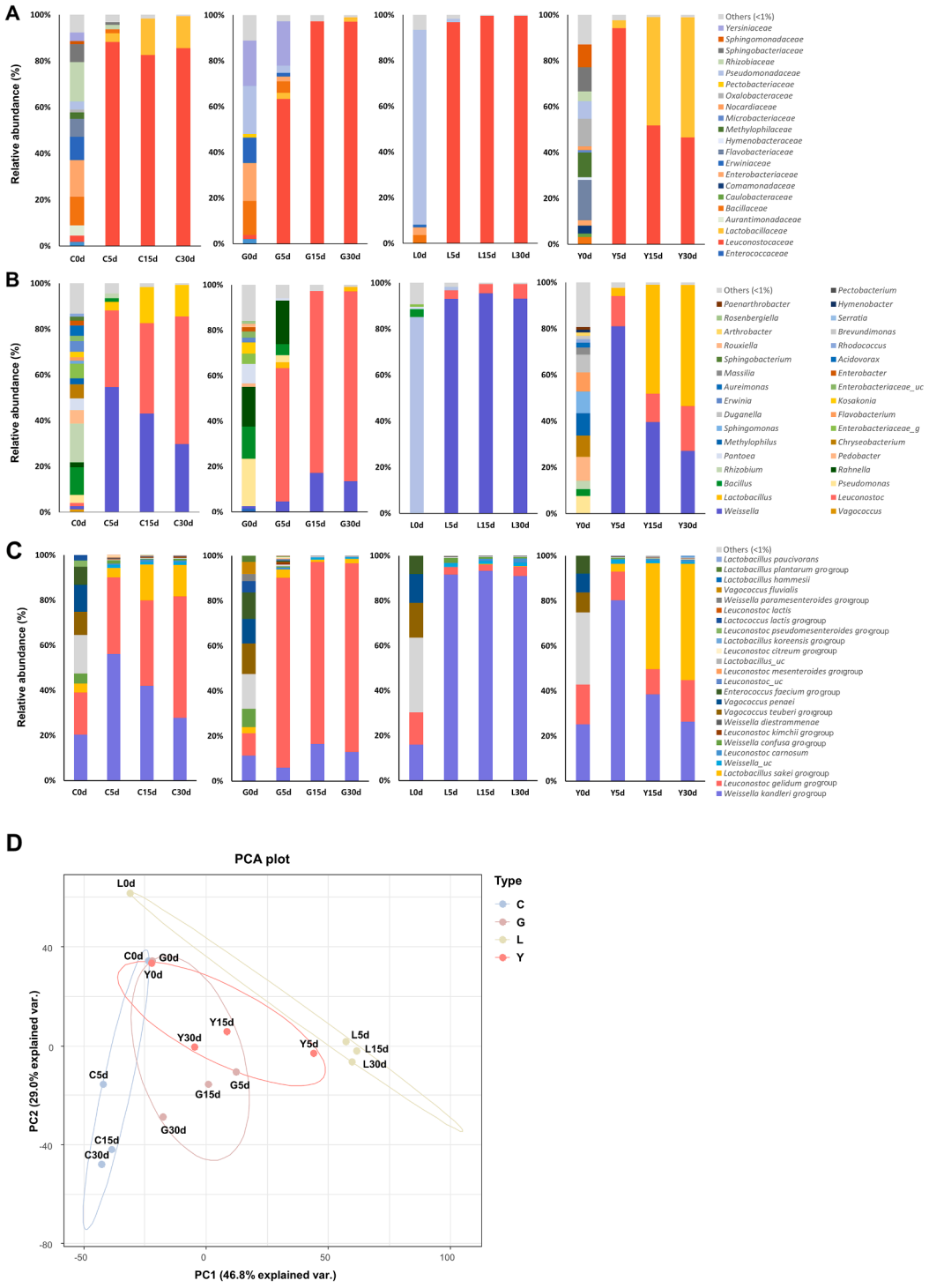

Fig. 3. Relative abundance of total bacteria at the family (A) and genus (B) level, and lactic acid bacteria at the species level (C); PCA plot displaying the bacterial communities in each type of the food (D) during fermentation. The bacterial composition was classified according to 16S rRNA gene sequence annotation. The x-axis represents the sampling day for each sample, and the y-axis represents the relative abundance of bacteria (A–C). C, Food prepared using kimchi cabbage; G, green onion; L, leaf mustard; and Y, young radish. The kimchi samples were collected at 0, 5, 15, and 30 days.

and C. During the initial phase of fermentation, the composition of the bacterial community was varied in samples C, G, and Y, whereas in sample L, *Pseudomonadaceae* comprised 85.2% of the bacterial population, thereby forming a simpler microbial community structure (Fig. 3A). Initially, the LAB were present in a small proportion; however, from day 5 of fermentation, the levels of *Leuconostocaceae* increased rapidly in all types of kimchi, and *Lactobacillaceae* levels gradually increased in samples C and Y during the late fermentation period. At the genus level, *Weissella*, *Leuconostoc*, and *Lactobacillus* primarily dominated samples C and Y, whereas *Leuconostoc* and *Weissella* gradually dominated samples G and L, respectively (Fig. 3B). Specifically, the relative abundance of *Weissella* members within the total bacterial population of sample L increased from <1% to >88.8%. At the species level of the LAB, the *Leuconostoc* (*Leu.*) *gelidum*, *Weissella* (*Wei.*) *kandleri*,

and *Lactobacillus* (*Lac.*) *sakei* groups were differentially predominant in the mid- and late fermentation stages (5–30 days) of samples C and Y (Fig. 3C). However, sample G was primarily occupied by the *Leu. gelidum* group, and the *Wei. kandleri* (*koreensis*) group was predominantly observed in sample L from day 5 post-fermentation and then remained stable. PCA was used to compare the difference between the four samples based on their microbial taxonomic profiles at the genus level (Fig. 3D). In the PCA plot, bacterial community profiles of each food sample differed, depending on the main ingredients, as fermentation progressed (with PC1 and PC2 accounting for a total of 46.8% and 29.0% of the variables, respectively).

3.4. Metabolite changes in four sample types

We investigated whether differences in the main ingredients of the food could affect the composition of the main metabolites during fermentation (Supplementary Fig. S1). Metabolite analysis results showed that each sample type contained various components, such as free sugar, organic acids, ethanol, amino acids, and vitamins (Figs. 4 and 5A, and Supplementary Fig. S2). The major free sugars present in each sample were glucose, fructose, and sucrose at the start of fermentation. As the fermentation progressed, the changing patterns in the concentrations of glucose, fructose, sucrose, ethanol, glycerol, and glycine were similar in samples C and Y; however, mannitol concentration was relatively higher in sample C, compared to that in sample Y. In sample G, the concentrations of fructose, sucrose, and glycine decreased, whereas that of mannitol, butyrate, and ethanol increased, with the concentrations of butyrate and ethanol being higher in sample G, compared to that in other samples. The changes in major metabolites during fermentation in sample L included a decrease in fructose and sucrose concentrations and an increase in mannitol, lactate, butyrate, and ethanol levels. Additionally, to understand the differences in the composition of metabolites between the samples, a heat map and PCA plot were constructed, showing the changes in major metabolite contents against the fermentation periods (Fig. 5). The heat map constructed using the concentrations of 39 metabolites showed the comprehensive difference between each food type during fermentation. Most primary metabolites were distributed similarly in all samples at the beginning of fermentation; however, as the fermentation progressed, the changes in the primary and secondary metabolite levels differed for each sample (Fig. 5A). Based on the metabolite profile at the end of fermentation (days 15 and 30), samples C, Y, and L were grouped into one cluster, whereas sample G was separated, because it exhibited different trends. In addition, various metabolites, such as several organic acids, amino acids, and vitamins, were produced at a relatively high concentration in sample Y than that in the other samples. In the PCA plot, samples C, Y, and L tended to move to the far right, in the opposite direction to the PC1 dimension, as fermentation progressed. The metabolic profile of sample G progressed lesser than that of the others (Fig. 5B). ANOSIM results showed no significant differences between the metabolite profiles of samples C and L; however, there were significant differences between the profiles of the other sample types (ANOSIM $p \leq 0.05$). In addition, one-way ANOVA and post-hoc tests were performed based on the metabolite content of each sample (Supplementary Fig. S3). After 30 days of fermentation, 12 metabolites exhibited significant differences ($p < 0.001$) in the metabolite composition analysis in all samples.

3.5. Relationships between bacterial community and metabolite profiles

The NMDS plot was generated to determine the association between the bacterial community composition and the metabolites for each sample type (Fig. 6A). The NMDS plot showed that the metabolite profiles correlated with 11 taxa of the bacterial populations (envfit, $p <$ 0.5). The stress value of the NMDS plot was 0.05. As fermentation

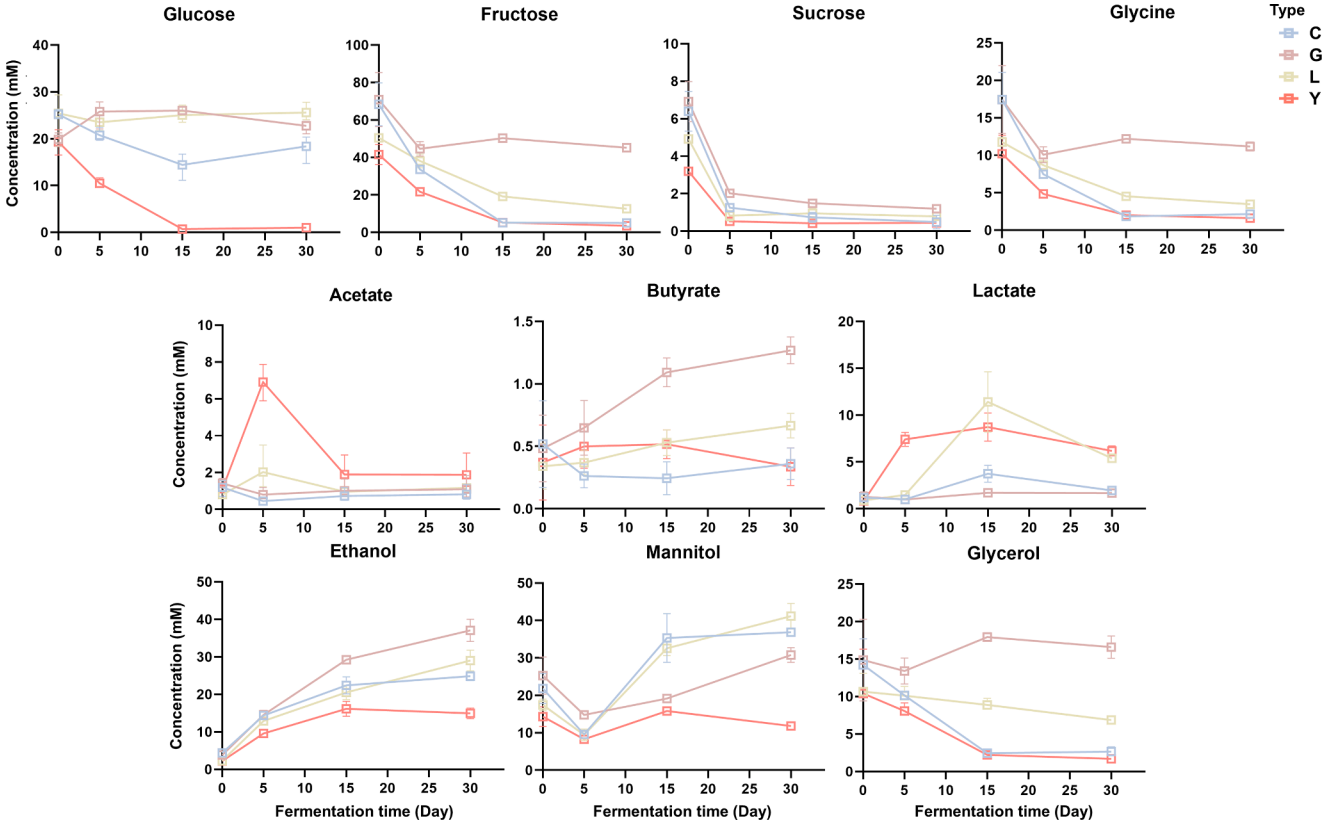

Fig. 4. Changes in the composition of metabolites in the food prepared using kimchi cabbage (C), green onion (G), leaf mustard (L), and young radish (Y) during the fermentation period.

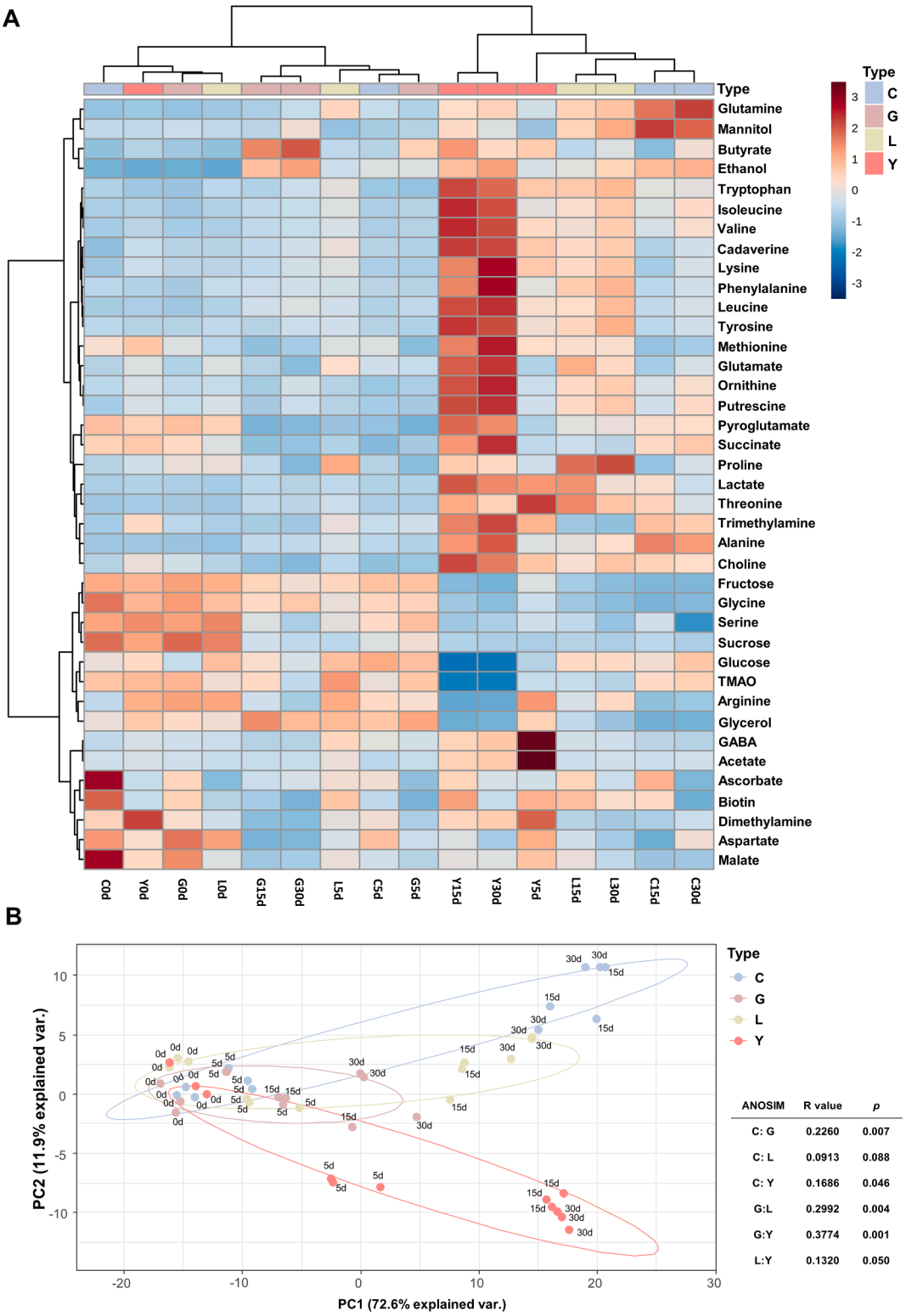

Fig. 5. (A) Heatmap of metabolite profiles in each type based on the fermentation period. (B) PCA plot of metabolite profiles in each food type. C, Food prepared using kimchi cabbage; G, green onion; L, leaf mustard; and Y, young radish. The kimchi samples were collected at 0, 5, 15, and 30 days.

progressed, the four sample types showed a fermentation pattern that moved to the right of NMDS1, involving the *Leu. gelidum*, *Lac. sakei*, and *Wei. kandleri* groups. The correlation between the top 11 taxa and the metabolite profiles of the samples was confirmed through a heatmap (Fig. 6B). The presence of the *Leu. gelidum* group negatively correlated with the utilization of fructose and sucrose as free sugars and positively correlated with the production of ethanol, mannitol, and butyrate. The presence of *Lac. sakei* group negatively correlated with the utilization glucose, fructose, and sucrose and positively correlated with the production of ethanol, butyrate, and lactate. The presence of *Wei. kandleri* group was correlated with the production of lactate, ethanol, and butyrate by utilizing fructose and sucrose, rather than glucose.

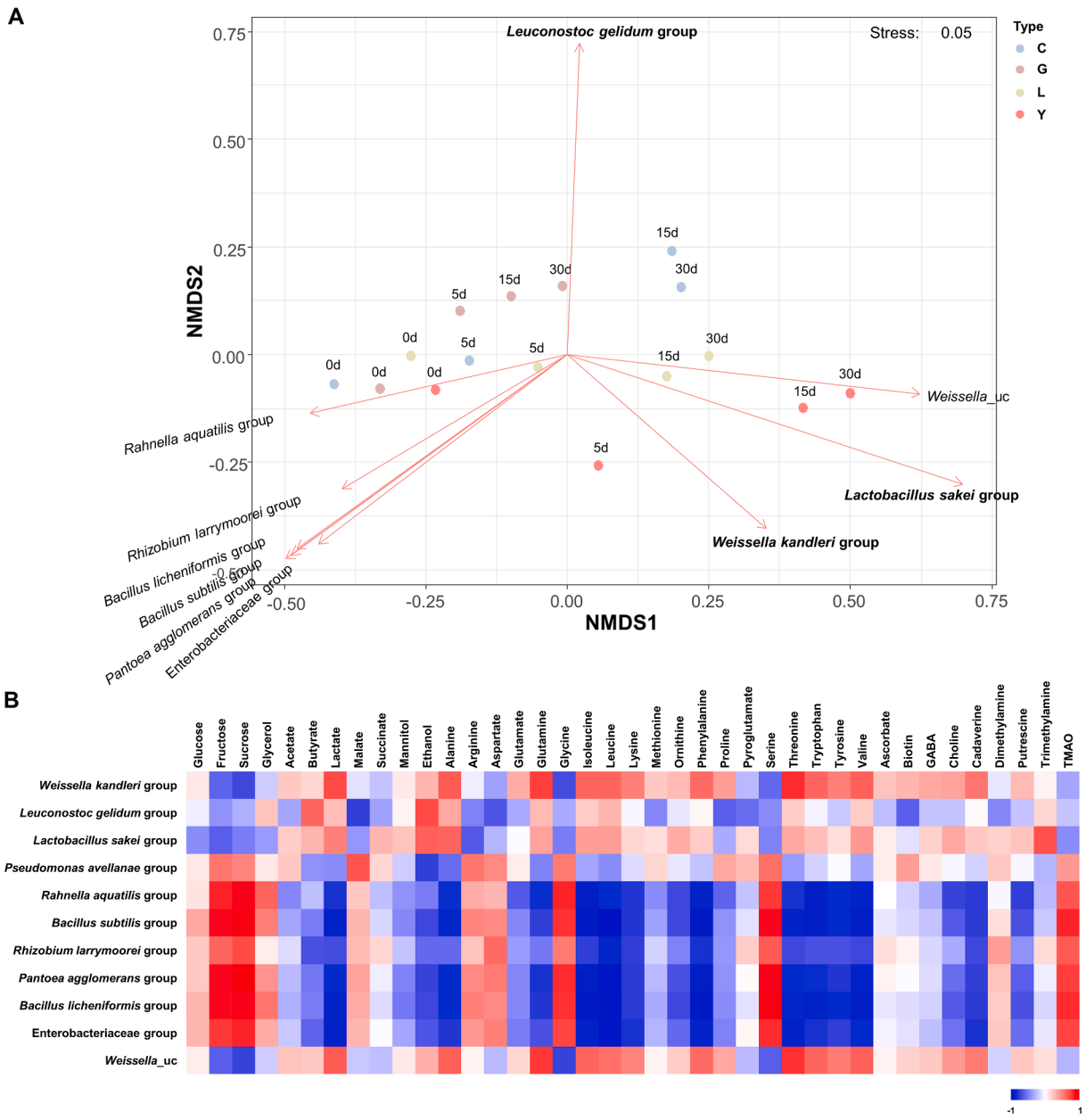

Fig. 6. Analysis of associations between bacterial composition and metabolite profile. (A) Nonmetric multidimensional scaling (NMDS) plot of the associations between bacterial composition and metabolite profile, conducted based on Bray–Curtis dissimilarities. The arrows indicate bacterial taxa variables with a significance factor of $p < 0.5$. (B) Correlation between the top 11 bacterial taxa and the metabolite composition during food fermentation. Heat map was generated using Spearman coefficients between the top 11 bacterial taxa and metabolite composition. C, Food prepared using kimchi cabbage; G, green onion; L, leaf mustard; and Y, young radish. The food samples were collected at 0, 5, 15, and 30 days.

4. Discussion

The fermentation of kimchi is greatly influenced by external factors, such as temperature, salt concentration, and the ingredients and manufacturing methods. The food type is determined by the main ingredients used, and the manufacturing method and minor ingredients are applied accordingly. The food fermentation is initiated by various microorganisms present in the major and minor ingredients; and therefore, main ingredients are the major factors that determine the microbial community and metabolite composition (Cheigh & Park, 1994; Song et al., 2020). Metabolite production and food quality depend on the type of LAB species present in the main ingredients of the food. Therefore, our study investigated whether differences in the main ingredients cause changes in the bacterial composition and metabolite production.

During fermentation, there were slight differences in the pH and LAB count between the samples. pH decreased faster in sample C, and LAB increased slower in sample G. In previous studies, the initial pH of the food was confirmed to range from 5.0 to 5.5 (Jeong, Lee, Jung, Choi & Jeon, 2013; Lee et al., 2015), whereas that in our study was initially approximately 4.5, and then it subsequently decreased after increasing to approximately 5.1–5.5. Sample G showed an increase in the production of butyrate, compared to that of lactate and acetate (Fig. 4), and the pH in sample G did not reduce like that in sample C. In contrast, in sample C, the pH was relatively lower than that in the other samples, probably due to a difference in the amount of amino acids produced, despite the low organic acid content (Fig. 4 and Supplementary Fig. S2). The slow change in pH during late fermentation is due to the buffering

action of free amino acids and inorganic ions (Kang, Kim & Byun, 1988). Sample C was sensitive to the changes in pH, possibly due to low amino acids production, inducing a relatively low pH value, compared to that of the other samples.

pH and LAB count are key indicators of the degree of food fermentation. During fermentation, the pH is reduced by the acids, such as organic and amino acids, produced by the LAB. Therefore, pH is influenced by the composition of the microbial communities in the food. Consequently, we studied the changes in LAB count, with respect to the fermentation period. We observed no difference in the LAB count between the samples on day 0; however, as fermentation progressed, the LAB count in sample Y increased most rapidly, and all samples reached $\geq 1 \times 10^8$ CFU/mL by the end of the fermentation period. These results suggest that a relatively large LAB population grew rapidly in the radishes in sample Y. Bacterial abundance testing results suggest that the main ingredients influence the diversity of the bacterial community in food. Therefore, the high microbial diversity of sample Y reflects the diversity of the bacteria that exist in young radish, the main ingredient of sample Y. There was an excessive growth of LAB in all samples; and therefore, the bacterial richness in the food decreased as the fermentation proceeded. The development of a highly acidic environment and low temperature hindered bacterial adaptation, resulting in a change in the composition of the microbial community.

At the beginning of fermentation, various bacteria comprised the community of each sample type; however, as fermentation progressed, the *Leu. gelidum*, *Lac. sakei* (recently reclassified as *Latilactobacillus sakei* (Zheng et al., 2020)), and *Wei. kandleri* groups were the most abundant phylotypes in each food type. During the fermentation process, the predominant LAB species were similar to those previously reported (Cho et al., 2006; Jung et al., 2014; Jung et al., 2011); however, the species and distribution of the predominant LAB differed depending on the food type. The microbial communities are affected by salt concentration, main ingredient used, fermentation period, sampling time, and nutritive components involved (Lee et al., 2015; Lee, Song, Jung, Lee & Chang, 2017; Lee, Song, Park & Chang, 2019). Red pepper closely affects the ontogeny of *Wei. cibaria* during food fermentation (Kang, Cho & Park, 2016). Therefore, the differences in the composition of the bacterial community food could be attributed to the differences in the intrinsic microorganisms present in the main ingredients (Lee et al., 2015; Song et al., 2020). In this study, during the early fermentation stage, the different food samples exhibited significant differences in the composition of the microbial community at the genus level (Fig. 3B). The genus *Lactobacillus* was more abundant in sample Y than that in sample C, although the sample groups C and Y harbored similar major bacterial community members at the genus level as the fermentation proceeded. The genus *Leuconostoc* was the most abundant in sample G, whereas *Weissella* was dominant in sample L. These results indicate that the dominant bacterial phylotypes differ with the main ingredient. Among the various endogenous microorganisms in the main ingredients, LAB lead the fermentation and are the dominant microorganisms (Chang, Shim, Cha & Chee, 2010).

Various LAB are involved in the kimchi fermentation process, and many metabolites are produced through fermentation, which determines the taste and quality of the food (Cho et al., 2006; Chun, Kim, Jeon, Lee & Jeon, 2017; Moon, Kim & Chang, 2018). The metabolic profiles of each type differed depending on the predominant LAB group, such as the *Leu. gelidum*, *Lac. sakei*, and *Wei. kandleri* members, during fermentation. Fructose and sucrose were considered the major free sugars used in samples C, G, and L, whereas glucose, fructose, and sucrose are all used as major free sugars in sample Y (Fig. 4), likely due to the difference in microbial community profiles between the food samples. The PCA and NMDS plots show the separation of each sample type based on the metabolites produced (Fig. 5B and Fig. 6A). In the NMDS plot, fermentation patterns were formed by the *Leu. gelidum*, *Lac. sakei*, and *Wei. kandleri* groups according to the sample type, and the three groups were identified as important microorganisms influencing the fermentation process of each food. In samples C and Y, *Lac. sakei* counts increased, and the glycerol levels decreased as the fermentation progressed (Fig. 3C and Fig. 4). In most *Lac. sakei* strains, the gene encoding the enzyme (glycerol kinase; EC2.7.1.30) that converts glycerol to *sn*-glycerol 3-phosphate has been identified; however, further research is needed to determine whether *Lac. sakei* degrades glycerol (McLeod et al., 2010). These results suggest that *Lac. sakei* can use glycerol as sugar for growth. Interestingly, when the *Lac. sakei* group dominated in a food sample, lactate production of that sample was higher, compared to that of the other samples, due to their homolactic fermentation; and therefore, the production of ethanol and mannitol was relatively low.

The *Leu. gelidum* group was the predominant LAB group in sample G, and it appeared to be involved in mannitol, ethanol, and butyrate production through heterolactic fermentation using fructose and sucrose; it barely influenced lactate or glycerol production. *Leu. gelidum* is well known for mannitol production (Chun et al., 2017; Jung et al., 2014). Furthermore, the heatmap showed no correlation between the glycerol content and the presence of the *Leu. gelidum* group in sample G. The PCA and NMDS plots showed that the metabolite profiles of sample G at the beginning and end of food fermentation were closely related, such that their fermentation seemed to proceed more slowly than that in the other samples. In sample L, where the *Wei. kandleri* group was dominant, ethanol, mannitol, and lactate were produced using fructose and sucrose rather than glucose, indicating that the preferred free sugars differed depending on the predominant LAB group, resulting in the production of different metabolites.

The one-way ANOVA results showed that the levels of 12 of the 39 metabolites, identified at 30 days of fermentation, differed significantly between the groups (Supplementary Fig. S3A). Among them, the glucose content in sample Y decreased faster than that in other samples (Fig. 4), probably because it was consumed as the major carbohydrate by the *Lac. sakei* group. The distribution of the *Lac. sakei* group increased as the fermentation progressed in sample Y, and it exhibited a negative correlation with the glucose content (Fig. 6B). In contrast, proline concentration increased in sample L as fermentation progressed, which was presumably due to the metabolism of the *Wei. kandleri* group. In addition, the content of amino acids essential for human metabolism tended to increase in samples L and Y, compared with that in the other samples, as the fermentation progressed. Essential amino acids in fermented foods can have direct or indirect effects on improving health (Steinkraus, 1997). The increase in free amino acid content in samples L and Y could be attributed to the extracellular proteolytic activity of the LAB present in the samples (Toe et al., 2019). The metabolite content of the other samples exhibited relative quantitative differences corresponding with the difference between their main ingredients. Therefore, our results suggest that kimchi of varying qualities can be prepared using different main ingredients.

5. Conclusions

In this study, we investigated the effects of the main ingredients of kimchi on the composition of the bacterial community and metabolite concentration during food fermentation. The composition of the bacterial community and their distribution differed between food prepared using kimchi cabbage, green onion, leaf mustard, and young radish. These differences in bacterial composition led to differences in the metabolites produced in the food. Therefore, the main ingredients were a major contributing factor to food quality. To improve the basic understanding of kimchi fermentation, further research is needed to investigate the influence of minor ingredients on food fermentation.

CRediT authorship contribution statement

Hye Seon Song: Investigation, Formal analysis, Visualization, Writing - original draft. **Se Hee Lee:** Investigation, Formal analysis, Visualization, Writing - review & editing. **Seung Woo Ahn:**

Investigation, Methodology. **Joon Yong Kim:** Formal analysis, Visualization. **Jin-Kyu Rhee:** Supervision, Writing - review & editing. **Seong Woon Roh:** Project administration, Supervision, Conceptualization, 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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2021.110668.

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