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Chemical composition and bacterial community changes during the fermentation of *yan yu*, a Chinese traditional fermented fish product

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Abstract

Yan yu is a traditional fermented fish product produced by the Dong people of Guizhou Province in southwestern China. However, despite its widespread regional consumption, little is known about the chemical characteristics and bacterial community changes involved during *yan yu* fermentation. Therefore, the present work assessed the changes in both the chemical and microbiota composition of *yan yu* during its fermentation. Glucose levels gradually decreased after an initial increase at the beginning of fermentation, whereas increase in lactic acid levels continued after 10 d of fermentation. A rapid increase in free amino acid levels was observed at the beginning, but either remained constant or slowly decreased later in the fermentation. In contrast, biogenic amine (BA), TVB-N, and TBARS levels remained low throughout the fermentation. Bacterial community analyses revealed that *Lactiplantibacillus* and *Tetragenococcus* dominated the bacterial community. Moreover, O2PLS-based correlation analysis indicated that these two genera significantly affected the chemical composition of *yan yu*. Furthermore, lactic acid and free amino acid contents (*i.e.*, two major quality parameters of fermented products) were highly correlated with the occurrence of *Lactiplantibacillus* and *Tetragenococcus*. These results are expected to establish a basis for the quality improvement of traditional fermentation of *yan yu*.

Introduction

Fresh fish cannot be easily handled and stored due to its rapid decay rates (Sallam, 2007). Therefore, several preservation techniques are often implemented to improve its shelf life, among which, fermentation is a traditional method that continues to be used worldwide. A number of approaches to make fermented fish products are used; some of those add carbohydrate sources to fish as substrate for fermentation of microorganisms, as the cases for *Suan yu* in China (Zang *et al.*, 2018) and *narezushi* (Kiyohara *et al.*, 2012) in Japan, and others add more than 10% salt to direct control the undesirable microorganisms, such as myeolchi-aekjeot (Lee *et al.*, 2015) in Korea and *plaa-som* in Thailand (Kopermsub and Yunchalard, 2010). The fermentation process of fish not only lowers pH values, which prevent the survival of bacteria responsible for food spoilage (Hu *et al.*, 2008), but also confer a unique flavour and texture, while also enhancing their nutritional value, all of which increases the appeal of these products around the world (Zang *et al.*, 2018).

The spontaneous fermentation process of traditional fermented fish leads to the development of numerous microorganisms. It is generally known that different microbial communities contribute to complex biochemical and physical reactions in fermented products, thus resulting in distinct sensorial properties in the final products (Alkema *et al.*, 2016). Among them, lactic acid bacteria (LAB) are mainly responsible for the accumulation of lactic acid, which not only makes fermented products safe to eat, but also provides a unique flavour profile to the fermented products, via sugar fermentation (Gänzle, 2015). Moreover, coagulase-negative staphylococci degrade both fatty acids and amino acids, which contribute to the aroma and flavour of the fermented products (Zeng *et al.*, 2013a). All these desirable biochemical changes are brought about by microorganisms (both environmental and from within the fish body itself) through fermentation, which greatly influences the development of texture and flavour. Specifically, this occurs via the production of small compounds (*e.g.*, amines, amino acids, peptides, aldehydes, and fatty acids), which are considered significant flavour compounds or flavour

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compound precursors (Leroy *et al.*, 2006). However, putrefying and pathogenic (opportunistic) bacteria such as Enterobacteriaceae, *Enterococcus*, and *Staphylococcus aureus* (Marty *et al.*, 2012) also occur widely in naturally fermented products, and therefore, their growth can compromise the safety of naturally fermented products. Moreover, certain bacteria decarboxylate amino acid precursors which could lead to hazardous amounts of biogenic amines. This poses a severe food safety concern in the fermented meat and fish industries (Anastasio *et al.*, 2010). Therefore, studying the microbial dynamics and chemical composition of fermented goods during their fermentation process is critical to produce high-quality and safe products.

Yan yu, which is made with carps cultured in paddy fields, is a traditional fermented fish product commonly prepared in every household of the Dong people in the Guizhou province of China. This product is not only consumed by locals, but is also sought-after by people from other regions, due to its unique organoleptic properties and preparation process. Currently, *Yan yu* is manufactured using traditional technologies through spontaneous fermentation, and therefore, little is known about the specific microbial dynamics and chemical changes during its fermentation. This study sought to explore the bacterial community succession and chemical changes during *Yan yu* fermentation, and assess the relationships between these two factors. Our findings could thus provide insights into the mechanisms that drive the *Yan yu* fermentation process, and contribute valuable knowledge to improve the quality of this product.

Materials and methods

Yan yu manufacturing and sampling

Yan yu (pickled sour fish) was processed following a modified traditional manufacturing method. A total of 25 fresh live carp (average weight: 1.5 ± 0.2 kg) were captured from a paddy field in Liping (Guizhou Province, China) in September. All fish were then quickly euthanised via a sharp blow to the head, following the guidelines issued by the Ministry of Science and Technology of the People's Republic of China, for the Treatment of Animals and Experimental Animal Management Regulation. The fish were eviscerated shortly thereafter, and the cleaned fish were cut into 4 - 6 cm cubes. The carp pieces then mixed with the following ingredients (w/w, g/100 g): paprika, 5%; cooked glutinous rice, 20%; Chiu-niang (Chinese rice wine), 8%; Chinese prickly ash, 1%; garlic, 2%; ginger, 1%; and star anise seeds, 0.5%. The fish was then cured with 15% salt (15 g salt/100 g total weight) for 24 h. Afterward, the mixtures were placed and sealed tightly in pickle pots and fermented at ambient temperature (25°C) for 60 d. Samples were taken from the pickle pots on days 10, 20, 30, 40, 50, and 60.

Determination of pH, glucose, and lactate

The determination of pH was performed by homogenising 10 g samples with 90 ml deionised water for 60 s, after which, pH was measured using a digital pH meter (PHSJ-5 pH Meters, Shanghai INESA Scientific Instrument Co., Ltd, China). Lactate and glucose concentrations were quantified via high-performance liquid chromatography as described by Vasilopoulos *et al.* (2008).

Determination of free amino acids

Amino acid extracts were prepared as described by Simon-Sarkadi and Holzapfel (1994). Briefly, 5 g samples were diluted with 15 mL of 10% trichloroacetic acid solution (TCA) and homogenised for 10 min. The extract was filtered through a double filter paper. The samples were then kept at -20°C for 24 h to allow the fat to separate, after which, they were centrifuged at 10,000 rpm and 4°C for 15 min. Supernatants were then collected and filtered through a 0.25-μm membrane filter. Free amino acids were analysed with an L-8900 Amino Acid Analyser (Hitachi, Japan).

Determination of biogenic amines, TVB-N, and TBARS

Biogenic amines were extracted as described by Zeng *et al.* (2013b) with some changes. Briefly, 5 g of samples were mixed into 10 mL of 0.6 M perchloric acid, then homogenised for 10 min. The homogenate was then centrifuged at 10,000 rpm and 4°C for 15 min. After double extraction, the supernatants were mixed with 0.6 M perchloric acid to a final 25 mL volume. A mixture was then formed by combining 0.4 mL of extract or a standard solution with 80 μL of 2 M sodium hydroxide and 120 μL of saturated sodium bicarbonate. Additionally, 0.4 mL of dansyl chloride (10 mg/mL) was added to each sample. The mixture was kept in an incubator at 40°C for 45 min, after which, 40 μL of 25% ammonium hydroxide was added to remove the dansyl chloride

residues. After incubating for 30 min at room temperature, acetonitrile was added to the mixture to a final 2 mL volume. Finally, the supernatants were filtered using a 0.22 mm Millipore membrane. The BA concentrations were measured via high-performance liquid chromatography. TVB-N was determined via the micro-diffusion method proposed by Cobb *et al.* (1973), whereas TBARS was analysed as described by Buege and Aust (1978).

DNA extraction and Illumina sequencing

Genomic DNA was extracted from seven samples using the EZNA Mag-Bind Soil DNA Kit (Omega, USA), following the manufacturer's instructions. The quality and quantity of the extracted DNA were assessed with a Qubit 2.0 Fluorometer (Invitrogen, USA). For MiSeq sequencing, the 341F (5′- CCC TAC ACG ACG CTC TTC CGA TCT G -3′) and 805R (5′- GAC TGG AGT TCC TTG GCA CCC GAG AAT TCC A -3′) universal primers were used to amplify the V3 - V4 region of the bacterial 16S rRNA gene. PCR amplification was performed as previously described (Zang *et al.,* 2018). Amplicons were purified and sequenced via Illumina MiSeq deep sequencing at Sangon Biotechnology Co., Ltd. Shanghai, China. Paired-end reads were joined by FLASH v1.2.3 to obtain raw tags. These tags were then filtered and quality checked prior to clustering. After comparing the fused tags with the primers, the tags exhibiting mismatches greater than 6 bp were disposed of using the PRINSEQ (PReprocessing and INformation of SEQuences) algorithm, whereas tags with fewer than 200 bp were eliminated. USEARCH 5.2.236 (Edgar, 2010) was used to identify potential chimeras.

Data analysis

In-silico sequence analyses were conducted using the Quantitative Insights into Microbial Ecology (QIIME) software package. Sequences with ≥ 97% similarities were clustered and treated as operational taxonomic units (OTU) using the UCLUST algorithm (Bokulich *et al.,* 2013). OTU taxonomic classifications were assigned using the SILVA database via the Ribosomal Database Project (RDP) classifier within QIIME (Quast *et al.,* 2013). O2PLS modelling was performed using SIMCA 14 (demo v.1.0.1) (Umetrics AB, Umeå, Sweden) to characterise the relationship between microbiota at the genus level and the chemical changes during *Yan yu* fermentation (Wang *et al.,* 2016). The O2PLS method consists of simultaneously projecting both the X and Y matrices on low dimensional hyperplanes (Trygg, 2002). The number of components in the respective set of the O2PLS model is evaluated via seven-fold cross-validation. Variable Importance in the Projection (VIP) was employed to identify potential functional microbiota in *Yan yu*. Terms with larger VIP values (> 1) are the most relevant for explaining Y variables. XLSTAT 2014 (Addinsoft, France) was used to assess the correlation between chemical content and microbial community in *Yan yu* samples.

Results and discussion

Analysis of pH, glucose, and lactic acid

The initial pH of the raw material was nearly 6.7. The pH values dropped quickly to 4.73 after 10 d, and declined slightly to 4.35 at 30 d, then remained relatively stable until the end of the fermentation process. In contrast, the pH values decreased more sharply at the initial fermentation period in another Chinese traditional fish product (*Suan yu*) (Zang *et al.,* 2018). These differences may have been because the higher salinity levels in *Yan yu* delayed the growth of microorganisms, which in turn, affected organic acid metabolism (Paludan-Müller *et al.,* 2002).

Glucose and lactate, two primary organic compounds produced during the fermentation of many fermented foods, were also analysed (Jung *et al.,* 2016; Li *et al.,* 2017). As shown in Table 1, glucose concentrations increased in the first 10 d, but gradually decreased as fermentation continued. In contrast, lactic acid remained under the detection limits in the fresh fish, but sharply increased after 10 d of fermentation, then remained stable after 40 d until the end of fermentation. These changes indicated that the glucose and other carbohydrates in the fish samples were converted into lactic acids and other organic acids, thus sharply decreasing the pH values of the fermented product (Zeng *et al.,* 2017). Similar results have been reported in other fermented products such as myeolchi-aekjeot, saeu-jeot, and *Suan yu* (Jung *et al.,* 2013; Lee *et al.,* 2015; Zeng *et al.,* 2017).

Free amino acids

Free amino acids, which are mainly produced from the proteolysis of proteins, are highly correlated with the flavour profile of certain foods, and are major metabolites in many fermented products (Lee

Table 1. pH values, and glucose and lactate contents during *yan yu* fermentation.

	Fermentation time (day)						
	Fresh	**10**	**20**	**30**	**40**	**50**	**60**
pH	6.71 ± 0.07^{a}	5.63 ± 0.10^{b}	4.73 ± 0.05^{c}	4.35 ± 0.14^{d}	4.26 ± 0.08^{d}	4.32 ± 0.07^{d}	4.26 ± 0.06^{d}
Glucose (mg/100 g)	1.79 ± 0.07^{b}	2.31 ± 0.10^{a}	1.52 ± 0.05^{c}	0.92 ± 0.05^{d}	0.41 ± 0.03^{e}	0.29 ± 0.03^{f}	0.31 ± 0.05^{ef}
Lactate (g/100 g)	-	0.70 ± 0.07^{a}	1.38 ± 0.14^{b}	1.82 ± 0.08^{c}	2.13 ± 0.12^{d}	2.27 ± 0.14^{d}	2.31 ± 0.09^{d}

Means with different letters within the same row indicate significant difference at $p < 0.05$.

et al., 2015; Li *et al.*, 2017). Therefore, determining the release of free amino acids throughout the fermentation process is critical. As shown in Figure 1, similar to previous studies in other fermented foods such as myeolchi-aekjeot (Korean fermented fish sauce) and saeu-jeot (Korean salted seafood), the concentrations of free amino acids sharply rose in the initial stage of fermentation, after which, they either remained constant or decreased gradually toward fermentation maturity (Jung *et al.*, 2013; Lee *et al.*, 2015). Free amino acid accumulation is likely due to the hydrolysis of proteins, which is stimulated by low pH values, as well as endogenous and microbial peptidases (Zeng *et al.*, 2015). In contrast, the reductions in free amino acid concentrations (*e.g.*, lysine, histidine, threonine, and asparagine) observed toward the end of the fermentation process, were likely due to the occurrence of other heterotrophic microorganisms such as fungi, which consumed the amino acids as an energy source (Jeong *et al.*, 2013).

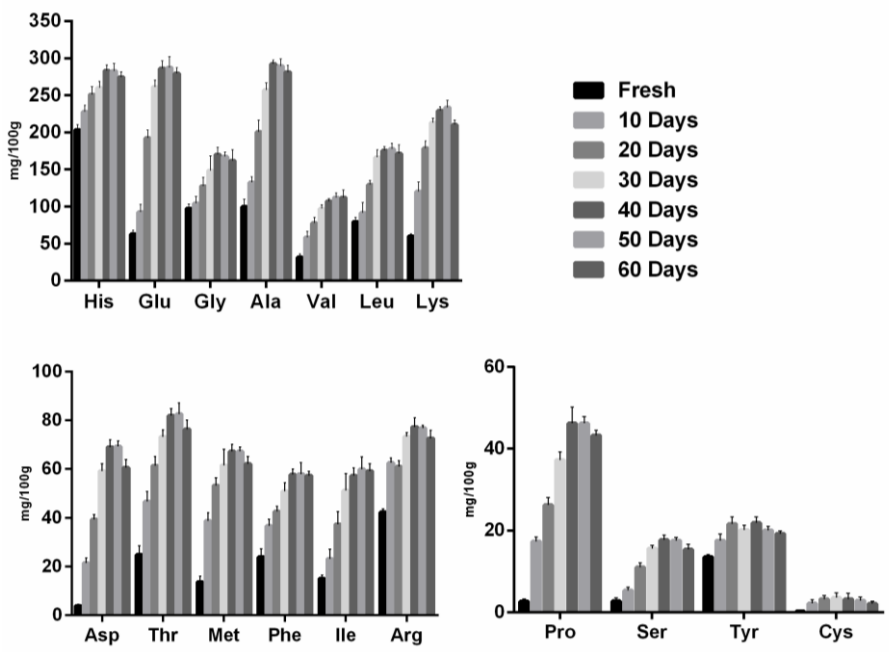

Figure 1. Changes in free amino acid contents during *yan yu* fermentation. Data are mean of triplicate (n = 3) measurements with error bars indicating ± standard deviations.

Biogenic amines, TVB-N, and TBARS

Biogenic amines (BAs) are small nitrogen-containing organic compounds, which are produced from the decarboxylation of amino acids and nitrogenous compounds by microbes. Therefore, these compounds often occur in protein-rich fermented foods (Lee *et al.*, 2015) and undermine their safety. Histamine, tyramine, cadaverine, and putrescine, which are well-acknowledged indicators of putrefactive microorganism contamination in fermented meat products (Ignatenko *et al.*, 2006), were analysed in this study. The concentrations of tyramine, putrescine, and cadaverine are shown in Figure 2A (no histamine was found in any of the samples). Tyramine concentrations varied from 3.50 to 14.10 mg kg^{-1} throughout the fermentation process (with the highest concentrations occurring at the end of the fermentation), which was significantly lower than the maximum permitted range of 100 - 800 mg kg^{-1} (Shalaby, 1996). Similar to tyramine, the concentration of putrescine, one of the most important BAs present in fermented products (Lee *et al.*, 2015; Jung *et al.*, 2016), was relatively low during the entire fermentation period, increasing slightly from 3.12 to 5.13 mg kg^{-1}. This low putrescine level (< 10 mg kg^{-1}) indicated that the *Yan yu* was of good quality (Křížek *et al.*, 2002). The concentrations of cadaverine also remained between 2.22 and 2.50 mg kg^{-1} throughout the fermentation process, which were lower than those reported by Zeng *et al.* (2013b) and Liu *et al.* (2010). These results indicated that the fermentation process effectively diminished the aggregation of BAs.

TVB-N has been widely used as an indicator of spoilage in fresh and fermented meat products. As shown in Figure 2B, the TVB-N values rose gradually from 12.36 to 14.33 mg/100 g, which was well below the maximum permitted value of 35 mg/100 g of fish flesh, specified by the EC guidelines (EC, 1995). On the other hand, TBARS values, which have been

extensively used to evaluate lipid oxidation in meat and meat products, reached 0.51 mg MDA/kg after the first 10 d of fermentation. These values decreased thereafter, and remained relatively stable until the end of the fermentation process. This was likely due to the degradation of aldehydes and the growth of microorganisms with antioxidant capacity during the fermentation process, which prevented the oxidation of unsaturated fatty acids (Zang *et al.,* 2018). The low TVB-N and TBARS values observed during *Yan yu* fermentation might be attributed to a suppression of putrefactive organism growth during the fermentation process (Zeng *et al.,* 2013b).

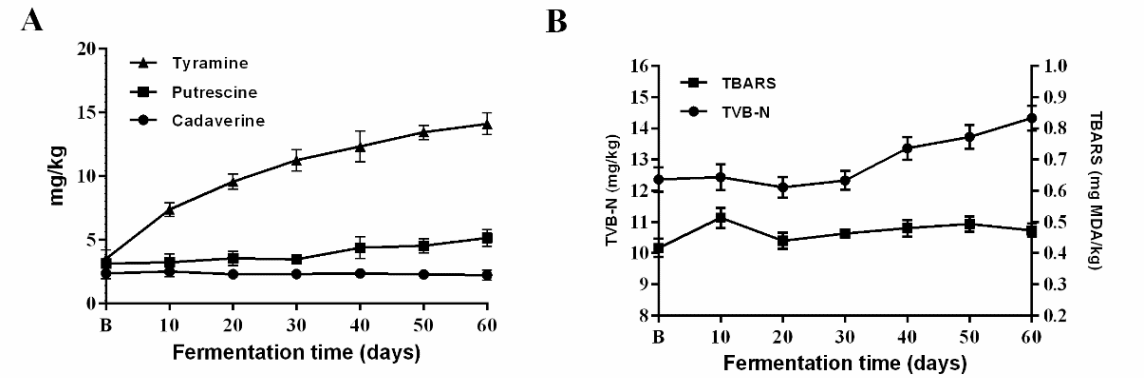

Figure 2. Changes in tyramine, putrescine, and cadaverine contents **(A)**, and TVB-N and TBARS values **(B)** during *yan yu* fermentation. Data are mean of triplicate (n = 3) measurements with error bars indicating ± standard deviations.

Bacterial community succession in Yan yu samples during fermentation

All samples analysed herein exhibited Good's coverage values above 90%, indicating that most of the bacterial phylotypes had been identified (*i.e.,* the identified sequences encompassed the majority of the microbiota in all samples).

To characterise the bacterial succession in *Yan yu* during its fermentation process, 16S rRNA gene sequencing reads were categorised into classes at the phylum and genus levels. At the phylum level, Firmicutes and Proteobacteria were found to be predominant throughout the fermentation process (Figure 3A). Specifically, the proportion of Firmicutes dramatically increased to 82.31% in the first week of fermentation, then steadily increased to 95.15%, and remained constant until the late fermentation stages. These results are consistent with previous studies, which characterised other fermented salted seafood (Jung *et al.,* 2013; Lee *et al.,* 2015).

To further assess the bacterial dynamics during *Yan yu* fermentation, bacterial compositions were determined at the genus level in all samples, and the top 10 genera are shown in Figure 3B. *Lactiplantibacillus*, *Companilactobacillus*, and *Tetragenococcus* were the predominant bacteria during the fermentation process. After 10 d of fermentation, there was a rapid increase in the proportion of *Lactiplantibacillus* and *Companilactobacillus*, two genera that were recently elucidated from a taxonomical classification of various lactobacilli (*i.e.,* a subgroup of LAB) (Zheng *et al.,* 2020). After 40 d, these genera respectively accounted for approximately 40 and 25%, respectively, of the total bacterial community. These two genera are widespread in various fermented foods and are common LAB. As their name implies, these bacteria produce lactic acid, and therefore, influence the quality of fermented products by decreasing their pH (Guo *et al.,* 2021). The high proportions of these bacteria during *Yan yu* fermentation highlighted their importance during the fermentation process. Furthermore, the *Tetragenococcus* genus, which encompasses typical halophilic lactic acid bacteria species, gradually increased to approximately 20% after 30 d, and remained constant as fermentation progressed. Previous studies demonstrated that members of the *Tetragenococcus* genus play vital roles in the fermentation of many other salty fermented products (Jung *et al.,* 2013; Lee *et al.,* 2015). Therefore, using a starter culture of this genus enhanced the flavour profile of the resulting fermented foods (Udomsil *et al.,* 2011). The rapid increase and predominance of *Lactiplantibacillus*, *Companilactobacillus*, and *Tetragenococcus* were likely due to the anaerobic and acidic conditions that

occur as fermentation progresses, which highlighted the capacity of these genera to adapt to the constant environmental changes during *Yan yu* fermentation. Furthermore, the bacterial community structure observed in the present study was slightly different from that reported in *Suan yu*, another Chinese traditional low-salt (3%, w/w) fermented freshwater fish, in which, *Lactobacillus* and *Macrococcus* were predominant during the fermentation process (Zang *et al.*, 2018). These slight variations might have been primarily due to the different salinities of these two fermented foods.

The comparative abundance of *Staphylococcus*, another dominant genus, rapidly increased and reached a peak of 9.72% after the first 10 d of fermentation, after which, it gradually declined to approximately 2 ~ 3% as fermentation progressed. This decrease in proportion might have been due to the decreasing pH values during fermentation, as *Staphylococcus* growth is known to be markedly pH-dependent (Aksu and Kaya, 2004). Therefore, partially acid-resistant strains might have survived. Interestingly, the abundance of this genus increased slightly to 3.37% in the final stages of fermentation. These results were consistent with those reported for *Suan yu*, where *Staphylococcus* proportions exhibited similar trends (Zeng *et al.*, 2015; Zang *et al.*, 2018). Although some members of the *Staphylococcus* genus are associated with diseases, other *Staphylococcus* species have frequently been identified in many fermented foods, that they were reportedly safe to consume (Jung *et al.*, 2013; Lee *et al.*, 2015). Due to their high NaCl tolerance, some *Staphylococcus* strains are often used as starters for the production of salted fermented food (Zeng *et al.*, 2013a). *Bacillus*, a genus that was also reported in other Asian fermented fish products (Zang *et al.*, 2018), was present as a major genus in our samples. Our data indicated that *Bacillus* abundance was relatively persistent during the fermentation process. This was likely because the anaerobic and high salinity conditions of *Yan yu* fermentation inhibited the growth or metabolism of *Bacillus*, and we inferred that the *Bacillus* identified during fermentation were spores. This is consistent with previous studies in doenjang (Jung *et al.*, 2016).

Other dominant genera found in our samples included *Leuconostoc*, *Weissella*, *Micrococcus*, *Enterococcus*, and *Vibrio*. Among them, *Leuconostoc* and *Weissella* are well-known LAB that are frequently discovered in various fermented foods (Jeong *et al.*, 2013; Zang *et al.*, 2018). *Vibrio* and *Micrococcus*, which are halotolerant bacteria, have been identified during fish sauce fermentation (Lee *et al.*, 2015). Enterococci, which form part of the LAB of importance in foods, are important for ripening and aroma development of various traditional fermented foods such as cheeses, fermented sausages, doenjang, and *Suan yu* (Franz *et al.*, 2003; Jung *et al.*, 2016; Zang *et al.*, 2018). However, the *Vibrio* genera may include some pathogenic strains (López *et al.*, 2012), and the detrimental activities of enterococci are associated with spoilage of foods, especially meats (Franz *et al.*, 2003). As shown in Figure 3B, *Vibrio* and *Enterococcus* were markedly inhibited throughout the fermentation process.

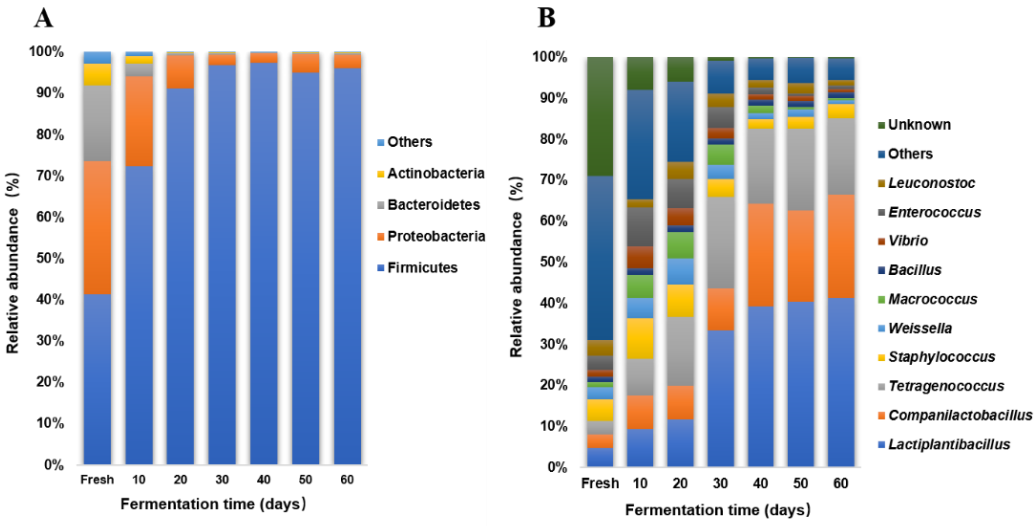

Figure 3. Relative abundance of bacteria at the **(A)** phylum and **(B)** genus level in the samples.

Analysis of the functional core bacterial genera for Yan yu fermentation

The O2PLS method was used to analyse the association between major bacterial communities and chemical components during *Yan yu* fermentation. The Q^2 and R^2 of this model were 0.801 and 0.850, respectively, confirming that the O2PLS method was well-suited for analysis and prediction. The VIP $_{(pred)}$ vector (VIP value for the predictive components) of the major bacterial genera analysed herein varied between 0.50 and 1.44, respectively (Figure 4A).

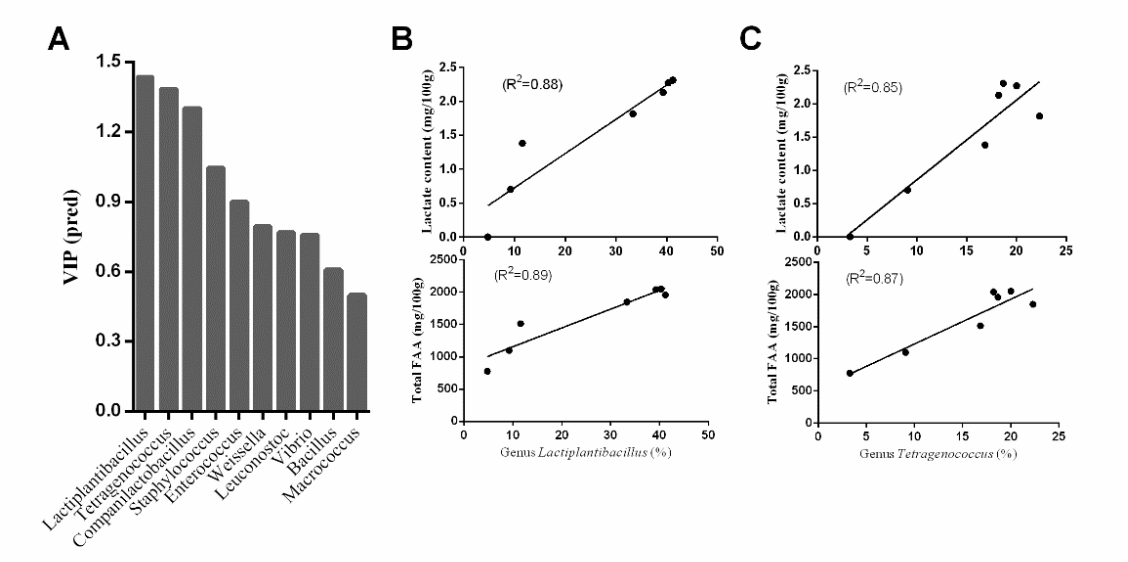

Figure 4. Correlation analyses between major bacterial genera and chemical components during y*an yu* fermentation. **(A)** VIP $_{(pred)}$ (variable importance for predictive components) plot of the major bacterial genera obtained by O2PLS modelling. **(B)** Linear regression curve of the relationship between the relative abundance of *Lactiplantibacillus* and the lactic acid and total free amino acid contents during *yan yu* fermentation. **(C)** Linear regression curve of the relationship between the relative abundance of *Tetragenococcus* and the lactic acid and total free amino acid contents during *yan yu* fermentation.

Among these, four genera ($VIP_{(pred)} > 1.0$) including *Lactiplantibacillus* ($VIP_{(pred)} = 1.44$), *Tetragenococcus* ($VIP_{(pred)} = 1.38$), *Companilactobacillus* ($VIP_{(pred)} = 1.30$), and *Staphylococcus* ($VIP_{(pred)} = 1.05$) had important effects on the chemical composition of *Yan yu*. These results were consistent with those of our bacterial community succession analyses, and thus confirmed the major fermentative role of these bacteria during *Yan yu* fermentation. The coefficient of determination based on the O2PLS results further demonstrated that the genus *Lactiplantibacillus* (Figure 4B) was highly correlated with the lactate ($R^2 = 0.89$) and total free amino acid contents of the fermented product ($R^2 = 0.88$), both of which are essential determinants of fermented product quality and flavour. Some members of the *Lactiplantibacillus* genus (such as *Lpb. plantarum*) have been reported to have a higher lactic acid production (Leroy and De Vuyst, 2004) compared to other LAB (Tranberg *et al.*, 2021), which is linked to amino acid metabolism. Importantly, our results also highlighted the key role of *Lactiplantibacillus* in lactic acid production and amino acid metabolism during *Yan yu* fermentation. Further, the genus *Tetragenococcus* (Figure 4C), which includes halophilic lactic acid bacteria, were also highly correlated ($R^2 = 0.85$) with lactate content. These results agree with those of Jung *et al.* (2016), who found that the dominance of *Tetragenococcus* coincided well with a rapid increase in lactate concentration, as well as a decrease in glucose levels during doenjang fermentation. Members of the *Tetragenococcus* genus have been reported to play important roles in taste and flavour enhancement during fermentation in various salty fermented foods. Particularly, *Tetragenococcus halophilus* has been used as a starter culture to produce desirable flavour compounds during anchovy sauce fermentation. This process relies on the hydrolysis of amino acids and peptides, and is facilitated by enzymes present in fish

or microorganisms (Udomsil *et al.,* 2011). Our data indicated that the occurrence of the *Tetragenococcus* genus was highly correlated (R^2 = 0.87) with that of free amino acids, thus highlighting the important role of *Tetragenococcus* in the flavour development of *Yan yu*. Taken together, our results demonstrated the key roles of *Lactiplantibacillus* and *Tetragenococcus* in the quality and flavour development of *Yan yu* during its fermentation process.

It is also worth noting that although low levels of tyramine were observed during fermentation, its variations were highly correlated with those of *Lactiplantibacillus* (R^2 = 0.85) and *Tetragenococcus* (R^2 = 0.82). Some members of these two genera have been reported to produce BAs during food fermentation (Satomi *et al.,* 2008). Therefore, future studies should focus on the selection of strains that do not produce these compounds.

Conclusion

The present work was the first to characterise the chemical changes and bacterial community succession during the fermentation of *yan yu*, as well as the correlation between these factors. Results indicated that *Lactiplantibacillus* and *Tetragenococcus* were the major drivers in *yan yu* fermentation. Therefore, additional efforts are required to select strains of these genera that not only render the best flavour profiles, but are also the safest for human consumption. These findings would facilitate the standardisation of *yan yu* production at an industrial scale. Future studies should also consider the optimisation of microbial composition to better control and promote flavour development in *yan yu* products.

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