ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Technol. Aliment. 24(2) 2025, 177–188
www.food.actapol.net pISSN 1644-0730 eISSN 1898-9594 http://doi.org/10.17306/J.AFS.001321

ORIGINAL PAPER

Received: 17.12.2024
Accepted: 10.02.2025

THE EFFECTS OF FERMENTATION TEMPERATURE AND STARTER CULTURE ON METABOLITE PROFILES AND ACIDITY CHANGES DURING THE STORAGE OF KIMCHI

Thi Trang Nguyen, Thi Hanh Nguyen✉

Department of Food Engineering, school of Chemistry and Life Sciences, Hanoi University of Science and Technology
1 Dai Co Viet, Hai Ba Trung, Hanoi 100000, **Vietnam**

ABSTRACT

Background. With the expansion of the kimchi market, maintaining an appropriate temperature and selecting a suitable starter culture for predicable fermentation are crucial for the production of kimchi of consistently high quality. The flavor of kimchi heavily depends on organic acids and mannitol. This study aims to investigate the effect of different fermentation temperatures and starter strains on the accumulation of these metabolites and changes in acidity during the storage of kimchi. Its findings will provide insights into the synergistic effects of temperature and starter cultures on kimchi fermentation, thus supporting the production of probiotic vegetable-based foods with desirable flavor profiles.

Materials and methods. A physiochemical analysis was conducted to measure changes in organic acids and mannitol in different kimchi samples fermented at different temperatures (10–30°C) and with different starter strains (*Leuconostoc mesenteroides*, *Lactobacillus fermentum* and *Lactobacillus plantarum*). Changes in acidity during 30 days of storage were also recorded.

Results. Within the examined temperature range, the most lactic acid was produced at 30°C in most of the starter culture-inoculated samples, suggesting that this temperature favored microbial pathways promoting the synthesis of lactic acid, which is the most characteristic organic acid contributing to the flavor of kimchi. Higher temperatures also fostered mannitol production in all samples.

Conclusion. Principle component analysis (PCA) of organic acid profiles indicates that the samples inoculated with starter cultures produced a more balanced and characteristic flavor at 30°C. At 30°C, *Leu. mesenteroides* exhibited the highest mannitol yield. During 30 days of storage, the most significant changes in acidity and pH were observed in the sample inoculated with *Leu. mesenteroides* and the control sample.

Keywords: fermentation, flavor, kimchi, metabolites, starter cultures, temperature

INTRODUCTION

Kimchi is a vegetable-based probiotic food made primarily of Chinese or napa cabbage and white radishes. It is seasoned with a mix of chili pepper flakes, garlic, ginger, scallions and fish sauce, giving it a unique and spicy flavor. The fermentation process, which can last from a few days to several months, allows beneficial bacteria to develop, enhancing the flavor while enriching the kimchi with probiotics that are good for digestion. Kimchi undergoes fermentation by various types of lactic acid bacteria (LABs) (Park et al., 2013). The addition of various sub-ingredients and the formation of LAB fermentation byproducts enhances the

✉hanh.nguyenthi@hust.edu.vn

fermentation process, helping to eliminate spoilage and harmful bacteria while increasing kimchi's functional properties (Park et al., 2014).

Kimchi offers a wide range of health benefits since it is rich in LABs, which contribute to its probiotic properties, promoting gut health and suppressing pathogenic bacteria (Park et al., 2014; Patra et al., 2016). The LABs in kimchi exhibit antioxidant and anti-inflammatory properties, which can help in managing conditions like obesity, cancer and atopic dermatitis (Cha et al., 2024; Park et al., 2014; Patra et al., 2016). With the expansion of the global kimchi market (Park et al., 2013), the use of starter cultures in kimchi production is essential to achieve consistent quality, especially in large-scale or commercial operations. Using LAB starter cultures can also affect the types and concentrations of metabolites produced. Starter cultures help in standardizing the flavor and quality of kimchi by controlling the microbial community and fermentation process, leading to the consistent production of metabolites (Jung et al., 2012; Lee et al., 2020; Lee et al., 2015). Moreover, certain LAB starter cultures can enhance the health-promoting properties of kimchi by enhancing its antioxidant and anti-cancer activity (Bong et al., 2013; Lee et al., 2018).

Many studies have been conducted to evaluate the effects of using starter cultures in kimchi fermentation. Previous results have shown that they have a positive impact on kimchi quality. LAB starter cultures are typically added at a relatively high inoculation rate (7–8 log CFU/g) at the beginning of fermentation (Lee et al., 2015). They can result in favorable properties, including improved sensory qualities (Ahn et al., 2015; Lee et al., 2011; Lee et al., 2020), improved functionality (Chae et al., 2006; Han et al., 2011; Lee and Lee, 2010), and greater accumulation of metabolites (organic acids, mannitol) (Jung et al., 2012; Kim et al., 2019; Moon et al., 2018). Therefore, LAB starter cultures directly influence the overall flavor of kimchi.

Fermentation driven by LABs is significantly influenced by temperature, which affects bacterial succession and metabolite profiles by altering glycolysis and the tricarboxylic acid cycle (Jung et al., 2024). Consequently, controlling fermentation temperature is crucial for the production of kimchi with desirable characteristics.

Microorganisms chosen as starter cultures for kimchi should exhibit specific properties, such as the ability to thrive in a specific fermentation environment (low temperature, acidic conditions, and the presence of NaCl). Different *Leuconostoc* and *Lactobacillus* spp. have been used as starter cultures in kimchi research and have proven to be well adapted to the fermentation environment. Among them, *Leu. mesenteroides*, *L. plantarum* and *L. fermentum* have been reported to enhance the functionality of kimchi and other fermented foods through their metabolite production (Lee et al., 2015; Naghmouchi et al., 2020).

However, existing studies have focused on the effects of temperature or starter culture strain on changes in the content of certain metabolites over a specific fermentation period. In this study, we investigated the effects of fermentation conditions (including starter culture, temperature and duration) on some metabolites that significantly influence the flavor of kimchi at the optimal ripening pH (around 4.1) (Hahn et al., 2002; Moon et al., 2020). Our goal was to provide insights into enhancing the flavor of kimchi while maintaining consistent quality. Changes in total acidity and pH, which are key factors influencing kimchi's characteristic sourness, were also monitored during storage. As kimchi is usually stored long-term in a low-temperature environment, fermentation proceeds slowly, allowing acid content and pH to serve as parameters for tracking fermentation progress during storage. Monitoring these parameters can help manage shelf life and provide indicators of storage stability.

MATERIALS AND METHODS

Preparation of kimchi

Kimchi samples were prepared at a ratio of 62.3:6.2:2.5:1.6:1.2:0.2:0.7:5.0:7.5:0.3:2.2:10.0:0.3 = chinese cabbage:salt:ginger:garlic:sugar:sodium glutamate:chili powder:green onion:carrot:fish sauce:glutinous rice flour:radish:chili, respectively. 12 kimchi batches were fermented under different conditions, as stated in Table 1.

After the samples reached the optimum pH (4.1–4.2), they were homogenized and then stored at –18 ±1°C for organic acid and mannitol analysis. Based on principal component analysis, the most promising sample from each group will be analyzed for changes in pH and acidity during storage at low temperatures (5 ±1°C) to assess its ability to maintain the optimal ripening state.

Table 1. Sample preparation

Group	Sample	Starter culture	Inoculation rate	Fermentation temperature °C
1	P-30	*L. plantarum*	10^7 CFU/g	30
	P-20	*L. plantarum*	10^7 CFU/g	20
	P-10	*L. plantarum*	10^7 CFU/g	10
2	M-30	*Leu. mesenteroides*	10^7 CFU/g	30
	M-20	*Leu. mesenteroides*	10^7 CFU/g	20
	M-10	*Leu. mesenteroides*	10^7 CFU/g	10
3	F-30	*L. fermentum*	10^7 CFU/g	30
	F-20	*L. fermentum*	10^7 CFU/g	20
	F-10	*L. fermentum*	10^7 CFU/g	10
–	O-30	Non-starter	0 CFU/g	30
	O-20	Non-starter	0 CFU/g	20
	O-10	Non-starter	0 CFU/g	10

Measurement of organic acids

The organic acid content was determined using an adjusted version of a procedure reported in a previous work (You et al., 2017). 5 g of each homogenized sample was added to a 50 mL centrifuge tube. Distilled water was added until the total volume reached 20 mL. Subsequently, the sample was centrifuged at 3500 rpm for 5 minutes, and the supernatant was filtered using filter paper (Advantec No. 5B, Japan). High performance liquid chromatography (HPLC) was conducted using a Nexera 40 system (Shimadzu, Kyoto, Japan) with a PDA detector (SPD-40V) at 200 nm. The injection volume was 10 µL. Organic acids were analyzed using the Shim-pack GIST C18-AQ (250 mm×4.6ID×5 µm). 0.1% H_3PO_4 in deionized water:acetonitrile = 97:3 served as the mobile phase for 15 minutes at a flow rate of 1 mL/minute. Malic, lactic, acetic, citric and succinic acid were identified in each sample by comparing their retention times with those of standard organic acids and were quantified using a calibration curve derived from the peak areas of the standards.

Measurement of mannitol content

The mannitol content of each sample was measured by a modified procedure of Choi et al. (2019). 5 g of homogenized sample was added to a 50 mL centrifuge tube. A total volume of 10 mL was obtained by adding distilled water. The suspension was heated in a water bath at 80°C for 15 minutes and then cooled to room temperature. Subsequently, the sample was centrifuged at 3500 rpm for 5 minutes, the solution was filtered using filter paper (Advantec No. 5B, Tokyo, Japan) and 20 µL was injected into the HPLC system for the analysis of mannitol. The measurement was performed using a HPLC Nexera 40 system (Shimadzu, Kyoto, Japan) equipped with a PDA (SPD-40V) detector at 200 nm. An amine-NH2 (250 mm×4.6ID×5 µm) column was used. The mobile phase was deionized water:acetonitrile = 25:75 at a flow rate of 1.2 mL/minute. Mannitol content was estimated by comparison with the retention time and peak area of the standard mannitol curve.

Measurement of pH and total acidity

A pH meter (Lab 845, SI Analytics) was used to determine pH and total soluble solids were quantified using a refractometer. The AOAC 942.15 method (AOAC, 2000) was adopted in order to measure the total titratable acidity.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) with the Student-Newman-Keuls test to determine statistical significance ($p < 0.05$) using SPSS Statistics 20. The data were expressed as means ±standard deviations (SD) which were calculated from triplicate measurements. Principle component analysis (PCA) was performed to determine correlations between the variables, and a heatmap was created using XLSTAT (Addinsoft, Paris, France).

RESULTS AND DICUSSION

The effects of temperature and starter culture strain on the time taken to reach the optimal ripening pH

The fermentation time to reach the optimal pH varied among the starter cultures and non-starter samples depending on the temperature (Fig. 1). At 30°C, the sample inoculated with *L. fermentum* exhibited the fastest fermentation, taking 11 ±1.0 hours to reach the optimal pH, followed by *Leu. mesenteroides* (14 ±0.5 hours). These relatively short fermentation times reflect high microbial activity at this temperature. The sample inoculated with *L. plantarum* took 17.5 ±1.0 hours at 30°C, while the non-starter sample required 18 ±1.0 hours, indicating slower microbial activity due to the lower initial microbial population. At 20°C, the required fermentation times increased for most samples, with *L. plantarum* sample reaching the optimal pH at 63 ±3.0 hours, demonstrating moderate adaptability, while other starter-inoculated samples took around 60 hours each. The non-starter sample exhibited slower fermentation, at 66 ±5.0 hours.

At 10°C, fermentation slowed significantly for all samples, with those inoculated with *L. plantarum* and *L. fermentum* taking the longest (approximately 432 hours). Kimchi with *Leu. mesenteroides* as a starter culture reached the optimal pH at 264 ±6.0 hours, while the non-starter required 336 ±20.0 hours. This analysis highlights the efficiency of starter cultures and the better adaptability of *Leu. mesenteroides* in a colder environment. The non-starter sample exhibits slower, less predictable fermentation due to its natural microbial diversity.

Mannitol production

During fermentation, free sugars are liberated from vegetables and are subsequently transformed into mannitol (Ha et al., 1989). In our study, there were moderate fluctuations of mannitol production in kimchi fermented with and without starter cultures when the temperature was changed (Fig. 2). The highest mannitol concentration was observed at 30°C regardless of

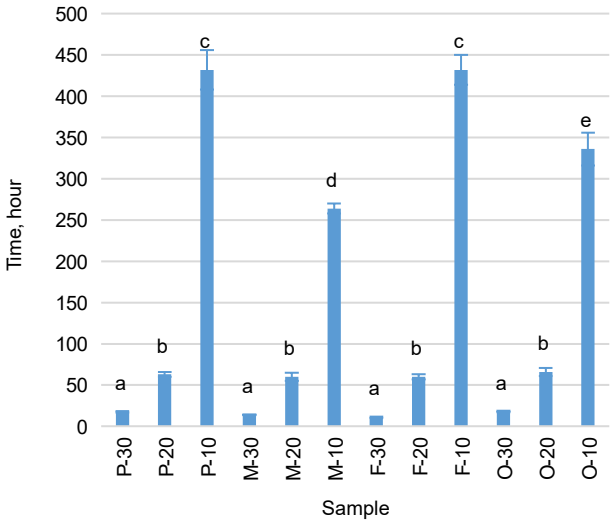

Fig. 1. Time to reach optimum pH of kimchi fermented by different starter strains and at different temperatures

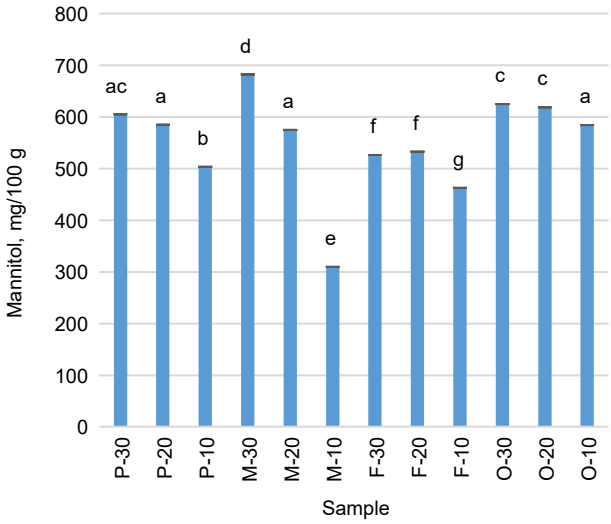

Fig. 2. Mannitol concentration in kimchi samples fermented by different starter strains and at different temperatures

starter culture and then decreased gradually as temperature decreased. This investigation suggests that within the examined temperature range, 30°C is the most favorable temperature for LAB growth and the accumulation of mannitol as a secondary metabolite. Among the starter-inoculated samples fermented at 30°C, *Leu. mesenteroides* exhibited the highest efficiency in mannitol production during fermentation, which is consistent with the results of a previous study (Lee et al., 2020).

The slightly lower concentration at 20°C may reflect a reduced metabolic rate despite a more prolonged fermentation period. The drop in mannitol level at 10°C can be attributed to the slower fermentation rate, which limits metabolic processes. The sample inoculated with *Leu. mesenteroides* showed the most significant decrease in mannitol, from 682.080 ±1.623 (M-30) to 310.017 ±13.989 mg/100 g (M-10).

Effects of temperature and starter culture on organic acid profiles

In this study, the levels of five organic acids were measured. The organic acid content of kimchi in various fermentation conditions is summarized in Table 2. The accumulation of organic acids at different temperatures shows notable variation, which reflects the influence of fermentation temperature on acid production and microbial activity.

Within the examined temperature range, the lactic acid content is highest at 30°C, regardless of starter culture, suggesting that this temperature favored microbial pathways that promote lactic acid synthesis. Most LABs grow optimally within a temperature range from 30°C to 45°C (Terpou et al., 2019) and produce lactic acid as the main metabolic end product in carbohydrate fermentation (Mozzi, 2015).

LABs primarily produce lactic acid through the homofermentative and heterofermentative pathways, with the homofermentative pathway yielding higher lactic acid output (Cho et al., 2014). This is why the sample inoculated with *L. plantarum*, which is a homofermentative LAB (Xu et al., 2019), produced the most lactic acid at 1264.488 ±17.636 mg/100 g.

The data also show that using starter cultures resulted in higher lactic acid production compared to the non-starter sample. This confirms the effectiveness of starter cultures in enhancing kimchi's flavor by

Table 2. Organic acid content of different kimchi models

Sample	Malic acid mg/100 g	Lactic acid mg/100 g	Acetic acid mg/100 g	Citric acid mg/100 g	Succinic acid mg/100 g
P-30	1 040.739 ±5.612^{ac}	1 264.488 ±17.636^{a}	370.626 ±8.685^{a}	12.306 ±4.212^{a}	29.942 ±8.453^{a}
P-20	1 022.384 ±59.725^{abc}	728.908 ±26.113^{bc}	392.591 ±20.057^{ab}	41.044 ±0.857^{b}	37.930 ±3.546^{a}
P-10	1 150.376 ±28.133^{c}	679.905 ±1.122^{d}	404.878 ±4.470^{ab}	73.739 ±1.314^{c}	20.503 ±0.572^{b}
M-30	1 105.417 ±46.611^{ac}	1 095.496 ±38.724^{e}	500.815 ±19.721^{c}	40.789 ±1.284^{b}	64.469 ±0.150^{cd}
M-20	920.667 ±1.807^{b}	823.867 ±4.883^{f}	473.506 ±2.582^{c}	49.771 ±0.948^{d}	72.020 ±1.246^{d}
M-10	1 165.782 ±17.183^{c}	639.729 ±7.266^{d}	379.654 ±1.492^{ab}	43.663 ±0.778^{bd}	32.786 ±0.653^{a}
F-30	709.495 ±0.022^{d}	1 019.969 ±7.980^{f}	492.114 ±0.741^{c}	47.416 ±2.725^{bd}	47.791 ±1.633^{c}
F-20	973.720 ±31.223^{ab}	749.587 ±21.579^{g}	414.386 ±11.903^{b}	43.585 ±1.411^{bd}	58.354 ±1.905^{c}
F-10	1 122.875 ±92.780^{c}	617.747 ±43.716^{d}	379.452 ±13.330^{ab}	84.141 ±0.660^{e}	31.745 ±2.294^{a}
O-30	1 303.751 ±36.608^{c}	963.729 ±10.193^{h}	517.114 ±7.434^{d}	34.396 ±0.571^{f}	63.762 ±0.940^{cd}
O-20	1 060.774 ±35.287^{ac}	616.926 ±22.978^{d}	407.407 ±11.968^{ab}	49.430 ±0.580^{d}	50.470 ±4.184^{e}
O-10	1 067.088 ±23.241^{ac}	699.085 ±9.204^{bc}	468.666 ±5.831^{c}	48.917 ±4.578^{d}	32.101 ±1.635^{a}

Means denoted with different letters within a column are significantly different from each other' ($p < 0.05$).

increasing the production of this major organic acid during fermentation.

Malic acid accumulation was significantly influenced by temperature across all samples inoculated with starter cultures. Within the examined temperature range, starter-inoculated samples tended to have higher levels of malic acid at lower temperatures. Meanwhile, the non-starter sample exhibited a relatively higher malic acid concentration at 30°C, with no statistically significant difference between the concentrations at 20°C and 10°C (around 1060 mg/100 g). The data indicate that naturally existing microorganisms contribute to elevated malic acid levels. The overall increase in malic acid production with decreasing temperature could be attributed to the prolonged fermentation time at lower temperatures. Generally, malic acid levels are inversely proportional to lactic acid levels due to the conversion of malic acid into lactic acid by malolactic acid enzyme in some LABs (Oguro et al., 2017; Sumby et al., 2019).

Acetic acid, which has a strong, pungent odor (Fugelsang, 1997; Li et al., 2022), is produced in larger quantities during fermentation at lower temperatures than during fermentation at higher temperatures (Park et al., 1993). This trend was observed in all the starter-inoculated samples. Both fermentation temperature and starter culture significantly influenced the acetic acid content in kimchi. More acetic acid was produced at higher temperatures in most samples, with the exception of *L. plantarum*, which showed no statistically significant difference across temperatures.

The acetic acid concentration in the sample inoculated with *Leu. mesenteroides* consistently decreased from 500.815 ±19.721 to 379.654 ±1.492 mg/100 g as the temperature decreased. Similarly, the sample fermented using *L. fermentum* as a starter culture had the lowest acetic acid level at 10°C, reflecting a decline in accumulation as temperatures dropped. Meanwhile, homofermentive fermentation of *L. plantarum* resulted in relatively stable acetic acid levels within the temperature range of 10°C to 30°C.

Overall, lower temperatures slow down the metabolic processes of microorganisms, leading to prolonged fermentation. The data show that low-temperature fermentation resulted in higher citric acid content in kimchi samples regardless of starter culture. When the temperature decreased from 30°C to 10°C, the most significant increase in citric acid was recorded in samples fermented using *L. plantarum* and *L. fermentum.* The extended time might allow certain metabolic intermediates, such as citric acid, to accumulate in greater amounts before being consumed or converted into other compounds. In contrast, higher temperatures speed up the fermentation process, resulting in the rapid depletion of these intermediates (Park et al., 2018). During the TCA cycle, citric acid is converted to lactic acid (Jung et al., 2024), which explains the higher lactic acid levels and lower citric acid levels of the samples.

The highest level of succinic acid was observed for *Leu. mesenteroides* at 20°C (72.020 ±1.246 mg/100 mg), suggesting that moderate temperatures optimize succinic acid synthesis. Other starter-inoculated samples showed a similar pattern, with the peak occurring at 20°C. Meanwhile, the highest succinic acid concentration at 30°C was recorded in the non-starter sample (63.762 ±0.940 mg/100 g).

Previous findings have demonstrated the significance of temperature in promoting bacterial succession (Jung et al., 2024; Park et al., 2018) and the influence of fermentation with starter cultures on organic acid levels (Lee et al., 2020; Oguro et al., 2017). However, the results of the current study underscore the combined effects of temperature and starter cultures on the accumulation of organic acids. A heatmap was created to visually represent the variations in organic acid content across the samples fermented under different conditions (Fig. 3). The results indicate that lower temperatures favor higher levels of certain acids, such as malic or citric acid, potentially due to slower but more diverse microbial activity that supports a range of metabolic pathways.

Principle component analysis (PCA) of organic acids

The PCA plots show the clear differences among samples fermented by distinct types of starter and at different temperatures (Fig. 4a–c). The organic acids in kimchi play a significant role in shaping its flavor profile. The primary organic acids contribute to its various sensory attributes, and they do so not only individually but also by interacting with other compounds to enhance the overall flavor complexity and sensory experience.

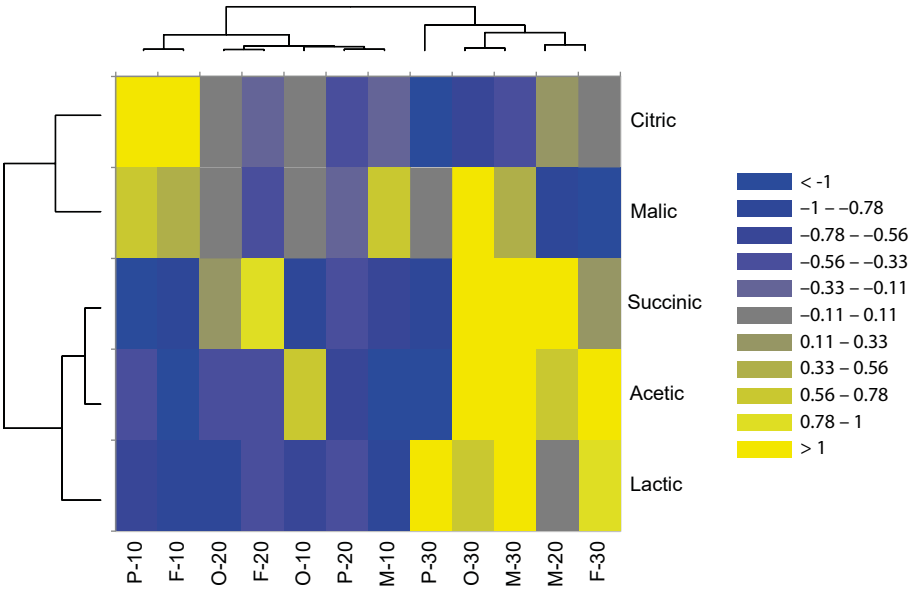

Fig. 3. Heatmap analysis of organic acids in kimchi models

Lactic acid is the most prominent organic acid in kimchi, and it is primarily responsible for the sour taste that is characteristic of well-fermented kimchi (Cho et al., 2015; Jeong et al., 2023), as well as enhancing its overall flavor complexity and mouthfeel (Christa et al., 2022). Meanwhile, malic acid mainly contributes to its initial tartness (Jeong et al., 2023).

Acetic acid provides a sharp, pungent flavor (Choi et al., 2019; Ryu et al., 1984), thus it should remain at moderate levels to maintain the traditional flavor balance in kimchi. A proper balance with lactic acid ensures that the kimchi retains its characteristic clean, mildly tangy sourness, without being overly harsh or vinegary. Meanwhile, succinic acid makes a key contribution to the umami taste of kimchi by interacting with amino acids to enhance umami and savory flavors. This interaction increases the fullness and depth of the taste (Christa et al., 2022; Lee et al., 2021).

F-10, positioned near the citric acid and malic acid vectors, likely has higher concentrations of these acids (Fig. 4a). This profile contributes to a fruity and mild sourness, with citric acid providing tangy notes and malic acid adding a smooth, apple-like flavor. However, its distance from lactic acid suggests that it may lack the dominant clean and mild sourness associated with kimchi. The position of sample F-20 near the succinic acid vector indicates a higher concentration of this acid, which imparts salty and umami notes rather than a strong sourness. While these flavors can complement fermentation, the proximity of F-30 to lactic and acetic acid vectors indicates its profile rich in these acids. The high lactic acid content provides the clean, mild tang essential to fermented products, while acetic acid adds a sharp, vinegary edge, balancing the overall sourness typical of kimchi.

The organic acid profiles of samples inoculated with *L. platarum* provide insights into their flavor characteristics, particularly for kimchi in which sourness is a defining feature (Fig. 4b). Sample P-10 is located closer to the acetic and citric acid vectors, indicating a profile with higher levels of these acids. This combination contributes to a sharp, vinegary sourness from acetic acid and a slightly fruity tang from citric acid, but it may lack the dominance of lactic acid typically found in fermented products. Sample P-20, positioned near the succinic acid vector, suggests a profile with a notable presence of succinic acid, which imparts mild umami and salty notes. However, its distance from lactic acid indicates that it may not deliver the clean sourness essential for traditional kimchi flavor. On the other hand, sample P-30 aligns closely with the lactic acid vector, suggesting

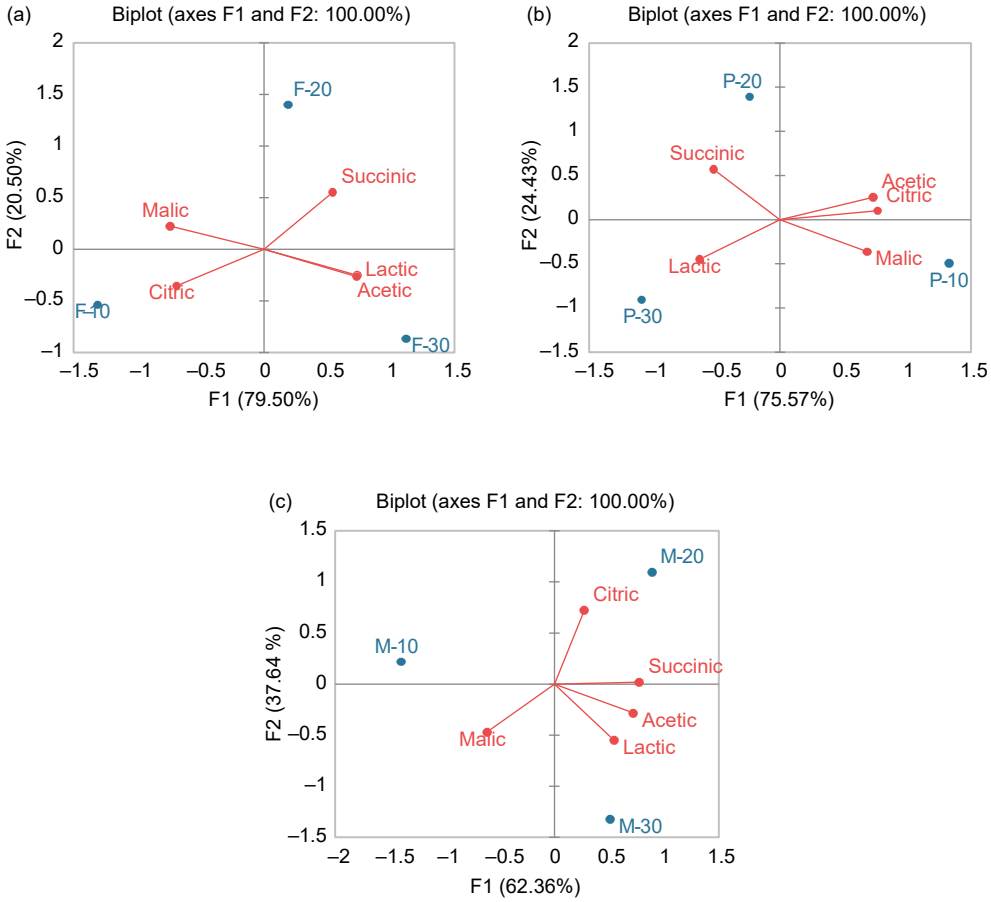

Fig. 4. Principal component analysis loadings for organic acids of kimchi samples fermented at different temperatures using *L. fermentum* (a), *L. plantarum* (b) and *Leu. mesenteroides* (c)

high levels of lactic acid, the dominant acid in kimchi fermentation. This high lactic acid content ensures a clean, mild, and well-rounded sourness characteristic of properly fermented kimchi, despite the sample being slightly farther from the acetic and citric acid vectors.

The flavor profiles of samples inoculated with *Leu. mesenteroides* differ based on their organic acid compositions, which are critical for kimchi's characteristic sourness (Fig. 4c). M-10 exhibits low acetic acid and moderate levels of other acids, resulting in a milder and less sharp sourness that may lack the distinctive fermented tang of traditional kimchi. M-20 has a high citric acid concentration, contributing a fruity and tangy note, but it deviates from the typical lactic fermentation profile of kimchi. In contrast, M-30 exhibits higher levels of lactic acid and moderate levels of acetic acid, providing a well-balanced sourness with the clean, mild tang of lactic acid and the moderate sharp edge from acetic acid, both of which are essential for an authentic kimchi flavor. As it best captures the characteristic sour and fermented notes expected in kimchi, M-30 is the most suitable choice among the three samples.

Changes in acidity and pH during 30 days of storage

The total acidity in all samples increased over 30 days of storage (Fig. 5a). During the first 15 days, the acidity tended to increase gradually from around 0.5 g/100 g. However, the acidity of the sample inoculated with

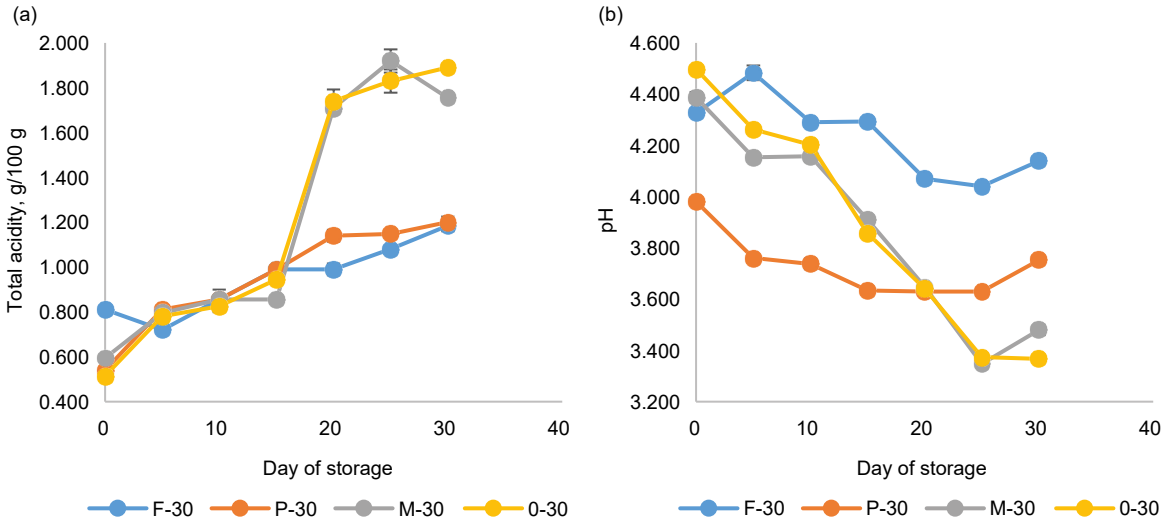

Fig. 5. Changes in acidity (a) and pH (b) during 30 days of storage

Leu. mesenteroides and the control sample increased significantly during the later stage of storage and respectively reached 1.755 ±0.000 g/100 g and 1.890 ±0.408 g/100 g on the 30th day. Regarding pH, the recorded data show a gradual decrease in all samples, with the fastest reduction – from 4.497 ±0.006 to 3.367 ±0.015 – observed in the control sample (Fig. 5b). Similar trends in pH and acidity caused by microbial activity have been recorded in previous studies (Jang et al., 2015; Jung et al., 2012; Moon et al., 2018). From 15 days of storage onwards, all samples entered the overacidification period, with the samples inoculated with *L. fermentum* and *L. plantarum* showing a slower change. The sample inoculated with *L. fermentum* exhibited the slowest rate of change toward the end of the storage period.

CONCLUSION

In this study, we evaluated the combined effects of temperature and starter culture on kimchi fermentation. At 30°C, lactic acid was the most abundant type of organic acid produced, whereas at 10 and 20°C, malic acid was the highest. The proportion of organic acids also varied depending on the starter culture used, significantly influencing the flavor of kimchi at optimal ripening pH. The *L. plantarum*-inoculated sample at 30°C had the most distinct organic acid profile according to the PCA map. *Leu. mesenteroides* exhibited the highest accumulation of mannitol when kimchi was fermented at 30°C. In the investigation into the stabilization of acidity and pH during storage for the most promising samples, samples inoculated with *L. fermentum* demonstrated the slowest rate of change. The results emphasize how starter cultures and temperature interact to influence key metabolites in kimchi fermentation, as well as changes in acidity during storage. These findings provide insights that could facilitate the production of kimchi with customized flavor characteristics.

ACKNOWLEDGEMENTS

This research is funded by Hanoi University of Science and Technology (HUST) under project number T2024-PC-068. The authors would like to express deepest gratitude to all those who supported us during the completion of this research.

DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval
Not applicable.

Competing Interests
The authors declare that they have no conflicts of interest.

OPEN ACCESS

REFERENCE

Ahn, G. H., Moon, J. S., Shin, S. Y., Min, W. K., Han, N. S., Seo, J. H. (2015). A competitive quantitative polymerase chain reaction method for characterizing the population dynamics during kimchi fermentation. J. Ind. Microbiol. Biotechnol., 42(1), 49–55. https://doi.org/10.1007/s10295-014-1553-x

AOAC (2000). AOAC Official Method 942.05.108.

Bong, Y.-J., Jeong, J.-K., Park, K.-Y. (2013). Fermentation Properties and Increased Health Functionality of Kimchi by Kimchi Lactic Acid Bacteria Starters. J. Korean Soci. Food Sci. Nutr., 42, 1717–1726.

Cha, J., Kim, Y. B., Park, S. E., Lee, S. H., Roh, S. W., Son, H. S., Whon, T. W. (2024). Does kimchi deserve the status of a probiotic food? Crit. Rev. Food Sci. Nutr., 64(19), 6512–6525. https://doi.org/10.1080/10408398.2023.2170319

Chae, M. H., Park, E. J., Oh, T. K., Jhon, D. Y. (2006). Preparation of Kimchi Containing *Bifidobacterium longum* BO-11. Korean J. Food Sci. Technol., 38(2), 232–236. https://www.researchgate.net/publication/263646752

Cho, J.-H., Lee, S.-J., Choi, J.-J., Chung, C.-H. (2015). Chemical and sensory profiles of dongchimi (Korean watery radish kimchi) liquids based on descriptive and chemical analyses. Food Sci. Biotechnol., 24, 497–506.

Cho, S., Kang, J. S., Cho, K. J., Lee, K. H., Kwon, C. H., Song, J., Lee, K., Kim, S. Y., Kim, E. J. (2014). Effect of Homofermentative and Heterofermentative Lactic Acid Bacteria on the Quality and Aerobic Stability of Silage: Meta-Analysis. J. Korean Soc. Grassland Forage Sci., 34(4), 247–253. https://doi.org/10.5333/kgfs.2014.34.4.247

Choi, Y.-J., Yong, S., Lee, M. J., Park, S. J., Yun, Y., Park, S.-H., Lee, M.-A. (2019). Changes in volatile and non-volatile compounds of model kimchi through fermentation by lactic acid bacteria. LWT – Food Sci. Technol., 105(2), 118–126.

Christa, P., Dunkel, A., Krauss, A., Stark, T. D., Dawid, C., Hofmann, T. (2022). Discovery and Identification of Tastants and Taste-Modulating N-Acyl Amino Acid Derivatives in Traditional Korean Fermented Dish Kimchi Using a Sensomics Approach. J. Agric. Food Chem., 70, 7500–7514. https://doi.org/10.1021/acs.jafc.2c02623

Fugelsang, K. C. (1997). Acetic acid bacteria. In: Wine Microbiology (p. 48–67). New York, NY: Springer. https://doi.org/10.1007/978-1-4757-6970-8_2

Ha, J., Hawer, W. S., Kim, Y. J., Nam, Y.-J. (1989). Changes of Free Sugars in Kimchi during Fermentation. Korean J. Food Sci. Technol., 21, 633–638.

Hahn, Y.-S., Oh, J.-Y., Song, J.-E. (2002). The Study on Amylolytic Enzyme and Protease Activities of Kimchi. Korean J. Food Sci. Technol., 34(2), 269–273.

Han, G. J., Choi, H. S., Lee, S. M., Lee, E. J., Park, S. E., Park, K. Y. (2011). Addition of starters in pasteurized brined baechu cabbage increased Kimchi quality and health functionality. J. Korean Soc. Food Sci. Nutr., 40(1), 110–115. https://doi.org/10.3746/jkfn.2011.40.1.110

Jang, J. Y., Lee, M. E., Lee, H. W., Lee, J. H., Park, H. W., Choi, H. J., Pyun, Y. R., Kim, T. W. (2015). Extending the shelf life of kimchi with *Lactococcus lactis* strain as a starter culture. Food Sci. Biotechnol., 24(3), 1049–1053. https://doi.org/10.1007/s10068-015-0134-8

Jeong, J. Y., Bae, M., Kim, M. J., Jang, H.-Y., Jung, S., Lee, J.-H., Hwang, I. M. (2023). Rapid Quantitative Analysis of Metabolites in Kimchi Using LC-Q-Orbitrap MS. ACS Omega, 8, 3896–3904. https://doi.org/10.1021/acsomega.2c06303

Jung, J. Y., Lee, S. H., Lee, H. J., Seo, H. Y., Park, W. S., Jeon, C. O. (2012). Effects of *Leuconostoc mesenteroides* starter cultures on microbial communities and metabolites during kimchi fermentation. Int. J. Food

Microbiol., 153(3), 378–387. https://doi.org/10.1016/j.ijfoodmicro.2011.11.030

Jung, S., Hwang, I. M., Lee, J. H. (2024). Temperature impact on microbial and metabolic profiles in kimchi fermentation. Heliyon, 10(5), e27174. https://doi.org/10.1016/j.heliyon.2024.e27174

Kim, M. J., Lee, H. W., Lee, M. E., Roh, S. W., Kim, T. W. (2019). Mixed starter of *Lactococcus lactis* and *Leuconostoc citreum* for extending kimchi shelf-life. J. Microbiol., 57(6), 479–484. https://doi.org/10.1007/s12275-019-9048-0

Lee, H., Yoon, H., Ji, Y., Kim, H., Park, H., Lee, J., Shin, H., Holzapfel, W. (2011). Functional properties of *Lactobacillus* strains from kimchi. Int. J. Food Microbiol., 145(1), 155–161. https://doi.org/10.1016/j.ijfoodmicro.2010.12.003

Lee, H. J., Lee, M. J., Choi, Y.-J., Park, S. J., Lee, M.-A., Min, S. G., Park, S.-H., Seo, H.-Y., Yun, Y.-R., Castro-Mejías, R. (2021). Free Amino Acid and Volatile Compound Profiles of Jeotgal Alternatives and Its Application to Kimchi. Foods, 10(2), 423. https://doi.org/10.3390/foods

Lee, J. J., Choi, Y. J., Lee, M. J., Park, S. J., Oh, S. J., Yun, Y. R., Min, S. G., …, Lee, M. A. (2020). Effects of combining two lactic acid bacteria as a starter culture on model kimchi fermentation. Food Res. Int., 136. https://doi.org/10.1016/j.foodres.2020.109591

Lee, K., Lee, Y. (2010). Effect of *Lactobacillus plantarum* as a Starter on the Food Quality and Microbiota of Kimchi. Food Sci. Biotechnol, 19(3), 641–646. https://doi.org/10.1007/s10068-010-0090-2

Lee, K. W., Shim, J. M., Yao, Z., Kim, J. A., Kim, J. H. (2018). Properties of kimchi fermented with GABA-producing lactic acid bacteria as a starter. J. Microbiol. Biotechnol., 28(4), 534–541. https://doi.org/10.4014/jmb.1709.09011

Lee, M. E., Jang, J. Y., Lee, J. H., Park, H. W., Choi, H. J., Kim, T. W. (2015). Starter Cultures for Kimchi Fermentation. J. Microbiol. Biotechnol., 25(5), 559–568. https://doi.org/10.4014/jmb.1501.01019

Li, S., Tian, Y., Sun, M., Liu, J., Bai, Y., Liu, X., Guo, Y. (2022). Characterization of Key Aroma Compounds in Fermented Bamboo Shoots Using Gas Chromatography-Olfactometry-Mass Spectrometry, Odor Activity Values, and Aroma Recombination Experiments. Foods, 11(14). https://doi.org/10.3390/foods11142106

Moon, E. W., Yang, J. S., Yoon, S. R., Ha, J. H. (2020). Application of colorimetric indicators to predict the fermentation stage of kimchi. J. Food Sci., 85(12), 4170–4179. https://doi.org/10.1111/1750-3841.15532

Moon, S. H., Kim, C. R., Chang, H. C. (2018). Heterofermentative lactic acid bacteria as a starter culture to control kimchi fermentation. LWT, 88, 181–188. https://doi.org/10.1016/j.lwt.2017.10.009

Mozzi, F. (2015). Lactic Acid Bacteria. In: B. Caballero, P. M. Finglas, F. Toldrá (Eds.), Encyclopedia of Food and Health (pp. 501–508). Cambridge, MA: Academic Press.

Naghmouchi, K., Belguesmia, Y., Bendali, F., Spano, G., Seal, B. S., Drider, D. (2020). *Lactobacillus fermentum*: a bacterial species with potential for food preservation and biomedical applications. Crit. Rev. Food Sci. Nutr., 60(20), 3387–3399. https://doi.org/10.1080/10408398.2019.1688250

Oguro, Y., Nishiwaki, T., Shinada, R., Kobayashi, K., Kurahashi, A. (2017). Metabolite profile of koji amazake and its lactic acid fermentation product by *Lactobacillus sakei* UONUMA. J. Biosci. Bioengin., 124(2), 178–183. https://doi.org/10.1016/j.jbiosc.2017.03.011

Park, J. M., Shin, J. H., Bak, D. J., Chang, U. J., Suh, H. J., …, Kim, J. M. (2013). Effect of a *Leuconostoc mesenteroides* strain as a starter culture isolated from the kimchi. Food Sci. Biotechnol., 22(6), 1729–1733. https://doi.org/10.1007/s10068-013-0273-8

Park, K.-Y., Jeong, J.-K., Lee, Y.-E., Daily, J. W. (2014). Health Benefits of Kimchi (Korean Fermented Vegetables) as a Probiotic Food. J. Med. Food, 17(1), 6–20. https://doi.org/10.1089/jmf.2013.3083

Park, S. E., Seo, S. H., Kim, E. J., Na, C. S., Son, H. S. (2018). Effects of different fermentation temperatures on metabolites of Kimchi. Food Biosci., 23, 100–106. https://doi.org/10.1016/j.fbio.2018.03.009

Park, Y.-S., Ko, C.-Y., Ha, D.-M. (1993). Effect of Temperature on the Production of Free Organic Acids during Kimchi Fermentation. J. Microbiol. Biotechnol., 3(4), 266–269.

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

Ryu, J.-Y., Lee, H.-S., Rhee, H.-S. (1984). Changes of organic acids and volatile flavor compounds in kimchis fermented with different ingredients. Korean J. Food Sci. Technol., 16(2), 169–174.

Sumby, K. M., Bartle, L., Grbin, P. R., Jiranek, V. (2019). Measures to improve wine malolactic fermentation. Appl. Microbiol. Biotechnol., 103(5), 2033–2051. https://doi.org/10.1007/s00253-018-09608-8

Terpou, A., Papadaki, A., Lappa, I. K., Kachrimanidou, V., Bosnea, L. A., Kopsahelis, N. (2019). Probiotics in food systems: significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. Nutrients, 11, 7, 1591. https://doi.org/10.3390/nu11071591

Xu, D., Ding, W., Ke, W., Li, F., Zhang, P., Guo, X. (2019). Modulation of metabolome and bacterial community in whole crop corn silage by inoculating homofermentative *Lactobacillus plantarum* and heterofermentative *Lactobacillus buchneri*. Front. Microbiol., 10. https://doi.org/10.3389/fmicb.2018.03299

You, S. Y., Yang, J. S., Kim, S. H., Hwang, I. M. (2017). Changes in the physicochemical quality characteristics of cabbage kimchi with respect to storage conditions. J. Food Qual., 2017. https://doi.org/10.1155/2017/9562981