JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

QUALITY STABILIZATION OF KIMCHI FERMENTED WITH *LACTOBACILLUS FERMENTUM* DURING STORAGE USING TRISODIUM CITRATE

Nguyen Thi Hanh[1], Cao Thi Ngoc Anh[1], Hoang Hong Son[1], Pham Thi Thu Uyen[1], Tran Nhat Le[1], Nguyen Van Hung[1], Nguyen Thi Trang[1]*

Address(es):
[1] Hanoi University of Science and Technology, School of Chemistry and Life Sciences, Department of Food Engineering, 100000 Hanoi, Viet Nam.

*Corresponding author: trang.nt232297m@sis.hust.edu.vn

https://doi.org/10.55251/jmbfs.12727

ARTICLE INFO

Received 26. 4. 2025
Revised 13. 10. 2025
Accepted 23. 11. 2025
Published 1. 12. 2025

Regular article

OPEN ACCESS

ABSTRACT

Renowned as a hallmark of Korean culinary heritage, kimchi represents a sophisticated fusion of complex flavor chemistry, probiotic functionality, and distinctive textural properties. Using lactic acid bacteria (LAB) as starter cultures in fermentation to increase the quality of kimchi has been proven in many previous studies. However, more research should be conducted to maintain the desirable characteristics of this product, especially during storage. This study examines the effectiveness of trisodium citrate as a quality-stabilizing agent for kimchi using *Lactobacillus fermentum* as a starter culture during low-temperature storage. Various concentrations of trisodium citrate (0%, 0.2%, 0.5%, 0.75%, and 1%) were tested to evaluate their effects on selected quality indicators of kimchi over a 30-day storage period at 4–6 °C. The result showed kimchi with 0.75% trisodium citrate exhibited the stable acidity and pH (4.019 ± 0.001), showed no presence of yeasts or molds and achieved the highest sensory evaluation score among all samples throughout the storage period. These findings contribute to enhancing and maintaining the overall quality of kimchi during storage, which is important in large-scale commercial distribution.

Keywords: fermentation, kimchi, trisodium citrate, metabolites, starter culture, temperature

INTRODUCTION

Kimchi is a flavorful and health beneficial food which is characterized by the fermentation of a mix vegetables and spices by lactic acid bacteria (LAB). These microorganisms are responsible for the characteristic flavor that sets kimchi apart from other fermented foods. Depending on environment conditions, the fermentation period may range from several days to months. The LAB involved in kimchi fermentation contribute significantly to its probiotic properties by promoting gut health and inhibiting harmful bacteria **(Park *et al.*, 2014; Patra *et al.*, 2016)**. Moreover, the LABs in kimchi exhibit antioxidant and anti-inflammatory properties, which may assist in managing conditions such as obesity, cancer, and allergic dermatitis **(Cha *et al.*, 2024; Park *et al.*, 2014)**.
Commercially available kimchi was fermented and consumed within a short time frame since fermentation continues during storage. Therefore, the quality can be changed due to over-fermentation, thus reducing consumer appeal. Although the addition of starter cultures can improve kimchi's functionality, it also accelerates the fermentation rate, thereby shortening the product's shelf life. Further investigation is needed into methods for preserving kimchi's desirable characteristics during storage **(Lee *et al.*, 2015)**. Previous studies have indicated that combining various preservation methods can help extend the shelf life of kimchi. Currently, there are many methods included storing kimchi under cold storage conditions with three modes: refrigerated, frozen, and ultra-frozen **(Park *et al.*, 2024; Lee *et al.*, 2021)**; using food additives: calcium lactate and calcium chloride **(Li *et al.*, 2024)**; and irradiation **(Song *et al.*, 2022)**. Among these, food additives can be a promising solution to solve the drawbacks of rapid fermentation and help maintain kimchi's quality and usability for a longer time.
Trisodium citrate is widely applied in food production because of its versatility and multifunctional properties. It not only helps stabilize pH and acids but also acts as a thickening agent to enhance the firmness of food production, such as dairy and vegetables. This food additive exhibited antimicrobial properties, attributed to its capacity to induce osmotic pressure through the generation of antimicrobial ions, thereby extending the shelf life of food products **(Williams *et al.*, 2023)**.
Our study aims to examine the effects of trisodium citrate at different concentrations on the quality stabilization of kimchi product that used *L. fermentum* as a starter culture which has been widely applied in food due to its probiotic and functional properties **(Naghmouchi *et al.*, 2020)**.

MATERIAL AND METHODS

Sample preparation

Kimchi samples were prepared at the ratio of 60: 2.4: 2.5: 1.2: 1.9: 1.2: 1.6: 6.0: 4.5: 3.9: 0.7 = Chinese cabbage: salt: ginger: garlic: sugar: chili powder: green onion: carrot: glutinous rice flour: radish: chili, respectively. The salt concentration of the mixture before fermentation was 1.854% which falls within the proper range 1 − 4% for LABs growth and suppresses undesirable microorganisms (Lee *et al.*, 2015). The starter culture *L. fermentum* SBS−1 (Custom Probiotics, Inc., California, USA) was added at a density of 7 log CFU/g. The fermentation was then conducted at 22 °C for 28 hours.

After samples reached optimum titratable acid content (0.5-0.6%), trisodium citrate was added into kimchi at different concentrations: 0% (control sample, O), 0.2% (sample A), 0.5% (sample B), 0.75% (sample C), and 1.0% (sample D). Then samples were stored at 4-6 °C for further analysis.

Measurement of pH and titratable acidity

pH meter (Lab 845, SI Analytics) used in pH determination. The AOAC method was adopted to measure the titratable acidity (Latimer, 2023).

Measurement of reducing sugars and total sugars content

Reducing sugars and total sugars content were determined using 3,5-dinitrosalicylic acid (DNS) **(Kim *et al.*, 1994)**. 0.5 mL of juice sample was mixed with 3 mL of DNS, then heated in a water bath at 100 °C for 5 minutes. To determine the total sugar content, the sample was first hydrolyzed in 5% hydrochloric acid for 2 hours and neutralized with 5% sodium hydroxide before being mixed with DNS.

Microbial analysis

The spread plate method was employed in microbial analysis. Kimchi juice was filtered and serially diluted with sterilized 0.85% NaCl solution. LAB were cultured on MRS agar and incubated anaerobically at 37 °C for 2 days **(Park *et al.*, 2013)**. Total viable bacteria were enumerated on PCA with incubation at 37 °C for

2 days (Park *et al*., 2018). Yeast and mold were counted on YGC agar after incubation at 30 °C for 3 days **(Park & Lee, 2012)**.

Measurement of the hardness of kimchi

The hardness of kimchi was analyzed following a modified method of a previous work **(Chang & Chang, 2010)**, using a TA-XT Plus texture analyzer (Stable Micro Systems, Godalming, UK). Chinese cabbage stalks were cut into 4 × 6 cm pieces. Two pieces of them were prepared for each experimental sample, and each piece was measured in triplicate. The operational conditions of the texture analyzer were as follows: probe (P2 - 6 mm), travel distance (65%), pre-test speed (5.0 mm/s), test speed (1.0 mm/s), and post-test speed (10.0 mm/s). The hardness of kimchi was expressed in gram.

Sensory analysis

The sensory analysis was conducted by 9 trained people at the Department of Food Engineering, School of Chemistry and Life Sciences, Ha Noi University of Science and Technology (Ha Noi, Viet Nam). Sourness, crispness, and overall acceptability were evaluated using a 9-point hedonic scale; 1 is very bad, 5 is moderate, and 9 is very good **(Chang & Chang, 2010)**.

Statistical analysis

Data were expressed as means ± standard deviations (SD) and subjected to a one-way analysis of variance with the Student-Newman-Keuls test to determine the statistical significance ($p<0.05$) using SPSS Statistics 20.

RESULTS AND DISCUSSION

pH and titratable acidity

The pH and acidity are crucial parameters that govern the quality of kimchi **(Park *et al*., 1995)**. The pH of all samples underwent fluctuations during 30-day storage. Samples with added trisodium citrate exhibited slight reduction during the first 3 days, that may due to intense activity of LABs. It was found that kimchi is optimal for consumption when it reaches a pH range of around 4.1 **(Hahn *et al*., 2002; Moon *et al*., 2020)**. However, it may vary based on ingredients, additives, and fermentation temperature **(Ku, 1988; Pathomrungsiyounggul *et al*., 2010)**. A pH level around 4 was deemed acceptable when additional starter cultures are used **(Ku, 1988)**.

Trisodium citrate was used to adjust the pH, as it functions as a buffering agent in the pH range of 2 to 8 **(Albert, 2012)**, depending on its concentration. Sample D exhibited the highest pH instability as its pH gradually declined from day 3 to 12, followed by an increase and eventual stabilization between days 18 and 30. In contrast, sample C was the most stable, maintaining a pH of around 4 during the period investigated.

Regarding titratable acidity, sample C displayed the smallest variation while other samples significantly fluctuated during 30 days. Possibly because the fermentation process not only produces lactic acid but also creates other types; however the formula was only quantified solely by lactic acid equivalents. In addition, trisodium citrate has a carboxylate functional group (-COO⁻) that can form chelate complexes with metal ions such as Mg^{2+}, Mn^{2+}. When citrate bound to metal ions, the concentration of free metals in the environment decreases.

(a) (b)

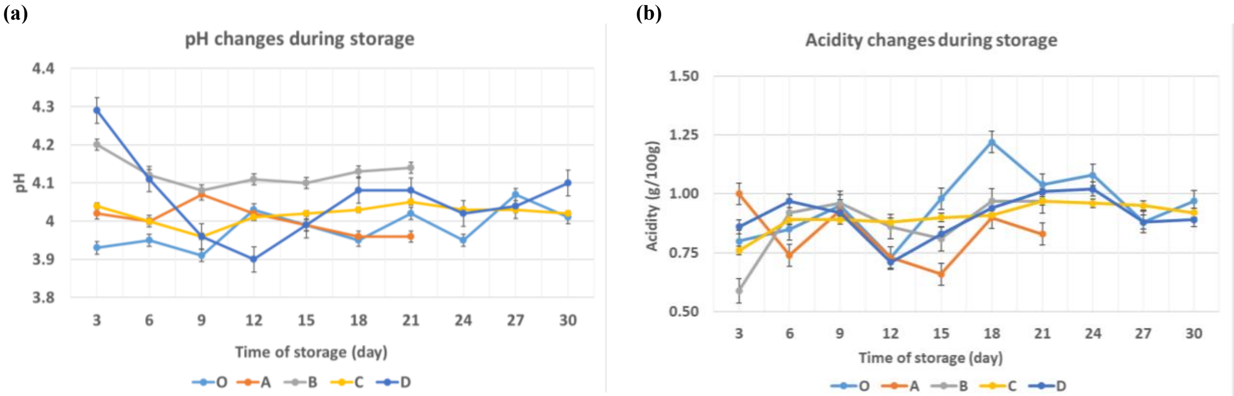

Figure 1 Effect of different trisodium citrate concentrations on the changes in pH (a) and titratable acidity (b) during 30-day storage of kimchi samples labeled O (control), A (0.2% trisodium citrate), B (0.5% trisodium citrate), C (0.75% trisodium citrate), D (1% trisodium citrate).

The phosphoketolase enzyme in some fermenting bacteria cannot function effectively since enzyme activity is impaired due to metal cofactor deficiency. This might lead to a reduction in the conversion of sugar into acetic acid, thereby reducing acidity. On day 21, the pH of sample A dropped below the optimal range, while yeast and mold growth was observed in sample B. Therefore, these two samples were not evaluated in the following days.

(a) (b)

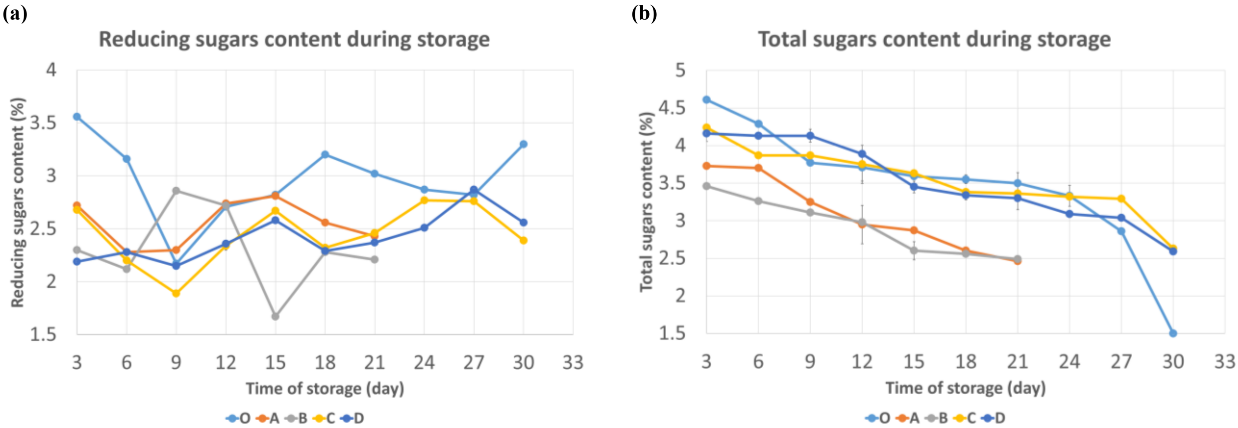

Figure 2 Changes in reducing sugars content (a) and total sugars content (b) during 30-day storage of kimchi samples labeled O (control), A (0.2% trisodium citrate), B (0.5% trisodium citrate), C (0.75% trisodium citrate), D (1% trisodium citrate).

Reducing sugars and total sugars content

During fermentation, polysaccharides undergo hydrolysis into monosaccharides which act as primary carbon sources for microbial catabolism. According to a study by Choi *et al*., glucose levels declined significantly during the early stages, whereas fructose degradation occurred predominantly in the later stages of fermentation. Mannitol biosynthesis was observed during the later stages, likely via heterolactic fermentation pathways, whereas galactose remained almost unchanged throughout the storage period **(Choi *et al*., 2016)**. Consequently, the reducing sugar concentrations may vary dynamically during storage due to internal transformations within the sample. The results showed a fluctuation in reducing sugars content during storage while the total sugars content gradually decreased, which is consistent with a previous finding **(Ryu *et al*., 1996)**.

The total sugars content of the sample D showed early indications of over-fermentation as titratable acidity exceeded 1.0% by day 24 , while sample C still maintained optimal acidity and pH level. During fermentation, total sugars undergo microbial conversion into organic acids via homo- or heterolactic pathways, thus their concentration gradually decreases. Additionally, the biochemical transformations occurring throughout fermentation appear to be predominantly shaped by microbial community composition, which dictate metabolic progressions over time. On the day 30, the rise in reducing sugars content of sample O might indicate an increase in activity of microorganisms degrading polysaccharide.

Hardness of kimchi

The temporary decline in this textural property was counteracted by the release of intracellular water from cabbage tissues **(Lee & Kim, 2003)**. The highest trisodium citrate trisodium citrate concentration (1.00%) promoted the retention of structural integrity and textural firmness of kimchi matrix by suppressing the activity of enzymes which drive firmness degradation such as pectinase and cellulase. Citrate possesses an ability to bind divalent metal ions **(Prywer *et al*., 2015)**, thus it can contribute to the reduction in activity of these enzymes. In contrast, at lower trisodium citrate concentrations (0.2%–0.75%), its interaction with metal ions might be weaker, allowing cell wall degrading enzymes to stay active during the early fermentation stage. As the fermentation environment became increasingly acidic (pH dropped to approximately 4.0–4.5), trisodium citrate enhanced its metal ion binding affinity, thereby intensifying its inhibitory effect on pectinase activity. Moreover, when the pH fell below 4.2, lactic acid bacteria proliferated and produced exopolysaccharides that contributed to gel formation and structural enhancement **(Sönmez *et al*., 2018; Silva *et al*., 2019)**.

Previous research examining kimchi inoculated with starter cultures **(Li *et al*., 2020; Chang & Chang, 2010)**, subjected to thermal and irradiation processing **(Song *et al*., 2008; Kim *et al*., 2008)**, or supplemented with calcium salts **(Song *et al*., 2008; Lee & Kim, 2003)**, has documented a progressive deterioration in the textural properties. Specifically, these treatments led to a substantial diminution in firmness, with a decline approaching 50% of the initial texture after the completion of the storage period.

Our result suggests that incorporating trisodium citrate at varying concentrations into kimchi fermented with *L. fermentum* may lead to a slight initial reduction in hardness, followed by a gradual recovery over time. At higher concentrations, trisodium citrate appeared to help maintain hardness more effectively.

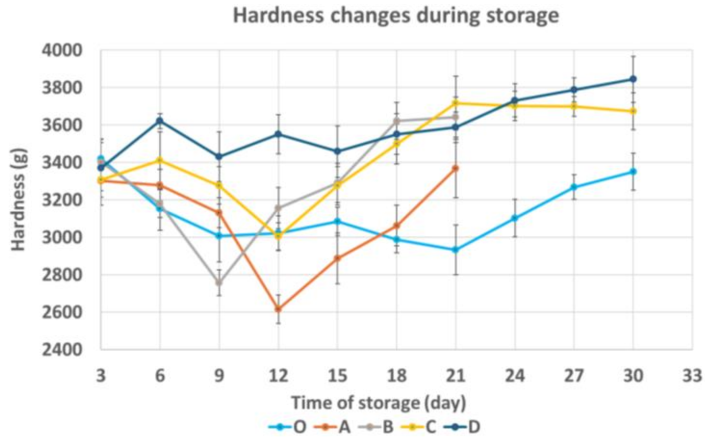

Figure 3 Changes in hardness during 30-day storages of kimchi samples labeled O (control), A (0.2% trisodium citrate), B (0.5% trisodium citrate), C (0.75% trisodium citrate), D (1% trisodium citrate).

Microbial analysis

The total viable bacteria count in all kimchi samples ranged from 7 to 8.8 log CFU/g, which aligns with previous work **(Park & Lee, 2012; Bang *et al*., 2008)**. Samples C and D indicated the absence of yeasts and molds. In contrast, yeasts and molds were detected at in sample B at 6.808 ± 0.140 log CFU/g and sample O at 7.600 ± 0.062 log CFU/g. Meanwhile, sample A displayed notable signs of spoilage as evidenced by its pH and acidity levels exceeding the acceptable threshold between days 18 and 21. Thus, it was excluded from microbial analysis at the end of the examined storage period.

Table 1 Microbial analysis of kimchi samples labeled O (control), A (0.2% trisodium citrate), B (0.5% trisodium citrate), C (0.75% trisodium citrate), D (1% trisodium citrate) on day 21 (sample B) and day 30 (sample O, C, D).

	O	B	C	D
Total viable bacteria (log CFU/g)	8.234 ± 0.067^{a}	8.215 ± 0.192^{a}	8.028 ± 0.096^{a}	8.032 ± 0.056^{a}
Lactic acid bacteria (log CFU/g)	8.300 ± 0.009^{a}	8.32 ± 0.048^{a}	7.996 ± 0.012^{b}	7.850 ± 0.006^{b}
Yeasts and molds (log CFU/g)	7.600 ± 0.062^{a}	6.808 ± 0.140^{a}	-	-

Different letters between samples in a row denote significant differences ($p<0.05$).

This data suggests that trisodium citrate at higher concentration (0.75-1%) may offer an advantage in inhibiting yeasts and molds under prolonged storage. Trisodium citrate possesses the capacity to chelate metal cations, thereby diminishing their catalytic role in oxidation reactions. Additionally, adding trisodium citrate can increase osmotic pressure, thus disrupting the homeostasis of metal ion concentrations and osmotic balance within microbial cellular processes. This disturbance adversely affects microbial growth and survival, resulting in bacteriostatic or fungistatic effects under stress-induced conditions **(Williams *et al*., 2023)**. This is consistent with previous studies demonstrating that trisodium citrate exhibits selective antimicrobial activity against gram-positive bacteria, including LAB **(Y. L. Lee *et al*., 2002; Ibrahim Sallam, 2007)**.

Microbiological analysis indicated that kimchi supplemented with suitable trisodium citrate levels can meet microbiological safety for consumption, with no detection of spoilage yeasts and molds.

Sensory analysis

A kimchi product that is evaluated well is one that has a good balance of sour, sweet, and salty tastes along with freshness and a crunchy texture **(Chang & Chang, 2010)**. Table 2 shows that the sourness and crispness of kimchi samples added trisodium citrate at different concentrations. Regarding sourness and crispness, sample C (0.75% trisodium citrate) demonstrated its quality significantly better than other samples, especially the control sample. In terms of overall acceptability, sample C also received higher and more stable evaluations compared with other samples. All kimchi samples containing trisodium citrate retained the characteristic kimchi flavor without exhibiting strange tastes.

According to Codex Alimentarius Commission, the maximum level of trisodium citrate supplementation in fermented products is followed Good Manufacturing Practice (GMP) **(Codex Alimentarius Commission, 2024)**. At addition levels below 1%, trisodium citrate did not cause undesirable flavors in the fermented kimchi and the food product was considered safe for consumer.

Table 2 Sensory evaluation during 30-day storage period of kimchi samples labeled O (control), A (0.2% trisodium citrate), B (0.5% trisodium citrate), C (0.75% trisodium citrate), D (1% trisodium citrate) on a 9-point hedonic scale.

		Day of storage									
		3	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**
Sourness	**O**	7.33 ±0.5^{ab}	6.89 ±0.78^{a}	6.56 ±0.67^{ac}	5.67 ±0.83^{a}	5.00 ±0.83^{a}	4.78 ±0.83^{ac}	4.44 ±1.11^{a}	4.43 ±1.01^{a}	4.29 ±1.22^{a}	3.71 ±0.53^{a}
	A	7.56 ±0.88^{ab}	6.89 ±0.33^{a}	6.11 ±0.66^{ab}	4.78 ±0.97^{b}	4.22 ±0.67^{a}	3.89 ±0.71^{a}	3.89 ±0.50^{a}	-	-	-
	B	6.89 ±0.33^{a}	6.56 ±0.72^{a}	5.44 ±0.33^{b}	4.78 ±0.66^{b}	4.44 ±0.72^{ac}	4.44 ±0.73^{ac}	4.33 ±1.0^{a}	-	-	-
	C	7.89 ±0.6^{b}	7.22 ±0.44^{a}	7.00 ±0.33^{ac}	6.89 ±0.60^{c}	6.56 ±1.24^{bc}	6.67 ±0.71^{b}	6.56 ±0.73^{b}	6.44 ±0.73^{b}	6.33 ±0.71^{b}	6.22 ±0.44^{b}
	D	7.33 ±0.87^{ab}	6.67 ±0.7^{a}	6.22 ±0.52^{abc}	5.89 ±0.71^{d}	5.67 ±1.31^{ac}	5.33 ±0.87^{c}	5.11 ±1.01^{ab}	4.89 ±1.22^{a}	4.22 ±1.27^{a}	3.78 ±0.60^{a}
Scripness	**O**	7.22 ±0.44^{ab}	7.00 ±0.86^{a}	6.78 ±0.5^{a}	5.89 ±0.33^{a}	5.22 ±0.83^{a}	5.00 ±0.83^{a}	4.56 ±1.12^{b}	4.50 ±1.01^{a}	4.13 ±1.22^{a}	3.63 ±0.53^{a}
	A	7.56 ±0.88^{ab}	7.0 ±0.5^{a}	6.67 ±0.71^{a}	5.44 ±0.88^{ab}	4.22 ±0.67^{a}	3.67 ±0.71^{a}	3.33 ±0.5^{a}	-	-	-
	B	6.89 ±0.33^{a}	6.67 ±0.87^{a}	5.56 ±0.53^{b}	5.00 ±0.71^{b}	4.44 ±0.73^{a}	4.33 ±0.71^{b}	4.22 ±1.09^{b}	-	-	-
	C	8.00 ±0.71^{b}	7.56 ±0.73^{a}	7.00 ±0.5^{a}	6.89 ±0.60^{c}	6.56 ±1.24^{b}	6.67 ±0.71^{c}	6.56 ±0.73^{c}	6.33 ±0.5^{b}	6.22 ±0.67^{b}	6.11 ±0.33^{b}
	D	7.33 ±0.87^{ab}	7.11 ±0.33^{a}	6.44 ±0.53^{a}	5.89 ±0.71^{b}	5.67 ±1.30^{b}	5.33 ±0.87^{b}	5.11 ±1.01^{d}	4.89 ±1.22^{a}	4.22 ±1.27^{a}	3.78 ±0.60^{c}
Overall - acceptability	**O**	7.33 ±0.5^{ab}	7.22 ±0.67^{a}	6.44 ±0.71^{a}	5.78 ±0.5^{a}	5.11 ±0.5^{a}	4.78 ±0.83^{a}	4.67 ±1.05^{a}	4.57 ±0.87^{a}	4.43 ±1.09^{a}	3.71 ±0.53^{a}
	A	7.56 ±0.88^{ab}	7.11 ±0.33^{a}	6.11 ±0.33^{ab}	4.89 ±0.93^{ab}	4.22 ±0.67^{b}	3.89 ±0.71^{ac}	3.89 ±0.5^{a}	-	-	-
	B	7.11 ±0.33^{a}	7.00 ±0.71^{a}	5.56 ±0.71^{b}	4.78 ±0.67^{b}	4.56 ±0.88^{ab}	4.44 ±0.73^{ac}	4.33 ±1.0^{a}	-	-	-
	C	8.11 ±0.78^{b}	7.44 ±0.53^{a}	7.11 ±0.53^{ac}	6.89 ±0.60^{ac}	6.78 ±1.09^{c}	6.67 ±0.71^{b}	6.44 ±0.73^{b}	6.33 ±0.87^{b}	6.22 ±0.67^{b}	6.00 ±0.71^{b}
	D	7.44 ±0.88^{ab}	7.00 ±0.71^{a}	6.33 ±0.71^{abc}	5.89 ±0.71^{ad}	5.78 ±1.22^{ac}	5.33 ±0.87^{ab}	5.11 ±1.01^{ab}	4.89 ±1.01^{a}	4.33 ±1.27^{a}	3.78 ±0.60^{a}

Different letters between samples in a column denote significant differences ($p<0.05$).

CONCLUSION

The study showed that the fermentation of kimchi with *L. fermentum* as a starter culture, combined with trisodium citrate supplementation, can contribute to pH stabilization and overall quality improvement. The pH of kimchi added trisodium citrate remained higher than that of the control sample throughout the storage periods. The sample with 0.75% trisodium citrate exhibited the most stable pH, maintaining around 4. It also effectively suppressed spoilage microorganisms and resulted in more desirable sensory characteristics. The findings suggest that using trisodium citrate is effective on enhancing the stability of kimchi fermented with *L. fermentum* as starter culture, improving its quality and potentially aiding storage in production.

Acknowledgments: This research is funded by Hanoi University of Science and Technology (HUST) under project number T2024-PC-068.

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