Fermentation parameters, antioxidant capacities, and volatile flavor compounds of tomato juice–skim milk mixtures fermented by *Lactobacillus plantarum* ST-III

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Abstract This study evaluated the influence of tomato juice enriched with the probiotic strain *Lactobacillus plantarum* ST-III on the flavor and health-promoting effects of fermented skim milk. Fermentation parameters, such as titratable acidity, viable cell counts, antioxidant activity, and volatile components, were examined. The viable bacterial cell counts of 40% tomato juice samples were significantly higher than those in the control group, peaking at 1.09×10^9 CFU/mL after 48 h, and the titratable acidity was increased by 2.76-fold versus the control value. The antioxidant ability of fermented milk was correlated with the tomato juice content in addition to fermentation time in the 2,2-diphenyl-1-picrylhydrazyl and ferric reducing/antioxidant power assays; for these methods, the scavenging activities of 40% samples were 1.18- and 1.28-fold higher than the control values, respectively, at 24 h. Moreover, abundant flavor components, especially aldehydes, were detected after the addition of *L. plantarum* ST-III-supplemented tomato juice.

Keywords *Lactobacillus plantarum* · Skim milk · Tomato juice · Fermentation · Antioxidant

Introduction

With the growing popularity of healthy diets, health, and functional foods have been increasingly produced by the global food manufacturing industry. Fermented foods such as cheese and yogurt contain various microbes (Evvie et al., 2017) and play an important role in digestive and immunomodulatory functions. Presently, probiotics possessing special hygienic functions are added to fermented food to enhance its value. Adequate amounts of probiotics exert a range of health-promoting activities, such as alleviation of lactose intolerance symptoms, adherence to human epithelial cell mucus, and improvement of microbial balance in the gastrointestinal tract (Argyri et al., 2013; Floros et al., 2012; Yoon et al., 2006).

Lactobacillus plantarum can survive under different environmental conditions because of its facultative heterofermentative characteristics. In addition, it is one of the few probiotics that can colonize the human gastrointestinal mucosa (Sabo et al., 2014). Pharmacologic studies suggested that *L. plantarum* can excrete plantaricin, which allows the bacterium to decrease the translocation rate of intestinal bacteria, compete with other harmful bacteria, and modulate immunologic and inflammatory factors (Song et al., 2014). Furthermore, fibrinogen and cholesterol concentrations in serum were significantly reduced via exposure to *L. plantarum* strains (Yadav et al., 2016).

The benefits of *L. plantarum* ST-III, which is isolated from kimchi, include cholesterol modulation and human gastrointestinal mucosa colonization (Chen et al., 2008; Chen et al., 2010). However, it does not multiply well in

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milk, and its acidification ability is too weak to clot milk (Wang et al., 2011). According to previous research (Ma et al., 2016), *L. plantarum* ST-III cannot grow well in milk without six key amino acids (Ile, Leu, Val, Tyr, Met, and Phe) and at least one purine. Conversely, most vegetables and fruits contain purines and free amino acids, making them good supplementary candidates for milk fermentation (Kuwaki et al., 2012). Different studies have explored vegetable and fruit juices such as pomegranate, apple, and cabbage fermented with probiotic bacteria including *Lactobacillus acidophilus*, *Lactobacillus delbrueckii*, *L. plantarum*, and *Lactobacillus casei* (Dimitrovski et al., 2015; Mousavi et al., 2011; Yoon et al., 2006). Pure fermented fruit or vegetable drinks cannot provide animal proteins that can be easily absorbed by the human body, and thus, the combination of fruit and milk double-fermented food could be more promising (de Vrese et al., 2011).

Tomato (*Lycopersicon esculentum*) is a popular and versatile fruit with high nutritive value. Because of its good taste and diverse uses, it has become one of the most popular crops (Ilahy et al., 2011). Tomato is rich in vitamins C and E, lycopene, and β -carotene, and it also contains certain phenolic compounds (Ilahy et al., 2011). Some researchers found that fruit–vegetable products fermented using *L. plantarum* can effectively control diseases induced by high fat-fructose diet consumption such as cancer and cardiovascular disease by attenuating dyslipidemia; altering levels of pro-inflammatory cytokines; and decreasing superoxide dismutase, catalase, and glutathione peroxidase activities (Huang et al., 2013; Noctor and Foyer, 1998).

There was study described pure tomato juice fermented using a mixture of probiotics (Sulhvir et al., 2016). However, no study discussed the parameters of *L. plantarum* in a tomato and milk mixture. In this study, tomato juice was added to milk to further investigate the fermentation effects of *L. plantarum* ST-III. The antioxidant activities and volatile compounds of fermented products were also examined.

Materials and methods

Preparation of tomato juice

Fresh tomatoes were purchased from a market, cleaned, and cut into pieces before extraction using a juicer (HR 1861, Philips China Investment Co., Ltd., Zhuhai, China), followed by filtration with gauze and centrifugation at $12,000 \times g$ for 10 min (5804R, Eppendorf, Hamburg, Germany). Then, the supernatant was pasteurized at 68 °C for 30 min and stored at 4 °C.

Preparation of *L. plantarum*

Lactobacillus plantarum ST-III was obtained from Bright Dairy & Food Co., Ltd. (Shanghai, China) and firstly cultured in MRS (Merck, Darmstadt, Germany) agar under anaerobic conditions (Bugbox Anaerobic System, Ruskinn, Bridgend, UK) with 95% N₂ and 5% CO₂ at 37 °C. The single colony was purified twice.

The purified *L. plantarum* ST-III was inoculated into MRS broth and cultivated to the logarithmic phase. The prepared cultures were centrifuged ($10,000 \times g$, 10 min), followed by washing twice with sterile saline solution (0.85%, w/w), and then using saline solution resuspended the cultures and adjusted the absorbance to OD₆₀₀ = 0.7 (about 1×10^8 CFU/mL).

Preparation of milk for fermentation with tomato juice

Skim milk powder (33.4% protein and 0.8% fat; Fonterra Ltd., Auckland, New Zealand) was reconstituted in distilled water. Different concentrations of skim milk samples were supplemented with 0%, 20%, or 40% (w/w) tomato juice to maintain a final skim milk concentration of 12% (w/w). The mixture was blended using an RW20 stirrer (IKA, Staufen, Germany) and then sterilized at 110 °C for 5 min, and the samples were inoculated with prepared bacteria at a final concentration of 1×10^7 CFU/mL (ten times dilution) after cooling to 37 °C.

Four replicates were created for each sample, and one set was used to monitor pH changes. The other samples were incubated at 37 °C for 24, 48, or 72 h followed by storage at 4 °C before analysis.

Fermentograph of *L. plantarum*

Sterile milk mixed with different amounts of tomato juice was prepared, and pH was monitored using the Cinac system (Alliance Instruments, Mery-Sur-Oise, France) during acidification for 72 h.

Enumeration of *L. plantarum*

The plate count method was used to enumerate the viable cells of *L. plantarum*. Samples were serially diluted using sterile saline to obtain 30–300 colonies on MRS agar plates. *Lactobacillus plantarum* was cultured anaerobically under 95% N₂ and 5% CO₂ at 37 °C for 48 h.

Determination of titratable acidity

The titratable acidities (TAs) of samples at different fermentation times were determined as described previously

(Yang et al., 2012) with slight modification. Briefly, 10 g samples were diluted with 20 mL of distilled water and titrated with 0.1 mM NaOH using phenolphthalein as an indicator. The amount of consumed NaOH was multiplied by 0.9 for conversion into % lactic acid.

Determination of antioxidant capacity

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity

The measurement method was based on a previously described protocol (Burda and Oleszek, 2001) with slight modification. Briefly, 0.1 mL of the test sample were added to 3.9 mL of 2,2 diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich, St. Louis, MO, USA) solution (0.004 g DPPH dissolved in 100 mL of 95% ethanol), and the mixture was incubated for 30 min at room temperature (26 °C) in the dark. The mixture was then centrifuged at $1500 \times g$ for 10 min, followed by measurements of absorbance at 517 nm against a blank control (ethanol) using a UV spectrophotometer (UV6x Series; Bluwave, Shanghai, China). The percent inhibition was calculated as follows:

$$\text{Percentage Inhibition (\%)} = \left[1 - \left(\frac{A_{\text{sample}}}{A_{\text{control}}} \right) \right] \times 100,$$

where A_{control} is the absorbance of 0.1 mL of the solvent. A standard curve was obtained by measuring the DPPH scavenging activities of 50, 100, 200, and 400 mg/L Trolox. All samples were required to reach 50% inhibition. Antioxidant capacity (AOC) was calculated relative to that of Trolox and expressed as Trolox equivalents- $\mu\text{mol TE}/100 \text{ g sample}$.

Ferric reducing/antioxidant power assay

Ferric reducing activity was detected according to Oyaizu (1986) with slight modification. Briefly, 1 mL of each sample was well mixed with 2.5 mL of phosphate-buffered saline (0.2 M, pH 6.6) and 1 mL of 1% (w/v) potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$) (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) solution. The mixture was incubated in a water bath at 50 °C for 20 min, followed by immediate cooling to room temperature. Then, 2 mL of 10% (w/v) trichloroacetic acid (Sinopharm Chemical Reagent Co., Ltd.) was added. After centrifugation at 3500 rpm for 10 min, 2.5 mL of supernatant were thoroughly blended with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (Sinopharm Chemical Reagent Co., Ltd.) for 2 min. The absorbance of Prussian blue solution was then determined at 700 nm against the blank solution. Higher absorbance values indicated stronger reducing activity. The standard curve was calculated as described for the DPPH assay.

Determination of volatile compounds

Fermented and unfermented milk containing 40% tomato juice and control samples were subjected to analyses of volatile compounds. The volatile components of the samples were extracted using headspace solid-phase micro-extraction and detected via gas chromatography–mass spectrometry. An Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) and Agilent 5975C mass selective detector were used for detection.

The samples were balanced for 35 min and extracted for 1 s at 50 °C. A DB-Wax column (30 m $\times$ 0.25 mm $\times$ 0.25 mm; Agilent Technologies) was used to separate the volatile compounds. The temperature of the column was held at 40 °C for 1 min, ramped up by 20 °C/min to 230 °C and held at this temperature for 5 min. The carrier gas was helium (1 mL/min) under pressure (2.4 kPa) and split (1:10) conditions. The mass spectrometer was run in the electron impact mode at 70 eV. The mass scan range was 35–500 m/z. Corresponding data were collected by comparing the retention times with those of the standards.

Statistical analysis

All experiments were organized in a completely randomized design. Each treatment was replicated three times. The data were presented as the mean or mean $\pm$ standard deviation and analyzed using Duncan's multiple range test ($p < 0.05$) and SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Results and discussion

Effect of tomato juice on the acidification of *L. plantarum* ST-III-fermented milk

The changes in pH during fermentation are shown in Fig. 1. The control group exhibited extremely weak acidification, and the pH values ranged from 6.51 to 6.06. With increasing tomato juice content, the pH values decreased rapidly to less than 5.00 within 24 h. The results suggested that tomato juice stimulated *L. plantarum* ST-III-mediated fermentation in skim milk.

The TA values of the samples during fermentation are illustrated in Fig. 2. The TA values in all tomato juice samples were significantly higher ($p < 0.05$) than those in the control. Additionally, the TA values increased with increasing fermentation time. Conversely, subtle changes were observed in the control group, and the values increased apparently when the juice content reached 40%. The maximum concentration had a TA value of 10.94%

Fig. 1 Changes of the pH of each sample with tomato juice (0%, 20%, 40%)

Fig. 2 The titratable acidity of samples during fermentation (24 h, 48 h, 72 h). Bars with different letters show different significant ($p < 0.05$) and denote standard error of the mean among samples with different contents of tomato juice

lactic acid at 72 h, which was 3.76-fold higher than the control value.

Lactobacillus plantarum was unable to grow well in reconstituted skim milk, in line with relevant report (Ma et al., 2016). The stimulatory effect on *L. plantarum* ST-III fermentation was possibly due to the components of the tomato juice, such as amino acids and trace amounts of nucleic acids. A study by Sorrequieta et al. (2010) reported that tomato had a certain amount of amino acids, and Johnson et al. (2013) found that tomatoes a certain amount of purines. Both amino acids and purines are essential for *L. plantarum* ST-III propagation (Ma et al., 2016). These results indicated that the trace amount of amino acids and purines in tomato juice can promote the growth of *L.*

plantarum ST-III in milk and increase lactic acid production.

Effect of tomato juice on the viability of *L. plantarum* ST-III cells

Overall, all tomato juice-containing samples had significantly ($p < 0.05$) higher cell counts than the control samples (Fig. 3). Cell counts also increased with increasing tomato juice content. The highest cell counts at 48 h were recorded in the 20% and 40% tomato juice groups (6.88×10^8 CFU/mL and 1.09×10^9 CFU/mL, respectively), but no differences were observed among the groups at 24 h. The cell counts declined after 48 h, especially in the 40% tomato juice group (4.48×10^8 CFU/mL) ($p < 0.05$), indicating that a short fermentation time was needed to ensure bacterial viability.

Several studies found that the decline of probiotic viability was attributable to the accumulation of substances such as organic acids, acetaldehyde, oxygen, and hydrogen peroxide as well as the storage condition (Sulhvir et al., 2016; Talwalkar and Kailasapathy, 2003). *Lactobacillus plantarum* is greatly resistant to low pH fruit juice (pH < 2.8–3.4), and it can be stored in a refrigerator for 1 month without losing viability (Molin, 2001). Therefore, the accumulation of lactic acid is not a main cause of decreased cell viability. The survival environment worsens during the latter part of fermentation, and the limitation of nutritive materials can lead to a lag or decline phase. Additionally, the accumulation of toxic oxygenic metabolites such as superoxide might cause the death of vulnerable cells, although *L. plantarum* possesses antioxidant ability (Das and Goyal, 2015).

Fig. 3 The viable cells of *Lactobacillus plantarum* ST-III in samples during fermentation (24 h, 48 h, 72 h). Bars with different letters show different significant ($p < 0.05$) and denote standard error of the mean among samples with different contents of tomato juice

Effect of tomato juice on the AOC of *L. plantarum* ST-III-fermented milk

The results for scavenging ability are shown in Fig. 4. DPPH scavenging activity and ferric reducing power both significantly increased with increasing tomato juice content ($p < 0.05$), and 40% tomato juice supplementation resulted in the greatest AOC at each fermentation time (including the unfermented group). All concentrations of tomato juice exhibited maximal inhibitory activity in the DPPH assay at 48 h. Compared with the findings in the control group, the scavenging activity improved from 44.12 ± 0.66 to 87.95 ± 2.10 $\mu\text{mol TE}/100\text{ g}$ (Fig. 4A). In the ferric reducing/antioxidant power assay, the reducing power was significantly ($p < 0.05$) increased by 1.29-fold versus the control finding at 24 h (Fig. 4B). Thereafter, the scavenging ability declined with time.

As stated previously, the viable counts of *L. plantarum* ST-III peaked at 48 h in the 20% and 40% tomato juice groups and decreased with increasing fermentation time. These findings were consistent with the DPPH assay results of previous findings (Das and Goyal, 2015) in which DPPH scavenging activity increased with increasing *L. plantarum* DM5 cell numbers. In addition to the antioxidant ability of *L. plantarum*, whey proteins in milk and their hydrolysates/peptides are natural antioxidants. Certain substances are produced during fermentation to participate in radical chain reactions and terminate the oxidation reaction. Some studies reported that proteins and peptides in cells were responsible for increasing the reducing activity during fermentation. The same results were found in soymilk and kefir fermented by *Saccharomyces* and lactic acid bacteria (Kesenkas et al., 2011; Wang et al., 2006).

Tomato is abundant in antioxidants, such as vitamin C, lycopene, and certain phenolic compounds. During

fermentation, phenolic compounds are mobilized to enhance the antioxidant ability (Chang et al., 2009). A similar study by Apostolidis et al. (2007) suggested that the polymerization of phenolic compounds results in increased antioxidant activity. Furthermore, tomato fragments had higher DPPH scavenging activity after fermentation using *Bacillus subtilis* A14h than unfermented samples (Moayedi et al., 2017), which might be attributable to the specific peptides generated during fermentation. The antioxidant activity of *L. plantarum* ST-III-fermented or unfermented tomato juice was further measured, as shown in Table 1. The AOC of tomato juice decreased with increased fermentation time in each method. This finding suggests that antioxidant substances in tomato play important roles in the amplification of *L. plantarum* ST-III in milk. Hervet-Hernandez et al. (2009) found that some antioxidants were substrates for microorganism growth. This also explained why antioxidant activity decreased during the latter phase of fermentation as indicated in the ferric reducing/antioxidant power assay.

Overall, the addition of tomato juice to milk influenced the AOC to a certain extent. The AOCs of tomato juice-fermented milk were much higher than those of pure tomato juice-fermented milk, possibly due to protein hydrolysis and antioxidant consumption during fermentation. The DPPH assay is more suitable for detecting lipophilic compounds. Triantis et al. (2007) suggested that the DPPH assay was not sensitive for amino acids, whereas it was suitable as a Cys or Cys peptides test. The DPPH assay is a radical scavenging capacity test, whereas the ferric reducing/antioxidant power assay focuses on total antioxidant ability. This also explains the differences in the values.

Fig. 4 Comparison of antioxidant capacity ($\mu\text{mol TE}/100\text{ g}$) of samples during fermentation (0 h, 24 h, 48 h, 72 h). Bars with different letters mean different significant ($p < 0.05$) and denote

standard error of the mean among samples with different contents of tomato juice. (A) DPPH assay. (B) Ferric reducing power assay

Table 1 Fermented tomato juice AOC abilities ($\mu\text{mol TE}/100\text{ g}$)

Method	0 h	24 h	48 h
DPPH	68.07 $\pm$ 0.23	47.62 $\pm$ 1.03	34.26 $\pm$ 0.18
Ferric reducing	224.01 $\pm$ 2.05	156.34 $\pm$ 1.49	98.36 $\pm$ 5.27

All values are mean of three replications $\pm$ SD

Volatile flavor compounds of milk fermented by *L. plantarum* ST-III

Because *L. plantarum* ST-III could not grow well in pure skim milk, we chose three groups for volatile organic compound detection. The volatile compounds detected in the control and tomato juice groups are presented in Table 2. The 20 detected compounds consisted of three alcohols, eight aldehydes, three carboxylic acids, three ketones, and three other ingredients.

In Table 2, pentanal, octanal, nonanal, acetic acid, heptanone, and nonanone were the main volatile compounds detected in both fermented (40% tomato juice) and control samples (40% tomato juice, not fermented). Additionally, alcohol and ketone levels were extremely lower than those in the unfermented samples ($p < 0.05$).

Conversely, the contents and species of aldehydes were significantly increased after fermentation ($p < 0.05$). *Lactobacillus plantarum* ST-III might utilize alcohols and ketones in tomatoes and milk, such as different organic acids and alcohols can generate different ester in the presence of different microbial enzymes. Acetaldehyde and benzaldehyde were found at the highest levels ($p < 0.05$) in pomegranate juice and goat milk fermented with *L. plantarum* (Di Cagno et al., 2017; Muelas et al., 2018), these findings are similar to our findings. Although high concentrations of aldehydes may cause off-flavors, the limitations are not sure and the interaction of fermented ingredients cannot be specifically detected. Major compounds are not always solely responsible for the main odor, pointing to clear interactions among volatile compounds to obtain unique sensations,

Tomato juice contains abundant volatile organic compounds including organic acids. After fermentation, acetic acid and decanoic acid levels were significantly higher than those in the corresponding control samples ($p < 0.05$). Moreover, octanoic acid was newly detected in the 40% tomato juice fermented group. Acids produced during fermentation are the major components causing milk coagulation and the resulting sour taste (Li et al., 2016). In

Table 2 Results of volatile compounds samples, showing the averaged peak areas (in arbitrary unit $\times 1000$)

Compound	Averaged peak area $\times 1000$		
	Sample 1	Sample 2	Sample 3
Hexanol	63.6 $\pm$ 1.8 ^b	162.7 $\pm$ 5.1 ^a	ND
Heptanol	20.1 $\pm$ 0.5	ND	ND
Octanol	103.6 $\pm$ 3.8 ^a	50.4 $\pm$ 2.6 ^b	ND
Pentanal	ND	2537.5 $\pm$ 45.3 ^b	6135.3 $\pm$ 43.5 ^a
Hexanal	205.9 $\pm$ 3.1 ^b	143.2 $\pm$ 6.5 ^c	336.4 $\pm$ 5.8 ^a
Heptanal	ND	365.3 $\pm$ 2.2 ^a	369.5 $\pm$ 1.1 ^a
Octanal	132.2 $\pm$ 5.6 ^c	998.8 $\pm$ 6.3 ^b	1220.9 $\pm$ 12.9 ^a
Nonanal	ND	816.3 $\pm$ 8.2 ^b	1187.3 $\pm$ 13.7 ^a
Decanal	ND	242.5 $\pm$ 6.5 ^b	366.9 $\pm$ 9.8 ^a
Vanillin	ND	ND	5252.1 $\pm$ 42.6
3,5-Di-tert-butyl-4-hydroxybenzaldehyde	ND	51.7 $\pm$ 0.8 ^b	76.2 $\pm$ 0.2 ^a
Acetic acid	ND	954.3 $\pm$ 13.5 ^b	6433.6 $\pm$ 66.1 ^a
Octanoic acid	ND	ND	6152.4 $\pm$ 481.3
Decanoic acid	ND	242.5 $\pm$ 16.5 ^b	1589.6 $\pm$ 69.7 ^a
Heptanone	3109.8 $\pm$ 48.5 ^a	2228.4 $\pm$ 14.4 ^b	1439.4 $\pm$ 30.7 ^c
Nonanone	3398.6 $\pm$ 54.8 ^a	1364.7 $\pm$ 25.4 ^b	462.3 $\pm$ 50.1 ^c
Undecanone	864.3 $\pm$ 18.9 ^a	475.9 $\pm$ 36.2 ^b	163.7 $\pm$ 3.4 ^c
Methyl butyrate	ND	162.7 $\pm$ 8.9 ^b	495.6 $\pm$ 20.1 ^a
Methyl stearate	ND	ND	138.8 $\pm$ 9.4
Limonene	ND	ND	2995.0 $\pm$ 41.6

All values are mean of three replications $\pm$ SD. Different superscript letters in the same row indicate significant differences at $p < 0.05$. ND, not detected; Sample 1, 0% tomato juice & not fermented; Sample 2, 40% tomato juice & not fermented; Sample 3, 40% tomato juice & fermented

addition to acids, samples fermented with tomato juice contained various volatile compounds such as vanillin, methyl stearate, and limonene. Vanillic and citric acids are important flavor compounds in tomato (Erdinc et al., 2018), and they both increased the AOC of the mixture and provided substrates to produce new flavor substances. Overall, tomato juice supplementation both changed the structure and increased the number of species of volatile compounds in milk.

In summary, tomato juice significantly stimulated the growth and acidification of *L. plantarum* ST-III in milk. Samples with the highest tomato juice concentration (40%, w/w) had the highest TA values and viable cell counts at 48 h. Additionally, the antioxidant abilities of fermented milk were increased. The findings illustrated that AOC increased with increasing tomato juice concentrations in the DPPH and ferric reducing/antioxidant power assays, and decreased with fermentation time for ferric reducing/antioxidant power assays. The DPPH activity increased significantly in the first 48 h of fermentation, but decreased thereafter. Moreover, tomato juice supplementation increased volatile organic compound levels in fermented milk. This work illustrated the potential combined application of vegetables, milk, and probiotics, especially *L. plantarum* ST-III, in an effort to meet the health and nutritional requirements of consumers. However, additional research is needed to clarify the substances in tomato that stimulate the growth of *L. plantarum* ST-III.

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