Food Control 137 (2022) 108973

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

NMR-based metabolomic investigation on antimicrobial mechanism of *Salmonella* on cucumber slices treated with organic acids

Chenxi Guo [a,b], Yun He [a,b], Yue Wang [a,b], Hongshun Yang [a,b,*]

[a] Department of Food Science and Technology, National University of Singapore, Science Drive 2, Singapore, 117542, Singapore
[b] National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou, Jiangsu, 215123, PR China

ARTICLE INFO

Keywords:
Sanitizer
Food metabolomics
Fresh-cut produce
Sanitization mechanism
Foodborne pathogen
Food safety

ABSTRACT

The potential of organic acids to inactivate foodborne pathogens in food industry has gained attention, while the sanitizing mechanism of acid stress has not been explained fully at metabolomics level. The aim of this study was to elucidate the antimicrobial mechanism of low-concentration organic acids (1.5% acetic acid, 1% citric acid, and 1.5% lactic acid) on *Salmonella enterica* strains (ATCC 6962, ATCC 13076, and ATCC 14028) inoculated on cucumber slices. Flow cytometry, nuclear magnetic resonance (NMR) combined with multivariate data analysis revealed that the population of *S. enterica* reduced 1.7–2.5 log CFU/g after different organic acid treatments. Bacterial membranes (2.37–19.00%) were destroyed and nucleic acid exposed, 40.27–85.33% of membranes was partially damaged, and 44 metabolites were identified from these three strains. The amino acids (Trp, Met, and Val) decreased significantly. Conversely, the intracellular organic acids and hydrocarbon grew obviously. The flux of marked metabolites showed that metabolisms of energy, amino acids, and carbohydrates pathways were disturbed which included TCA cycle, glycolysis, and biosynthesis of amino acids. The antibacterial mechanism on *S. enterica* provides clue to enhance the application of organic acids in controlling foodborne pathogens on fresh produce.

1. Introduction

Salmonella is one of the common foodborne pathogens in the world. As estimated by Centers for Disease Control and Prevention (CDC) that there are approximately 1.35 million infections and 420 deaths cases caused by salmonellosis in the United States per year. Meanwhile, the infections of *S. enterica* are not only spread through food of animal origin but also transmitted by vegetables, fruits, and other plant products (Jackson, Griffin, Cole, Walsh, & Chai, 2013). To avoid suffering from salmonellosis, many regulations of food safety have been published by government and food safety regulatory department (Hanning, Nutt, & Ricke, 2009). The typical serotypes of *Salmonella* such as Newport, Typhimurium, and Enteritidis are commonly found in vegetables. Cucumber has been confirmed as a vehicle contaminated by *Salmonella*. In 2014, a *Salmonella* outbreak involved 275 patients among 29 states in US which linked with cucumber (Angelo et al., 2015). During the whole procedure from farm to fork, postharvest cleaning and sanitizing of vegetables is the main intervention step to remove field pollution and pathogens. Adding antimicrobial agents to washing water reduces microbiology efficiently. Hypochlorite (sodium hypochlorite), chlorine-based sanitizers, peroxide (peracetic acid, hydrogen peroxide), and natural extractions (Yemmireddy, Cason, Moreira, & Adhikari, 2020) such as essential oils have been applied to inactive microorganisms (Beuchat, Pettigrew, Tremblay, Roselle, & Scouten, 2005; Xu & Wu, 2014; Fuzawa et al., 2016; da Costa Lima & de Souza, 2021).

Organic acids are not only commonly used as environmentally-friendly, low-cost sanitizers in food industry, but also serve as natural food additives to enhance the taste, flavor, and quality of food products (Shimizu, Takahata, & Kato, 1995). The United States Food and Drug Administration (FDA) considered that organic acids are generally recognized as safe (GRAS). Acetic acid, citric acid, and lactic acid have been applied to various food products such as eggplant dip, Greek salad, and tabbouleh salad (Tassou, Samaras, Arkoudelos, & Mallidis, 2009; Osaili, Al.Nabulsi, Nazzal, & Shaker, 2017; Al-Rousan et al., 2018). A previous study showed that acetic acid, citric acid and lactic acid could inactive foodborne pathogens efficiently (Amrutha, Sundar, & Shetty, 2017) in fresh produce. Besides, chlorogenic acid has been demonstrated to cause irreversible changes in cell membrane permeability (Lou, Wang, Zhu, Ma, & Wang, 2011), moreover the antibacterial mechanism of phenyllactic acid and lactic acid against *Bacillus cereus*

* Corresponding author. Department of Food Science and Technology National University of Singapore, Singapore, 117542, Singapore.
E-mail address: fstynghs@nus.edu.sg (H. Yang).

https://doi.org/10.1016/j.foodcont.2022.108973
Received 30 October 2021; Received in revised form 12 March 2022; Accepted 17 March 2022
Available online 20 March 2022

were elucidated at the proteomics level (Ning et al., 2021). However, the antibacterial mechanism of organic acids at metabolomics level has not yet been illustrated in detail.

Nuclear magnetic resonance (NMR) spectroscopy is one of main equipment to identify the metabolites of bacteria (Li et al., 2021) and determine the concentration of metabolites (Wang et al., 2012). Meanwhile, according to our previous studies, NMR is useful and accurate tool to be the basis of multiple data analysis at the level of bacterial metabolism, it explained well the metabonomic changes of *Listeria monocytogenes*, *Escherichia coli* and other pathogen under the treatment of low-concentration acidic electrolyzed water, heat, and nisin (Chen et al., 2022; Liu et al., 2020; Wang, Wu, & Yang, 2022; Wu, Zhao, Lai, & Yang, 2021; Zhao, Chen, Wu, He, & Yang, 2020). Thus, it is a powerful method to analyze the change of metabolites and illustrate the response of bacteria cells under acid stress.

Pervious research indicated that different organic acids (acetic acid, citric acid and lactic acid) with the same initial pH and molar concentration had different antibacterial effects on *Shigella* species (In, Kim, Kim, & Oh, 2013). The aim of this research was to distinguish the difference among three organic acids (acetic acid, citric acid and lactic acid) and unravel the antimicrobial mechanism of organic acids at metabolic level. The metabolic profile, main types of metabolites and the change of pathways were determined by observing bacterial cell membrane integrity and metabolites' concentration of *S. enterica* under different low-concentration organic acid treatments.

2. Materials and methods

2.1. Materials and treatment solutions

The concentrations of different organic acids were prepared based on the previous methods with slight modifications (Chen et al., 2019; Mani-Lopez, Garcia, & Lopez-Malo, 2012; Park et al., 2011; Zhao, Zhao, Phey, & Yang, 2019). The pH value of three organic acids should be in a certain range. Acetic acid (AA, 1.5 mL, $\geq$ 99%, Sigma-Aldrich, St Louis, MO, USA) was diluted in 98.5 mL sterilized deionized water (pH 2.32 $\pm$ 0.14), 1 g citric acid (CA, $\geq$ 99.5%, Sigma-Aldrich, St Louis, MO, USA) was dissolved in 100 mL of sterilized deionized water (pH 2.80 $\pm$ 0.09), DL-Lactic acid solution (pH 2.24 $\pm$ 0.14) was prepared by diluting 1.5 mL of DL-Lactic acid (LA, Sigma-Aldrich, St Louis, MO, USA) into 98.5 mL sterilized deionized water. All these organic acid solutions should be freshly prepared before use to avoid loss of the free hydrogen ions.

2.2. Bacteria strains and culture condition

S. Typhimurium (ATCC 14028), *S.* Enteritidis (ATCC 13076), *S.* Newport (ATCC 6962) were collected from Department of Food Science & Technology, National University of Singapore, all strains (100 μL) were activated by transferring into a test tube containing 10 mL sterile Luria-Bertani broth (Sigma-Aldrich, St Louis, MO, USA) at 37 °C for 24 h, nalidixic acid was used to cultivate drug-resistance of *S. enterica*, which was based on a previous method (Lepaus & Rocha, 2020). The bacterial cell pellets were obtained by centrifugation at 8000×*g*, 10 min at room temperature, and the cell pellets were washed by 0.1% (w/v) peptone water twice and then re-suspended in 50 mL 0.1% (w/v) peptone water, divided into 5 × 10 mL in 15 mL sterile centrifuge tubes for the following experiments.

2.3. Inoculation on cucumber pieces

The fresh cucumbers were purchased from Fair Price (Singapore) and washed with flow tap water to clean the surface, then cut into slices with a surface area of about 1 cm^2 and a thickness of 1 mm. Cucumber slices (10 $\pm$ 0.3 g) were weighed in petri dishes. In order to sterile the surface of slices to eliminate interference from other microorganisms, all samples were exposed to UV light for 15 min and then turned over via a sterile tweezer for another 15 min (Hadjok, Mittal, & Warriner, 2008). The resuspended culture from section 2.2 was inoculated on 10 g cucumber slices. The strains inoculated for about 24 h and the culture on the slices increased to about 8.0 log CFU/g on cucumber slices (Liu et al., 2018; Zhao et al., 2020).

2.4. Sanitizing treatments of different organic acids

The inoculated cucumber slices were transferred into 50 mL tubes by using sterile tweezers. Ten milliliters DI water (control), 1.5% (v/v) AA, 1% (w/v) CA, and 1.5% (v/v) LA solution was added into four tubes and vortexed 5 min to make sure all the slices surface immersed into the solutions. After that, 20 mL neutralizing buffer (Becton, Dickinson and Comp, Sparks, MD, USA) was added into the tubes to stop sanitization, and then centrifuged to remove the solution (8000×*g*, 10 min, 20 °C). The treated cucumber slices mixed with 10 mL 0.1% peptone water and the mixture was homogenized in the stomacher bags (IUL Instruments, Germany) for 2 min.

2.5. Enumeration of surviving cells

Xylose lysine deoxycholate (XLD, Sigma-Aldrich, St Louis, MO, USA) is mainly used to isolate *Salmonella* (Nye et al., 2002). XLD agar with 5% sodium chloride (XLD-SC) was served as selective medium to enumerate the injury bacteria cells (Lan, Zhang, Zhang, & Shi, 2019). The nalidixic acid (100 μg/L) was added into XLD and XLD-SC. Peptone water (0.1%, w/v) was used for serial dilution (Peng, Bitsko, & Biswas, 2015) and 100 μL diluent was plated on the XLD agar and XLD-SC agar. The culture was incubated for 48 h at 37 °C and the bacteria cell population was indicated by log CFU/g.

2.6. Staining procedure and flow cytometry analysis

SYTO9 (a green-fluorescent nucleic acid dye) and propidium iodide (PI, a red-fluorescent dye) were used to evaluate bacterial membranes after treatments. The stained method was based on previous study (Berney, Hammes, Bosshard, & Weilenman, 2007) and instructions of the LIVE/DEAD™ BacLight™ Bacterial Viability and Counting Kit for flow cytometer (Thermo Scientific, Waltham, MA, USA) with slight modifications. The final concentration of SYTO9 and PI dyes was 5 μmol/L. The 0.22 μm-filtered cells were stained in dark for 30 min and stored on the ice, all the samples were tested by FCM in 30 min. The images of FCM analysis were performed by BD LSRFortessa™ Flow Cytometer. The excitation/emission of SYTO9 and PI was 488/500 nm and 490/635 nm, respectively. The ratio of Dead/Live cells without treatments was served as the control group.

2.7. Extraction of Salmonella metabolites

The metabolites of *S. enterica* were extracted (Zhao et al., 2020; Wu et al., 2021; Wang et al., 2022) and divided into four groups: Control (I), AA (II), CA (III) and LA (Ⅳ). To remove and cell debris, all the cells obtained from section 2.4 were centrifuged at low speed (1500×*g*, 1 min, 4 °C) to get the supernatant. The obtained cell supernatant was then centrifuged (12,000×*g*, 20 min, 4 °C) to get the cell pellets. And the collected cell pellets was mixed well with 750 μL ice methanol-d4 and immediately frozen using liquid nitrogen and freeze-thawed twice to destroy the cell membrane. The metabolites in ice methanol-d4 were stored overnight at −20 °C, the supernatant after centrifugation (12, 000×*g*, 20 min, 4 °C) was used for NMR analysis (Chen et al., 2020). The final concentration of 1 mmol/L 3-trimethylsilyl [2,2,3,3-d4] propionate (TSP, Sigma-Aldrich, St Louis, MO, USA) was added into the supernatant to serve as internal chemical standard. Metabolite's extraction of different groups was prepared in triplicate.

Table 1
Effects of different organic acids treatments on reduction of three *S. enterica* strains.

Treatment		Reduction (log CFU/g)		
		ATCC 6962	ATCC 13076	ATCC 14028
Acetic acid	Dead	2.3 ± 0.08^{a}	2.3 ± 0.76^{b}	1.9 ± 0.66^{a}
	Injury	2.4 ± 0.32^{b}	2.5 ± 0.10^{a}	2.1 ± 0.40^{a}
Citric acid	Dead	2.0 ± 0.10^{b}	2.5 ± 0.66^{a}	1.7 ± 0.77^{b}
	Injury	2.2 ± 0.31^{a}	2.7 ± 0.55^{a}	1.9 ± 0.35^{a}
Lactic acid	Dead	2.1 ± 0.11^{b}	2.2 ± 1.00^{b}	2.0 ± 0.65^{a}
	Injury	2.2 ± 0.03^{b}	2.4 ± 0.18^{a}	2.2 ± 0.09^{a}

Note: Within the same row, means with different letters are significantly different ($P < 0.05$).

2.8. NMR spectroscopic analysis and spectral analysis

The NMR samples were tested at 298 K with a frequency of 500.23 MHz via a Triple Inverse Gradient probe (Bruker DRX-500 NMR spectrometer) (Chen et al., 2020). The data of ^{1}H spectrum was acquired by 3.3 s with the standard Bruker NOESY pulse sequence (noesypr1d) and collected after 100 ms mixing. Furthermore, the data of spectrum was recorded by using 128 transients and 2 s relaxation delay. Moreover 1 Hz line broadening factor was used to process the spectra of all samples before the Fourier transform. For identification and quantification of metabolites, 2D ^{1}H–^{13}C heteronuclear single quantum coherence spectroscopy (HSQC) of *S. enterica* sample was acquired by using the Bruker hsqcedetgpsisp2.3 pulse sequence at 298 K. The ^{1}H obtained by F2 channel with a 10-ppm spectral width, and ^{13}C was recorded on the F1 channel with a spectral width of 175 ppm.

The baseline and phase of one-dimensional ^{1}H spectrum was manually adjusted by Topspin 3.6.0 (Bruker) and corrected the highest peak of TSP to zero position. The metabolites were identified by the 2D ^{1}H–^{13}C spectrum and 1D ^{1}H referring to the Biological Magnetic Resonance Data Bank (http://www.bmrb.wisc.edu/metabolomics/) (Shaykhutdinov, MacInnis, Dowlatabadi, Weljie, & Vogel, 2009). Mestrenova (Mestreab Research SL, Santiago de Compostela, Spain) was used to process the adjusted ^{1}H spectral and manually cut off resonance of known solvent (methanol-d4) from 3.29 to 3.35 ppm. 0.02 ppm was set as a unit and the range area of TSP was normalized from −0.01 ppm to 0.01 ppm. Moreover, the obtained spectrum (−0.01–10.0 ppm) was normalized to the total intensity and binned into a basket with an integration width of a standard unit (0.02 ppm) by using the software Mestrenova (Zhao et al., 2020). For quantitative analysis, the concentration variation of metabolites were determined by comparing the integrals of each metabolite to that of TSP, as an internal reference at a known concentration of 1 mmol/L (Jégou, Kervarec, Cérantola, Bihannic, & Stiger-Pouvreau, 2015).

The binned data was deeply probed by principal component analysis (PCA) to display the dissimilarity among different treatments and the responsible compounds. Furthermore, the fold changes (FCs) and *P* value were calculated by the binned data, the value of FCs compared with control group (FCs ≥1.50 or FCs ≤0.67) was considered as statistically significant (Dalman, Deeter, Nimishakavi, & Duan, 2012). The pathway analysis was conducted by MetaboAnalyst 5.0 (http://www.metaboanalyst.ca/) with the screened metabolites. And Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/pathway.html) cooperatively.

2.9. Statistical analysis

Each experiment was performed in triplicate independently. Analysis of variance (ANOVA) was performed by using Duncan's multiple range test in SPSS Statistics (IBM Corp., Armonk, NY, USA) to compare the effects of different treatments on the three *S. enterica* strains inoculated on cucumber slices. Difference with $P < 0.05$ was considered significant. The volcano plots and heatmap were drawn by TBtools software.

3. Results and discussion

3.1. Reduction and injured bacteria cells after treatments

The antimicrobial effects of three *S. enterica* strains under organic acid pressure were investigated by enumerating colonies on XLD and XLD-SC. Acetic acid, citric acid and lactic acid effectively reduced the population of *S. enterica* on the cucumber slices. The amount of *S. enterica* increased to 8.0 log CFU/g after overnight inoculation. The reduction of three treatments were approximately 1.7–2.5 log CFU/g on three *S. enterica* strains (Table 1). Although three organic acids showed great antibacterial effects, it indicated that *S.* Enteritidis (ATCC 13076) was the mostly vulnerable to acid stress after comparing with other strains. On the contrary, the reduction of the *S.* Typhimurium (ATCC 14028) was the least. As shown in Fig. 1(A-C), there was significant differences ($P < 0.05$) among three organic acids on XLD and XLD-SC agar. These results were similar with the previous study (Park et al., 2011), demonstrating that the populations of pathogens on apple surface were reduced by 2.8, 3.4 and 2.9 log CFU/g after 10 min organic acid (2% acetic acid, 2% lactic acid and 2% citric acid) treatments.

For *S.* Typhimurium (ATCC 14028), *S.* Enteritidis (ATCC 13076) and *S.* Newport (ATCC 6962), there was no significant difference ($P < 0.05$) after treatments of acetic acid and lactic acid. Whereas citric acid treatment led to maximum alive population of ATCC 6962 and ATCC 14028, meanwhile, it exhibited the best bactericidal effect for ATCC 13076 compared to other two organic acid treatments. However, some of cells stayed in injury situation when exposed to the unfavorable external environment, which still held the ability of infection by salmonellosis (Amano, 1999). Both the dead and injury cells could not grow on XLD-SC agar, Fig. 1 revealed that the sublethal effect of AA, CA and LA had no significant difference ($P < 0.05$) between ATCC 13076

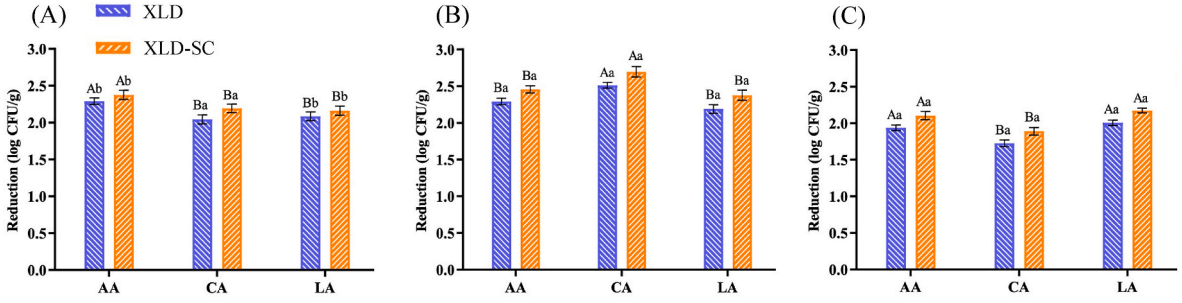

Fig. 1. The antibacterial effect of Acetic acid (AA), Citric acid (CA) and Lactic acid (LA) to *Salmonella* Newport ATCC 6962 (A), *S.* Enteritidis ATCC 13076 (B), *S.* Typhimurium ATCC 14028 (C) Data are displayed as means ± standard deviation. Different lowercase letters represent statistical differences between the population of *Salmonella* on XLD and XLD-SC with the same treatment. Different capital letters represent statistical differences between different treatment methods with the same media.

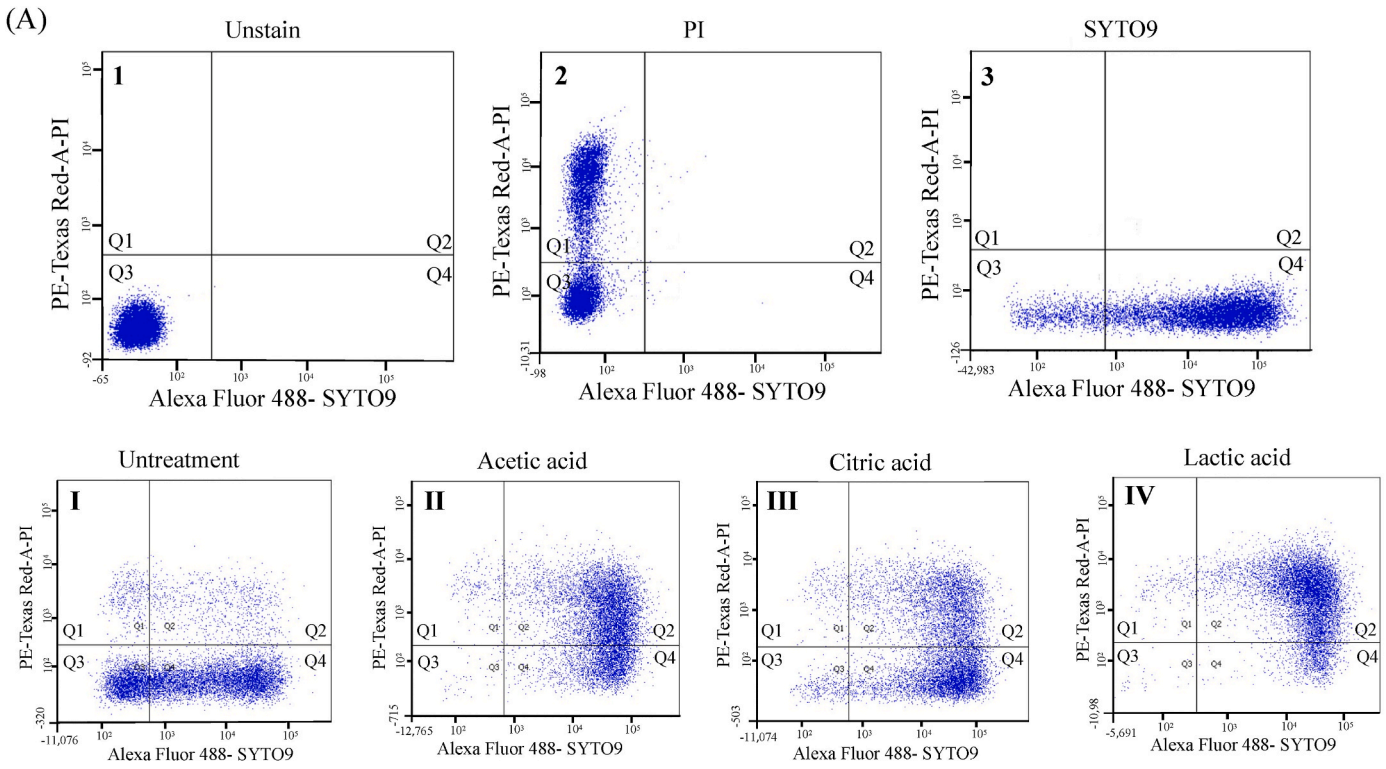

Fig. 2. The flow cytometry images after treatments represented by *Salmonella* Newport ATCC 6962. Note: I: Control; II: Acetic acid; III: Citric acid; IV: Lactic acid; 1-3 decide the baseline; Points in Q1: totally damaged; Q2: partly damaged.

Table 2
Effects of different organic acids on damaging of three *S. enterica* strains on cell membrane.

Strains	Treatment	Totally damaged	Partly damaged	Membrane damaged
ATCC 6962	Control	1.93 ± 0.25^{a}	11.23 ± 2.56^{a}	13.07 ± 2.65^{a}
	Acetic acid	2.47 ± 0.31^{a}	65.60 ± 1.85^{b}	68.07 ± 1.55^{b}
	Citric acid	2.40 ± 0.26^{a}	40.27 ± 0.40^{c}	42.07 ± 0.15^{b}
	Lactic acid	2.37 ± 0.49^{a}	85.33 ± 0.83^{a}	87.70 ± 0.56^{a}
ATCC 13076	Control	8.00 ± 0.53^{b}	17.83 ± 0.96^{b}	25.83 ± 1.36^{b}
	Acetic acid	15.53 ± 0.61^{a}	60.80 ± 3.77^{b}	71.83 ± 2.43^{b}
	Citric acid	15.43 ± 0.64^{a}	56.40 ± 0.46^{b}	76.00 ± 1.56^{c}
	Lactic acid	12.90 ± 1.40^{a}	74.17 ± 1.51^{a}	87.07 ± 0.92^{a}
ATCC 14028	Control	4.67 ± 0.61^{b}	11.47 ± 2.55^{a}	16.13 ± 3.15^{a}
	Acetic acid	12.93 ± 5.14^{a}	69.63 ± 1.05^{ab}	82.57 ± 5.95^{ab}
	Citric acid	16.33 ± 1.67^{a}	66.77 ± 1.10^{b}	85.77 ± 5.01^{b}
	Lactic acid	19.00 ± 2.45^{a}	76.03 ± 2.43^{a}	92.37 ± 1.35^{a}

Note: Within the same row, means with different letters are significantly different ($P < 0.05$).

and ATCC 14028.

3.2. Result of the damaged bacterial cell membrane

Flow cytometry (FCM) is a useful tool to detect the integrity of cell membrane and analyze different metabolic activities of bacterial cells (Soejima, Iida, Qin, Taniai, & Yoshida, 2009). As shown in Fig. 2 and Fig. S1, Bacteria with intact cell showed green color, and the images presented red fluorescence indicated the cell membranes were totally damaged after treatment. Moreover, bacteria with partly damaged membranes exhibited less green fluorescence and fluoresced red. PI alone, SYTO9 alone and unstained cell plots were used to determine the baseline. The proportions of the damaged *S. enterica* membranes after treatments were calculated by the flow cytometry density plots of three *S. enterica* strains cells.

Because of bacterial cell apoptosis, the damage of cell membrane occurred to a certain extent in the control groups, according to Table 2, there was 15.00–25.00% cell membranes has been damaged before treatments, it was because that the bacteria cells faced plenty of pressure in real environment (Adhikari et al., 2016). After different organic acids treatments, it showed that 2.37–19.00% of cell membranes was destroyed and bacterial nucleic acid exposed, besides, 40.27–85.33% of membranes was partially damaged. As for the three strains, lactic acid had the strongest ability to damage the cell membrane and made the nucleic acid expose. Moreover, ATCC 6962 cells showed the strongest resistance to the organic acids compared with other two strains, which was consistent with the results obtained in the above antimicrobial part. Overall, three organic acids damaged the bacteria membrane validly, assessment of the cell membranes' integrity laid the foundation for organic acid molecules to disrupt the normal biochemical activities in bacteria cells by breaking the cell barrier and entering the cell.

3.3. Metabolic changes and comparison of three Salmonella strains

The identification of metabolites was based on NMR data and previous studies (He, Zhao, Chen, Zhao, & Yang, 2021; Lou, Zhai, & Yang, 2021), a total of 44 identified metabolites were showed in Table S1 including amino acids, organic acids, nucleotides, alcohols, sugars and other derivatives. Fig. S3 and Fig. S4 show the distribution of signals from three *Salmonella* strains of representative ^{1}H NMR spectrums.

To deeply illustrate the differences among three strains after treatments, PCA was used to investigate the discrepancies of metabolites. The model quality parameter R^2 X = 0.980 and Q^2 = 0.873 (Fig. 3A) indicated that the model had good interpretation and prediction capabilities (Chen et al., 2020). Three *S. enterica* strains of the control group were

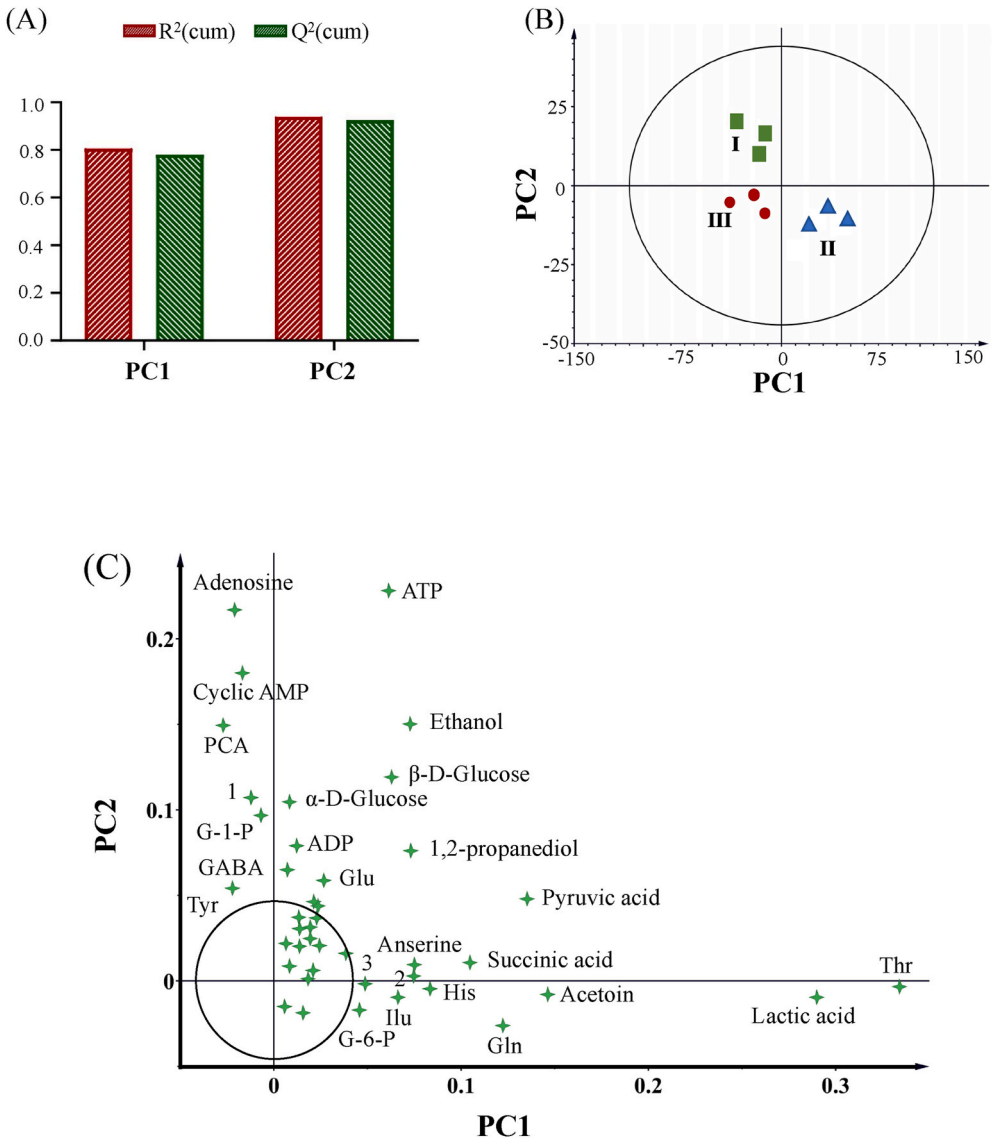

Fig. 3. Principal component analysis (PCA) of ^{1}H NMR spectra of three *Salmonella enterica* strains in the control groups. The variances are explained by principal components in PCA (A); the score plot of PCA (B); the loading plot of PCA (C) Note: I: ATCC 6962; II: ATCC 13076; III: ATCC 14028; 1: Acetic acid; 2: Uridine; 3: Phe.

clustered in three parts and recognizable separations by score plot (Fig. 3B). ATCC 13076 stood in the right side of score plot, ATCC 6962 and ATCC 14028 appeared in the opposite side. The discriminative metabolites to separate the variables were indicated in the loading plots (Fig. 3C), the metabolites from three strains had high similar trend in loading plot. Histidine, lactic acid, acetoin, threonine, cyclic AMP, and isoleucine had a larger loading on PC1, while ATP, ethanol, β-D- glucose, glutamate, G-1-P, adenosine, and pyruvic acid made more contribution to PC2.

3.4. Alternative metabolites during treatments of organic acids

First three components (PC1, PC2 and PC3) of three *S. enterica* strains by constructing PCA were used to illustrate all of metabolite's discrimination under acetic acid, citric acid and lactic acid treatments. The model quality and clustering information are shown in Fig. 4. (A1-C1). For ATCC 6962, 98.0% (PC1:77.2%, PC2: 16.8%, PC3: 4.0%) of all variances was explained by the first three components. For ATCC 13076, the first three components explained for 95.2% with PC1 (73.7%), PC2 and PC3 explained 18.5% and 3.0%. For ATCC 14028, 54.0%, 27.2% and 7.7% were explained by PC1, PC2 and PC3. And the Q^2 values of the three strains were 0.828, 0.873 and 0.906. All the PCA parameters of different strains were proper models with good fitness and predictability. Based on score plots of metabolome-separation, it was obviously observed that after each of the three different treatments, all groups were separated distinctly from the control group (Fig. 4. A1-C1). The loading plots of three strains (Fig. 4. A2-C2) after treatments showed presentively that cyclic AMP, threonine, adenosine, and pyruvic acid occupied most part in PC1, besides, ATP, fructose, ethanol and glucose devoted more parts on PC2. All the metabolites marked on the loading plots could be the representative biomarkers to elucidate the response of different organic acids treatments.

In order to reveal the changes of metabolites under different acid stress, 27 kinds of total metabolites were quantified by drafting heatmap (Fig. 5). The concentration of metabolites was transferred into $\log_{10}$ Changes to reflect the changed metabolites and presented by the shades of deep blue and red in the heatmap, representatively. Amino acids in cells represented by phenylalanine and cysteine increased significantly, similar results were also observed in previous study on *L. monocytogenes* strains (Wu et al., 2021), and the organic acids in bacterial cells such as lactic acid and acetic acid also grew.

All in all, three *S. enterica* strains had high similarity on changes of

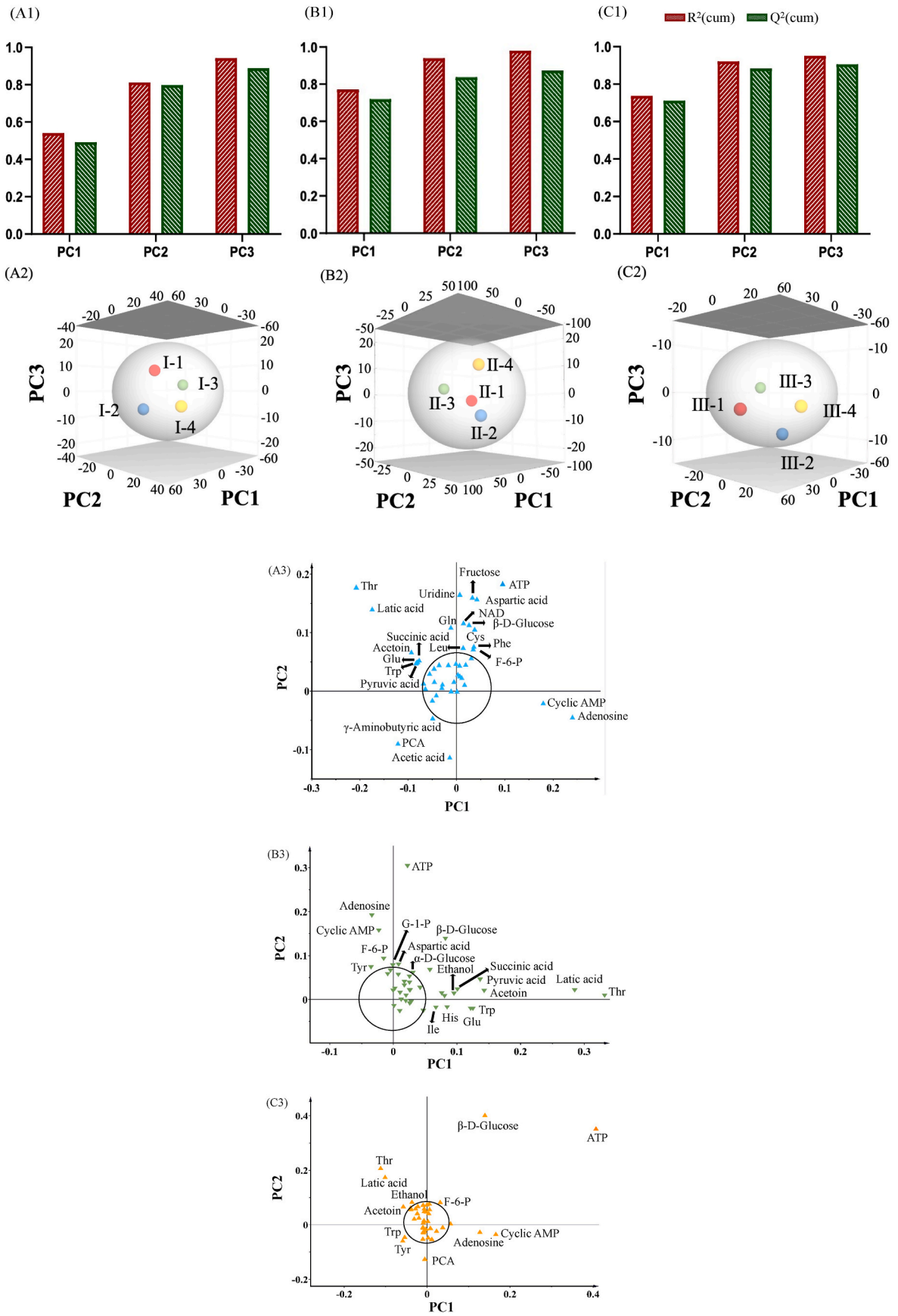

Fig. 4. Principal component analysis (PCA) of ^{1}H NMR spectra of three *Salmonella. enterica* strains under different treatments. The variances are explained by principal components in PCA (A1-C1); the score plot of PCA (A2-C2); the loading plot of PCA (A3-C3). Note: I: ATCC 6962; II: ATCC 13076; III: ATCC 14028; 1: Control; 2: AA treatment; 3: CA treatment; 4: LA treatment; A3: ATCC 6962; B3: ATCC 13076; C3: ATCC 14028.

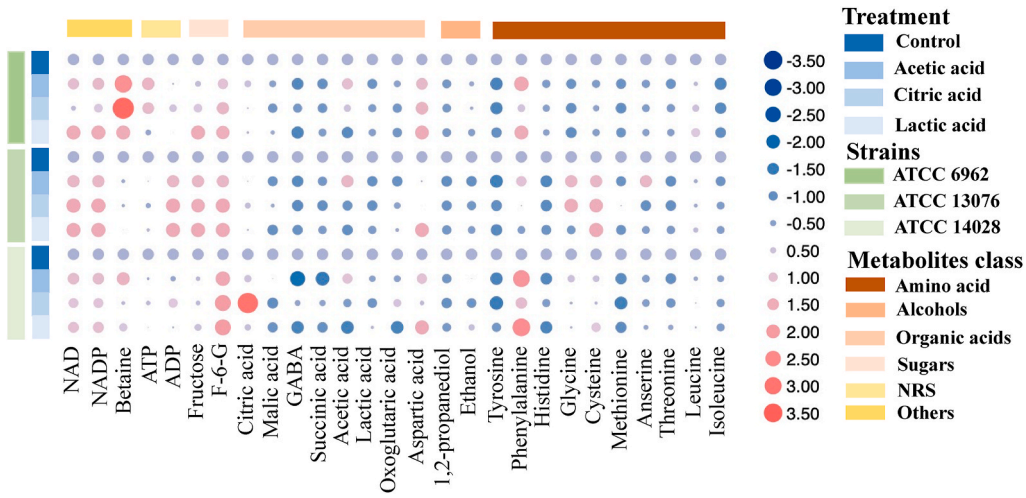

Fig. 5. Heatmap of metabolites in *S. enterica* under Control, Acetic acid (AA), Citric acid (CA) and Lactic acid (LA) Note: NRC: nucleotide-related compounds.

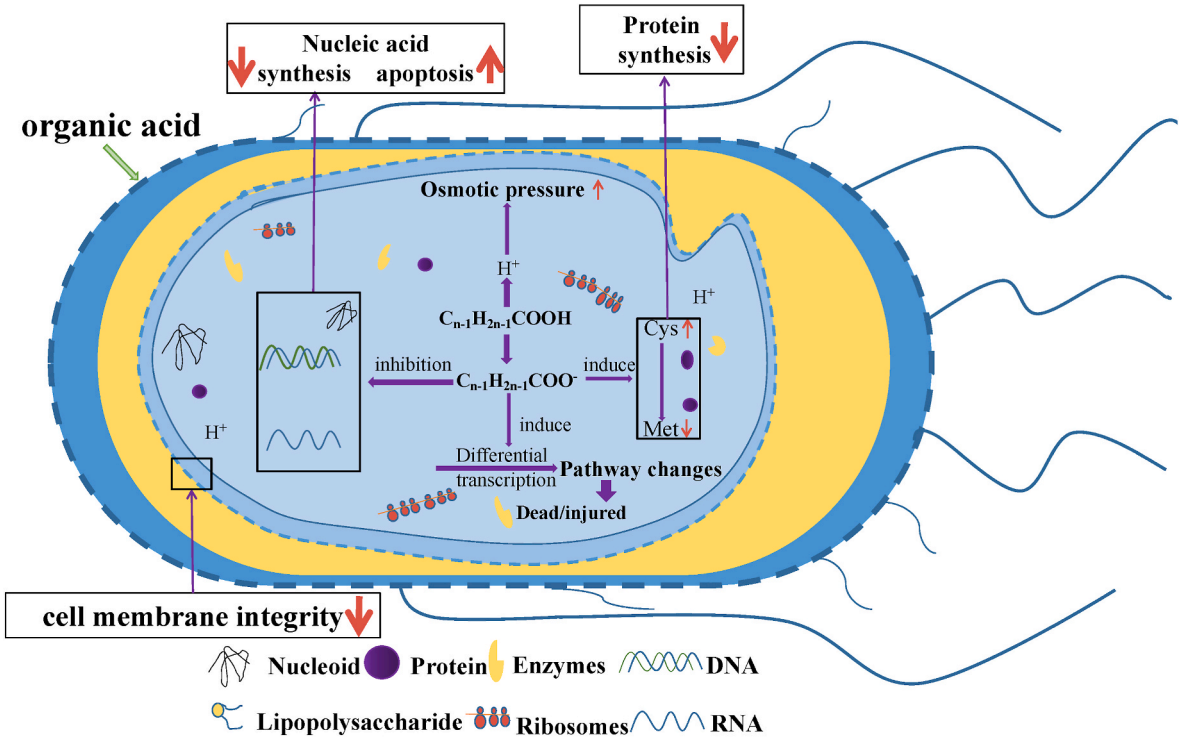

Fig. 6. Proposed mechanisms of bactericidal effects of organic acid treatments. Note: Arrow up: the growth of metabolites' concentration; the increase of pressure; the acceleration of apoptosis. Arrow down: the decrease of metabolites' concentration; the deceleration of synthesis; the damage of integrity.

metabolites under acetic acid, citric acid and lactic acid treatments. Compared with the control group, the great majority of amino acids (Tyr, Gly, Trp, Phe and Gln) and organic acids such as aspartic acid, γ-aminobutyric acid and succinic acid decreased obviously. As shown in Fig. 5, the acid-stress treatments were associated with higher levels of glucose, fructose, cyclic AMP, NAD, NADP, ATP and few amino acids (Lushchak, 2011; Rangel-Vargas et al., 2018).

3.5. Pathway analysis

Organic acid molecules broke the barrier and caused the fluctuating concentration of metabolites. The affected metabolic pathways were investigated based on the changes of main screened metabolites, which provided a deeper understanding of metabolic network activities. Volcano plots were constructed by *P*-Value, fold changes (Tables S2, S3, and S4) and correlation coefficients. The metabolites (*P* Value < 0.05, FCs >1.50) were determined with significance of statistics (Zhao et al., 2020).

According to Table (S2–S4) and Fig. 7 (A1-C1), it showed that 22 (ATCC 6962), 22 (ATCC 13076) and 23 (ATCC 14028) metabolites located on the volcano plots changed after acetic acid treatment, representatively. The orange points of marked metabolites occupied on the right side of the volcano plots indicated that the growth of contents, reversely the green points showed the decrease of the metabolites. The marked metabolites (Fig. 7. A1-C1; Fig. S2. A1-F1) had statistical significance under acid pressure. By categorizing the flux of metabolites, the reduction of Trp, Ala, Gly, Met and Tyr have been identified among three *S. enterica* strains.

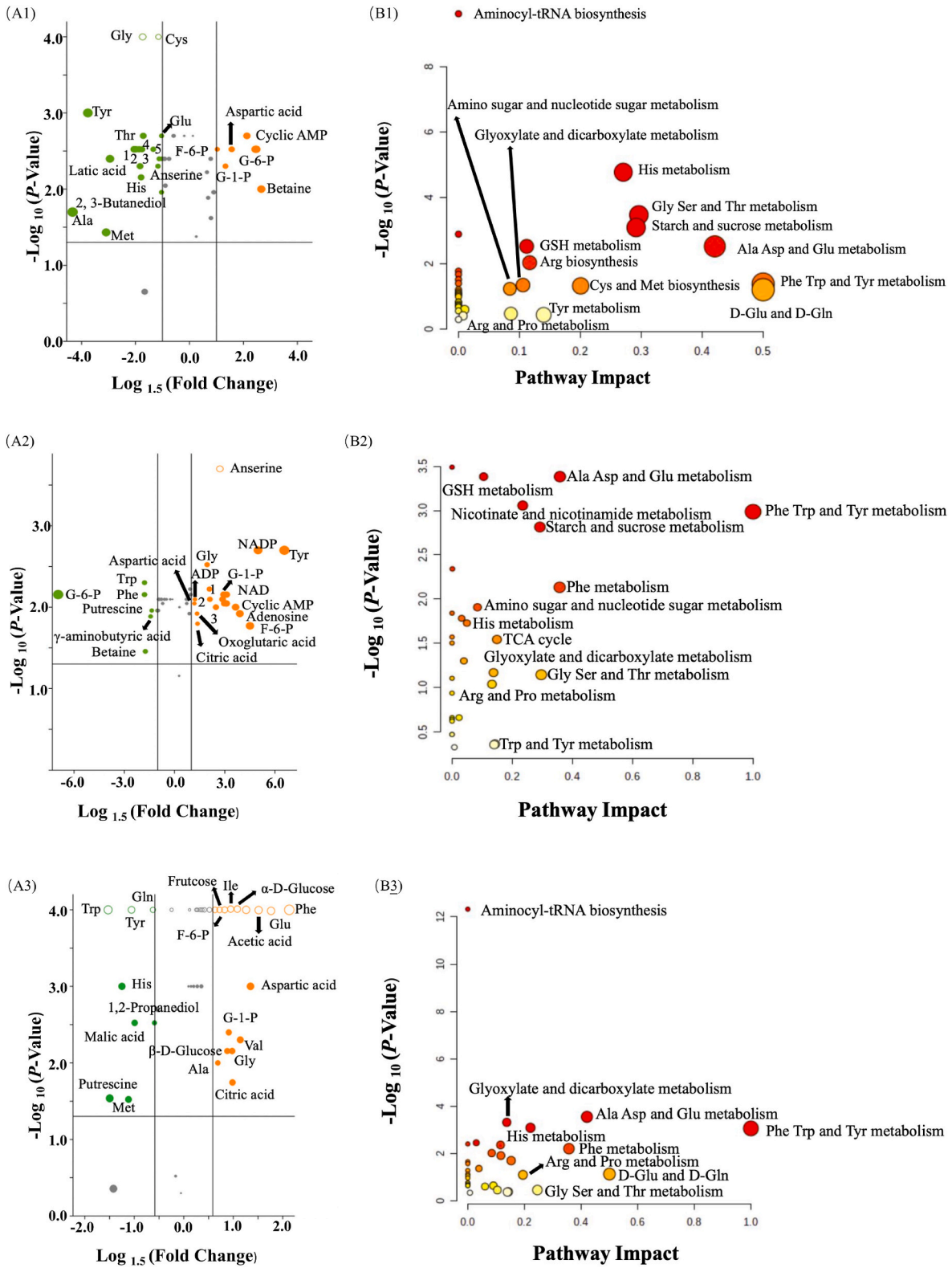

Fig. 7. Volcano plots of acetic acid groups (A1-A3); pathway analysis for acetic acid groups (B1–B3). Note: the volcano plots show the marked metabolites (*P* value < 0.05, FCs >1.50), pathways analysis show the affected pathways after treatment; green color in the volcano plot shows decreased amount after treatment; orange color in the volcano plot shows increased amount after treatment. A1: 1. Pyruvic acid; 2. Glu; 3. Acetoin; 4. Succinic acid; 5. 1,2-Propanediol A2: 1. β-D-Glucose; 2. α-D-Glucose; 3. Pyroglutamic acid.

Table 3
List of metabolic pathway analysis for screened metabolites in three *S. enterica* strains under the same treatment.

Treatment	Metabolite set	Raw *P* (ATCC 6962)	Raw *P* (ATCC 13076)	Raw *P* (ATCC 14028)
Acetic acid	**Aminoacyl-tRNA biosynthesis**	0.000000	0.000000	0.000003
	Starch and sucrose metabolism	0.000809	0.013886	0.000549
	Glutathione metabolism	0.003033	0.032306	0.002079
	Alanine, aspartate and glutamate metabolism	0.003033	0.000000	0.028584
	Glycolysis/ Gluconeogenesis	0.021822	0.028112	0.024852
	Phenylalanine, tyrosine and tryptophan biosynthesis	0.043196	0.040694	0.038188
Citric acid	**Aminoacyl-tRNA biosynthesis**	0.000003	0.000472	0.001802
	Starch and sucrose metabolism	0.000549	0.000350	0.000963
	Glycolysis/ Gluconeogenesis	0.024852	0.018845	0.035115
	Phenylalanine, tyrosine and tryptophan biosynthesis	0.038188	0.000386	0.000754
	Amino sugar and nucleotide sugar metabolism	0.004679	0.036660	0.007986
Lactic acid	**Aminoacyl-tRNA biosynthesis**	0.000000	0.003864	0.000000
	Phenylalanine, tyrosine and tryptophan biosynthesis	0.000842	0.000273	0.000450
	Phenylalanine metabolism	0.006045	0.001999	0.003273

However, the slight differences appeared among the three groups. As for acetic acid, the metabolites of three strains changed roughly in the same trend, while the concentration of Cys in ATCC 13076 increased obviously which was three times of that of other two strains. Besides, a significant elevated level was observed in betaine concentration in ATCC 6962 with a FC of 2.93. On the contrary, the concentration of Tyr and Gly reduced evidently. Under the interference of lactic acid, although the content of carbohydrate mounted, the accumulation of fructose, glucose and F-6-P in ATCC 14028 were less than that of other two strains. Furthermore, the concentration of Phe and ethanol fluctuated slightly in ATCC 13076 under citric acid treatment by comparing the results of the three groups. After acetic acid treatment, the concentration of anserine and oxoglutaric acid increased significantly.

Furthermore, 14, 17 and 14 pathways (Fig. 7. A2-C2) were influenced after acetic acid treatment. Citric acid affected 16, 10 and 13 pathways (Fig. S2. A2-C2). Lactic acid was proved effectively with changing 8, 10 and 11 pathways (Fig. S2. D2-G2) for ATCC 6962, ATCC 13076, and ATCC 14028, respectively. It suggested that about 9 pathways related to amino acids biosynthesis were affected such as histidine metabolism; cystine and methionine biosynthesis and phenylalanine metabolism and tyrosine metabolism. Furthermore, the carbohydrate and energy metabolisms in the cells have been disturbed in starch and sucrose metabolism under different treatments. Most of the interfered pathways related with energy supply, amino acid biosynthesis and gene expression were identified.

Tables 3 and 4 reveal the disrupted pathways by strains and treatments, respectively. It was found that aminoacyl-tRNA biosynthesis and glycolysis/gluconeogenesis changed obviously in all groups. While the trifling discriminations existed among three treatments. For acetic acid, TCA cycle in ATCC 13076, Pantothenate and CoA biosynthesis of ATCC 6962 and amino sugar and nucleotide sugar metabolism in ATCC 14028 were impacted markedly. Besides, purine metabolism and arginine biosynthesis was altered by citric acid particularly. The effect of lactic acid for valine, leucine and isoleucine degradation, alanine, aspartate and glutamate metabolism were noticeable. In addition, the amino acid synthesis pathways of ATCC 6962 were unstable, which more easily affected by organic acids.

Analysis of pathways provided the basis for bacterial metabolism of organic acids. According to the changed pathways, the proposed mechanism is shown in Fig. 6. Organic acid molecules damaged the cell membrane and accelerated entry into the cell. Intracellular osmotic pressure increased obviously with the accumulation of ions, which led to the destruction of the intracellular microenvironment and disruption of metabolic pathways. Besides, the transcription of DNA decelerated, and differential transcription appeared. The synthesis of macromolecules and nucleic acid were hindered, meanwhile, the apoptosis of nucleic acid molecules accelerated.

3.6. Discussion on the bactericidal mechanism of organic acids

It was inferred that the three organic acids had no significant differences in affecting the metabolism of three *S. enterica* strains based on the pathway analysis. A schematic diagram of biochemical pathways was proposed in Fig. 8. Organic acid molecules diffused freely into the cell. The extracellular ionized hydrogen ions entered through active transport, the accumulation of hydrogen ions led to the change of neutral and slight alkaline microenvironment. Intracellular hydrogen ions were released through active transport to maintain the neutral environment and ensure enzymes activity, which consumed plenty of ATP and competed with the bacterial growth. Meanwhile, organic acids inhibited the normal growth of bacteria by reducing bacterial

Table 4
List of metabolic pathway analysis for screened metabolites in three *S. enterica* strains after treatment.

Strain	Metabolite set	Raw *P* (Acetic acid)	Raw *P* (Citric acid)	Raw *P* (Lactic acid)
ATCC 6962	**Aminoacyl-tRNA biosynthesis**	0.000000	0.000323	0.000000
	Histidine metabolism	0.000017	0.018719	0.000792
	Starch and sucrose metabolism	0.000809	0.001536	0.019384
	beta-Alanine metabolism	0.001292	0.031451	0.026030
	Alanine, aspartate and glutamate metabolism	0.003033	0.000414	0.000275
	Arginine biosynthesis	0.009539	0.014428	0.011871
	Glycolysis/Gluconeogenesis	0.031535	0.004564	0.003400
	Phenylalanine, tyrosine and tryptophan biosynthesis	0.043196	0.001033	0.000842
ATCC 13076	**Aminoacyl-tRNA biosynthesis**	0.000000	0.000472	0.003864
	Phenylalanine, tyrosine and tryptophan biosynthesis	0.040694	0.000386	0.000273
ATCC 14028	**Aminoacyl-tRNA biosynthesis**	0.000003	0.001802	0.000000
	Glycolysis/Gluconeogenesis	0.024852	0.035115	0.021761
	Phenylalanine, tyrosine and tryptophan biosynthesis	0.038188	0.035115	0.021761

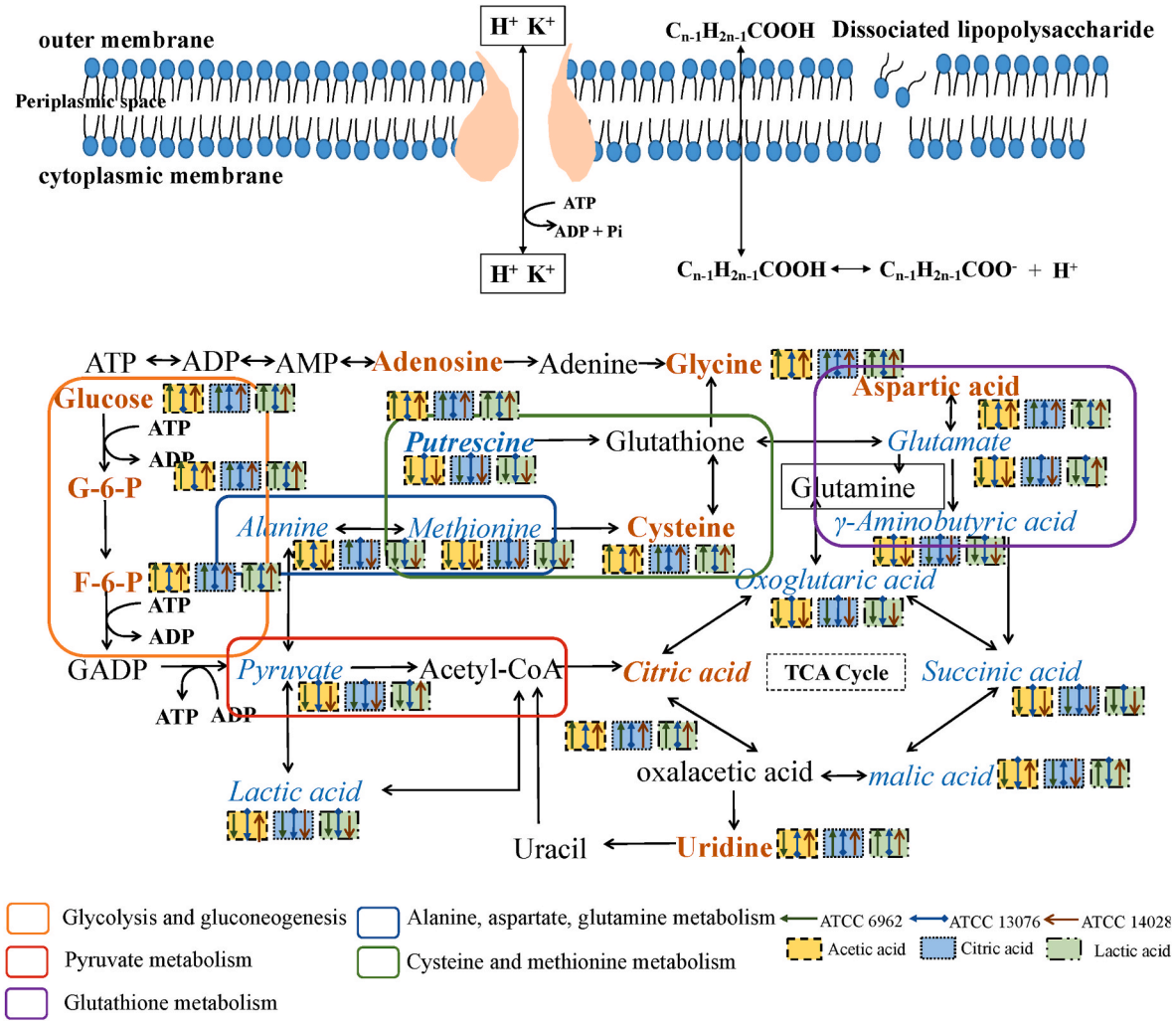

Fig. 8. Metabolic pathway of *Salmonella. enterica* altered by stress of organic acid, Notes: Italic letters show the decrease of metabolites, bold letters show increase of metabolites. Arrow up: the growth of metabolite concentration; arrow down: the decrease of metabolite concentration. Different types of arrow styles represent different *Salmonella. enterica* strains, and different dashed lines represent different organic acid treatments.

production of energy efficiency (Ricke, 2003). Moreover, organic acid molecules damaged the integrity of cell membranes which was observed by flow cytometry, previous study elucidated the organic acid molecules could damage the membrane by entering the periplasmic space (Alakomi et al., 2000). The incomplete cell membranes further combined with the organic acids played the synergistic effect on the bactericidal effect (Chaveerach, Keuzenkamp, Urlings, Lipman, & Knapenvan, 2002). Besides, the damage of the outer membrane caused sublethal damage to bacterial cells and achieved antibacterial effects (Brul & Coote, 1999).

Furthermore, the intracellular defense mechanism was activated because the acid radical ions made an obvious difference in the osmotic pressure. Amino acids served as the main sources of osmoregulation solutes, leading to the high concentrations of Tyr, Ile and Leu detected in the metabolites (Wei, Liao, Wang, Yang, & Kong, 2015; Ahn, Jung, Jang, Madsen, & Park, 2016; Ezraty, Gennaris, Barras, & Collet, 2017). As for the synthesizing of macromolecule, the acid pressure interfered or blocked DNA synthesis in the cell nucleus via inhibiting the activity of ribonucleic acid reductase to affect the bacterial division and proliferation. Besides, the low concentration of acetoin indirectly showed the metabolism and normal life of *S. enterica* was altered (Romick & Fleming, 1998).

The disruption of energy metabolism was deeply investigated. In glycolysis, initial phosphorylation increased the reactivity of the molecule and accelerated the transformation from G-6-P to F-1-P/F-6-P, the energy was consumed. It elucidated that EMP has been obstructed based on the reduction of pyruvate and the growth of G-6-P and NAD, which was consistent with the previous research results (Doi, 2019). The elevated level of β-D-Glucose and α-D-Glucose and reduction of lactic acid and acetic acid (De Pablo, Nilsson, Pekna, & Pekny, 2013) revealed the weakness of phosphoketolase pathway. In addition, the accumulation of pyruvate led to the growth of citric acid, which was linked by Acetyl-CoA. Moreover, the transformation from arginine to putrescine was strengthened by glutamate, indicating the start of the γ-aminobutyric acid shunt pathway, previous studies (Richard & Foster, 2004; Wu et al., 2017) has proved that the succinic acid gained from putrescine could keep the normal metabolism even the bacteria was injured. Meanwhile, succinic acid derived from γ-aminobutyric acid could compensate for the suppression of the TCA cycle. The decrease in malic acid content further demonstrated that organic acids interfered the metabolic activities of oxidation and energy release in *S. enterica* from citric acid to succinic acid inter the TCA cycle.

Overall, organic acid molecules damaged the integrity of cell membrane. The previous studies showed that the organic acid molecules depolymerized lipopolysaccharides of the bacteria outer membranes (Brul & Coote, 1999; Alakomi et al., 2000). Furthermore, organic acid exacerbated intracellular energy competition, and the transmission of genetic information was inhibited. Besides, the undulation of

metabolites demonstrated the inconsistency of decomposition and synthesis after organic acids treatments. In addition, after organic acid treatments, even if bacteria cells had the integrity of membranes, the ability of self-repair weakened, but they were also potentially pathogenic (Fleischmann, Robben, Alter, Rossmanith, & Mester, 2021).

4. Conclusion

This study illustrated that the antibacterial effect and mechanism of different low-concentration organic acids (acetic acid, citric acid and lactic acid) on three *S. enterica* strains on cucumber slices. Three organic acids had the similar effect on inhibiting *S. enterica* by analyzing the affected pathways and fluctuation of metabolites. Organic acid molecules damaged the integrity of cell membrane. Furthermore, a total of 44 metabolites were identified in *S. enterica* through the qualitative and quantitative analysis of spectrums. Amino acids increased significantly and the lower concentration of organic acids such as aspartic acid, γ-aminobutyric acid and succinic acid were detected after treatments. The fluctuation of the marked metabolites and changes of pathways revealed that the energy metabolism (TCA cycle, glycolysis), protein synthesis and DNA expression and transcription of *S. enterica* were influenced. This study analyzed the antibacterial mechanism of different low-concentration organic acids on *S. enterica* from the perspective of bactericidal effect and metabolic mechanism, which provides theoretical support for the research of using low-concentration organic acids in fresh produce to control foodborne pathogens.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Chenxi Guo: Conceptualization, Methodology, Investigation, Software, Recourses, Visualization, Writing – original draft. **Yun He:** Formal analysis, Investigation, Software. **Yue Wang:** Formal analysis, Writing – review & editing. **Hongshun Yang:** Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing.

Acknowledgments

This study was funded by Applied Basic Research Project (Agricultural) Suzhou Science and Technology Planning Programme (SNG2020061), Singapore Ministry of Education Academic Research Fund Tier 1 (R-160-000-A40-114) and an industry grant from Shanghai Cenwang Food Co., Ltd. (R-160-000-011-597).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2022.108973.

References

Adhikari, A., Bary, A., Cogger, C., James, C., Ünlü, G., & Killinger, K. (2016). Thermal and starvation stress response of *Escherichia coli* O157:H7 isolates selected from agricultural environments. *Journal of Food Protection, 79*, 1673–1679.

Ahn, S., Jung, J., Jang, I. A., Madsen, E. L., & Park, W. (2016). Role of glyoxylate shunt in oxidative stress response. *Journal of Biological Chemistry, 291*, 11928–11938.

Al-Rousan, W. M., Olaimat, A. N., Osaili, T. M., Al-Nabulsi, A. A., Ajo, R. Y., & Holley, R. A. (2018). Use of acetic and citric acids to inhibit *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Staphylococcus aureus* in tabbouleh salad. *Food Microbiology, 73*, 61–66.

Alakomi, H. L., Skyttä, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K., & Helander, I. M. (2000). Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Applied and Environmental Microbiology, 66*, 2001–2005.

Amano, F. (1999). Pathogenicity of *Salmonella* in the resting state: Research toward VBNC (viable but non-culturable) state of *Salmonella* in the environment. *Microbes and Environments, 14*, 107–121.

Amrutha, B., Sundar, K., & Shetty, P. H. (2017). Effect of organic acids on biofilm formation and quorum signaling of pathogens from fresh fruits and vegetables. *Microbial Pathogenesis, 111*, 156–162.

Angelo, K. M., Chu, A., Anand, M., Nguyen, T. A., Bottichio, L., Wise, M., et al. (2015). Outbreak of *Salmonella* Newport infections linked to cucumbers — United States, 2014. *Morbidity and Mortality Weekly Report, 64*, 144–147.

Berney, M., Hammes, F., Bosshard, F., & Weilenman. (2007). Assessment and interpretation of bacterial viability by using the LIVE/DEAD BacLight Kit in combination with Flow Cytometry. *Applied and Environmental Microbiology, 73*, 3283–3290.

Beuchat, L. R., Pettigrew, C. A., Tremblay, M. E., Roselle, B. J., & Scouten, A. J. (2005). Lethality of chlorine, chlorine dioxide, and a commercial fruit and vegetable sanitizer to vegetative cells and spores of *Bacillus cereus* and spores of *Bacillus thuringiensis*. *Journal of Industrial Microbiology and Biotechnology, 32*, 301–308.

Brul, S., & Coote, P. (1999). Preservative agents in foods: Mode of action and microbial resistance mechanisms. *International Journal of Food Microbiology, 50*, 1–17.

Chaveerach, P., Keuzenkamp, D. A., Urlings, H. A. P., Lipman, L. J. A., & Knapen, F.van (2002). In vitro study on the effect of organic acids on *Campylobacter jejuni/coli* populations in mixtures of water and feed. *Poultry Science, 81*, 621–628.

Chen, L., Liu, Q., Zhao, X., Zhang, H., Pang, X., & Yang, H. (2022). Inactivation efficacies of lactic acid and mild heat treatments against *Escherichia coli* strains in organic broccoli sprouts. *Food Control, 133*, Article 108577.

Chen, L., Zhang, H., Liu, Q., Pang, X., Zhao, X., & Yang, H. (2019). Sanitizing efficacy of lactic acid combined with low-concentration sodium hypochlorite on *Listeria innocua* in organic broccoli sprouts. *International Journal of Food Microbiology, 295*, 41–48.

Chen, L., Zhao, X., Wu, J., Liu, Q., Pang, X., & Yang, H. (2020). Metabolic characterization of eight *Escherichia coli* strains including "Big Six" and acidic responses of selected strains revealed by NMR spectroscopy. *Food Microbiology, 88*, Article 103399.

da Costa Lima, M., & de Souza, E. L. (2021). A systematic quantitative analysis of the published literature on the efficacy of essential oils as sanitizers in fresh leafy vegetables. *Critical Reviews in Food Science and Nutrition, 61*, 2326–2339.

Dalman, M. R., Deeter, A., Nimishakavi, G., & Duan, Z. H. (2012). Fold change and p-value cutoffs significantly alter microarray interpretations. *BMC Bioinformatics, 13* (Suppl 2). S11–S11.

De Pablo, Y., Nilsson, M., Pekna, M., & Pekny, M. (2013). Intermediate filaments are important for astrocyte response to oxidative stress induced by oxygen–glucose deprivation and reperfusion. *Histochemistry and Cell Biology, 140*, 81–91.

Doi, Y. (2019). Glycerol metabolism and its regulation in lactic acid bacteria. *Applied Microbiology and Biotechnology, 103*, 5079–5093.

Ezraty, B., Gennaris, A., Barras, F., & Collet, J. F. (2017). Oxidative stress, protein damage and repair in bacteria. *Nature Reviews Microbiology, 15*, 385–396.

Fleischmann, S., Robben, C., Alter, T., Rossmanith, P., & Mester, P. (2021). How to evaluate non-growing cells-current strategies for determining antimicrobial resistance of VBNC bacteria. *Antibiotics Basel, 10*, 115.

Fuzawa, M., Ku, K., Palma-Salgado, S. P., Nagasaka, K., Feng, H., Juvik, J. A., et al. (2016). Effect of leaf surface chemical properties on efficacy of sanitizer for rotavirus inactivation. *Applied and Environmental Microbiology, 82*, 6214–6222.

Hadjok, C., Mittal, G. S., & Warriner, K. (2008). Inactivation of human pathogens and spoilage bacteria on the surface and internalized within fresh produce by using a combination of ultraviolet light and hydrogen peroxide. *Journal of Applied Microbiology, 104*, 1014–1024.

Hanning, I. B., Nutt, J. D., & Ricke, S. C. (2009). Salmonellosis outbreaks in the United States due to fresh produce: Sources and potential intervention measures. *Foodborne Pathogens and Disease, 6*, 635–648.

He, Y., Zhao, X., Chen, L., Zhao, L., & Yang, H. (2021). Effect of electrolysed water generated by sodium chloride combined with sodium bicarbonate solution against *Listeria innocua* in broth and on shrimp. *Food Control, 127*, Article 108134.

In, Y., Kim, J., Kim, H., & Oh, S. (2013). Antimicrobial activities of acetic acid, citric acid and lactic acid against *Shigella* Species. *Journal of Food Safety, 33*, 79–85.

Jackson, B. R., Griffin, P. M., Cole, D., Walsh, K. A., & Chai, S. J. (2013). Outbreak-associated *Salmonella enterica* serotypes and food commodities, United States, 1998-2008. *Emerging Infectious Diseases, 19*, 1239–1244.

Jégou, C., Kervarec, N., Cérantola, S., Bihannic, I., & Stiger-Pouvreau, V. (2015). NMR use to quantify phlorotannins: The case of cystoseira tamariscifolia, a phloroglucinol-producing brown macroalga in Brittany (France). *Talanta, 135*, 1–6.

Lan, L., Zhang, R., Zhang, X., & Shi, H. (2019). Sublethal injury and recovery of *Listeria monocytogenes* and *Escherichia coli* O157:H7 after exposure to slightly acidic electrolyzed water. *Food Control, 106*, Article 106746.

Lepaus, B. M., & Rocha, J. S. (2020). Organic acids and hydrogen peroxide can replace chlorinated compounds as sanitizers on strawberries, cucumbers and rocket leaves. *Ciência e Tecnologia de Alimentos, 40*, 242–249.

Li, S., Tian, Y., Jiang, P., Lin, Y., Liu, X., & Yang, H. (2021). Recent advances in the application of metabolomics for food safety control and food quality analyses. *Critical Reviews in Food Science and Nutrition, 61*, 1448–1469.

Liu, Q., Chen, L., Laserna, A. K. C., He, Y., Feng, X., & Yang, H. (2020). Synergistic action of electrolyzed water and mild heat for enhanced microbial inactivation of *Escherichia coli* O157:H7 revealed by metabolomics analysis. *Food Control, 110*, Article 107026.

Lou, Z., Wang, H., Zhu, S., Ma, C., & Wang, Z. (2011). Antibacterial activity and mechanism of action of chlorogenic acid. *Journal of Food Science, 76*, M398–M403.

Lou, X., Zhai, D., & Yang, H. (2021). Changes of metabolite profiles of fish models inoculated with *Shewanella baltica* during spoilage. *Food Control, 123*, Article 107697.

Lushchak, V. I. (2011). Adaptive response to oxidative stress: Bacteria, fungi, plants and animals Comparative biochemistry and physiology. *Toxicology & pharmacology, 153*, 175–190.

Mani-Lopez, E., Garcia, H. S., & Lopez-Malo, A. (2012). Organic acids as antimicrobials to control *Salmonella* in meat and poultry products. *Food Research International, 45*, 713–721.

Ning, Y., Fu, Y., Hou, L., Ma, M., Wang, Z., Li, X., et al. (2021). iTRAQ-based quantitative proteomic analysis of synergistic antibacterial mechanism of phenyllactic acid and lactic acid against *Bacillus cereus*. *Food Research International, 139*, 109562–109562.

Nye, K. J., Fallon, D., Frodsham, D., Gee, B., Graham, C., Howe, S., et al. (2002). An evaluation of the performance of XLD, DCA, MLCB, and ABC agars as direct plating media for the isolation of *Salmonella enterica* from faeces. *Journal of Clinical Pathology, 55*(4), 286–288.

Osaili, T. M., AlNabulsi, A. A., Nazzal, D. S., & Shaker, R. R. (2017). Effect of storage temperatures and stresses on the survival of *Salmonella spp.* in halva. *Letters in Applied Microbiology, 65*, 403–409.

Park, S., Choi, M., Park, J., Park, K., Chung, M., Ryu, S., et al. (2011). Use of organic acids to inactivate *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on organic fresh apples and lettuce. *Journal of Food Science, 76*, M293–M298.

Peng, M., Bitsko, E., & Biswas, D. (2015). Functional properties of peanut fractions on the growth of probiotics and foodborne bacterial pathogens. *Journal of Food Science, 80*, M635–M641.

Rangel-Vargas, E., Luna-Rojo, A. M., Cadena-Ramírez, A., Torres-Vitela, R., Gómez-Aldapa, C. A., Villarruel-López, A., et al. (2018). Behaviour of 11 foodborne bacteria on whole and cut mangoes var ataulfo and kent and antibacterial activities of hibiscus sabdariffa extracts and chemical sanitizers directly onto mangoes contaminated with foodborne bacteria. *Journal of Food Protection, 81*, 743–753.

Richard, H., & Foster, J. W. (2004). Escherichia coli glutamate and arginine dependent acid resistance systems increase internal pH and reverse transmembrane potential. *Journal of Bacteriology, 186*, 6032–6041.

Ricke, S. C. (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science, 82*, 632–639.

Romick, T. L., & Fleming, H. P. (1998). Acetoin production as an indicator of growth and metabolic inhibition of *Listeria monocytogenes*. *Journal of Applied Microbiology, 84*, 18–24.

Shaykhutdinov, R. A., MacInnis, G. D., Dowlatabadi, R., Weljie, A. M., & Vogel, H. J. (2009). Quantitative analysis of metabolite concentrations in human urine samples using 13C1H NMR spectroscopy. *Metabolomics, 5*, 307–317.

Shimizu, T., Takahata, T., & Kato, M. (1995). Antibacterial activity of several organic acids commonly used as food additives. *Shokuhin Eiseigaku Zasshi, 36*, 50-54_1.

Soejima, T., Iida, K., Qin, T., Taniai, H., & Yoshida, S. (2009). Discrimination of live, anti-tuberculosis agent-injured, and dead *Mycobacterium tuberculosis* using flow cytometry. *FEMS Microbiology Letters, 294*, 74–81.

Tassou, C. C., Samaras, F. J., Arkoudelos, J. S., & Mallidis, C. G. (2009). Survival of acid-adapted or non-adapted *Salmonella* Enteritidis, *Listeria monocytogenes* and *Escherichia coli* O157:H7, in traditional Greek salads. *International Journal of Food Science, 44*, 279–287.

Wang, B., Wang, H., Wang, E., Yan, W., Ye, W., & Li, P. (2012). Isolation and structure characterization of related impurities in Sodium Tanshinone IIA Sulfonate by LC/ESI-MSn and NMR. *Journal of Pharmaceutical and Biomedical Analysis, 67*(68), 36–41.

Wang, Y., Wu, J., & Yang, H. (2022). Comparison of the metabolic responses of eight *Escherichia coli* strains including the "big six" in pea sprouts to low concentration electrolyzed water by NMR spectroscopy. *Food Control, 131*, Article 108458.

Wei, D., Liao, S., Wang, J., Yang, M., & Kong, L. (2015). Cholestatic liver injury model of bile duct ligation and the protection of Huang-Lian-Jie-Du decoction by NMR metabolomic profiling. *RSC Advances, 5*, 66200–66211.

Wu, M., Li, X., Guo, S., Lemma, W. D., Zhang, W., Ma, J., et al. (2017). Enhanced succinic acid production under acidic conditions by introduction of glutamate decarboxylase system in *E. coli* AFP111. *Bioprocess and Biosystems Engineering, 40*, 549–557.

Wu, J., Zhao, L., Lai, S., & Yang, H. (2021). NMR-based metabolomic investigation of antimicrobial mechanism of electrolyzed water combined with moderate heat treatment against *Listeria monocytogenes* on salmon. *Food Control, 125*, Article 107974.

Xu, W., & Wu, C. (2014). Different Efficiency of ozonated water washing to inactivate *Salmonella enterica* Typhimurium on green onions, grape tomatoes, and green leaf lettuces. *Journal of Food Science, 79*, M378–M383.

Yemmireddy, V. K., Cason, C., Moreira, J., & Adhikari, A. (2020). Effect of pecan variety and the method of extraction on the antimicrobial activity of pecan shell extracts against different foodborne pathogens and their efficacy on food matrices. *Food Control, 112*, Article 107098.

Zhao, X., Chen, L., Wu, J., He, Y., & Yang, H. (2020). Elucidating antimicrobial mechanism of nisin and grape seed extract against *Listeria monocytogenes* in broth and on shrimp through NMR-based metabolomics approach. *International Journal of Food Microbiology, 319*, 108494.

Zhao, L., Zhao, M. Y., Phey, C. P., & Yang, H. (2019). Efficacy of low concentration acidic electrolyzed water and levulinic acid combination on fresh organic lettuce (Lactuca sativa Var Crispa L.) and its antimicrobial mechanism. *Food Control, 101*, 241–250.