



Effect of electrolysed water generated by sodium chloride combined with sodium bicarbonate solution against *Listeria innocua* in broth and on shrimp

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ABSTRACT

Electrolysed water (EW) generated using sodium chloride (NaCl) and sodium bicarbonate (NaHCO₃) as electrolytes shows strong bactericidal effects on pathogens. However, the sanitising effect of EW generated by adding NaHCO₃ before and after electrolysis was not clear. The difference of the bactericidal mechanism in broth medium and real food system was not elucidated as well. This present study aimed to compare the antibacterial effect and inactivation mechanism of EW generated by the addition of NaHCO₃ before and after electrolysis against *Listeria innocua* ATCC 33090 in broth and on shrimp. The addition of NaHCO₃ before electrolysis enhanced both *in vitro* and *in vivo* antibacterial activity with greater bacterial reduction (2.9 log CFU/mL and 1.7 log CFU/g, respectively) compared with adding NaHCO₃ after electrolysis. The antibacterial mechanism was further investigated by NMR-based metabolomics. The EW generated by adding NaHCO₃ before electrolysis mainly affected amino acid metabolism and carbohydrate and energy metabolism in broth. The reduction of fewer types of amino acids and sugars was observed on the shrimp attributing to the different nutritional compositions of shrimp. Moreover, the accumulation of γ -aminobutyric acid and glutamate in *L. innocua* on shrimp compensated for the disrupted TCA cycle caused by the EW stress. Therefore, *L. innocua* was less sensitive to generated EW on shrimp as compared with in broth. The results provide comparative information on the responses of *L. innocua* to EW stress in broth and on shrimp and could serve as the basis to develop strategies to better control pathogens in food.

1. Introduction

Listeria monocytogenes is a typical foodborne pathogen commonly found in the environment of seafood processing. The occurrence of *L. monocytogenes* in shrimp and shrimp products has been well demonstrated (Knudsen et al., 2016). It has strong adaptability to the external environment and can lead to invasive listeriosis in susceptible populations which has high associated mortality (Olaimat et al., 2018). *L. innocua* is used as a surrogate for *L. monocytogenes* because of their high similarity in ecological cohabitation, genomic synteny, and physiological properties (Costa et al., 2018).

The utilisation of effective sanitisers is the most common method to control contamination by microorganisms. The application of electrolysed water (EW) has several advantages over traditional sanitising systems, including cost-effectiveness, convenience of application, effective sanitisation, and posing no threat to human beings and the environment (Rahman et al., 2016; Zhao et al., 2021). In addition to

using sodium chloride (NaCl) as an electrolyte to produce EW, recent studies have found that many inorganic and organic salts could be used to improve the electrolytic efficiency when generating EW (Simon et al., 2014; Youssef et al., 2014). Among them, sodium bicarbonate (NaHCO₃), a kind of additives in many commodities, attracted our interest. NaHCO₃ can act as an effective electrolyte because of its high ionic mobility (Simon et al., 2014). In addition, the electrolysed NaHCO₃ can produce peroxycarbonate and derivatives, acting as strong disinfectants which lead to oxidative stress to the organism (Fallanaj et al., 2016). Moreover, Zhang, Lai, and Yang (2018) have found that the near neutral electrolysed water (NEW) can be directly generated by combining NaCl with NaHCO₃ electrolysis. The electrolysis of NaHCO₃ could enhance the ·OH production in EW, the radicals that could exhibit extra disinfecting ability against bacteria.

Besides, there are still some problems relating to the application of EW. The current equipment used to generate EW is quite huge and not convenient for household use or food industries. Therefore, it is

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necessary to develop a portable EW generator to meet the demand and solve these safety problems. In our previous work, several portable sanitising units generating near NEW were developed (Zhang et al., 2018a, 2018b). The previously developed portable electrochemical sanitising generator exhibited good antibacterial efficacy. However, two electrolytic cells were used simultaneously, leading to low current efficiency (Zhang, Yang, & Chan, 2018). Therefore, a portable generator using NaHCO_3 and NaCl as electrolytes using one electrolytic cell was further developed. Previous research was mainly focused on the physical properties of the unit and the *in vitro* sanitising effect of the generated EW (Zhang, Lai, & Yang, 2018). However, the effects of the addition of NaHCO_3 before and after electrolysis were unknown, and the difference of the antibacterial mechanism in broth medium and real food system was also unclear.

The objectives of the study were to identify the mode of action of the electrolysis of NaHCO_3 on the sanitising effect of generated EW against *L. innocua* and to compare the antibacterial mechanism in broth and on shrimp. The changes in reactive oxygen species (ROS), ATP contents, and cell morphology of *L. innocua* were analysed to preliminarily investigate the bactericidal mechanism of different treatments. NMR-based metabolomics was further applied to determine the metabolic changes and inactivation mechanism of the generated EW. Moreover, the bacterial reduction and pathway alternations in *L. innocua* in broth were compared with those of *L. innocua* inoculated on shrimp.

2. Materials and methods

2.1. Electrolysed solution preparation and assessment of EW

The electrolysis was conducted using 1% (w/v) NaCl (Tokyo Chemical Industry Co., Ltd, Singapore) solution in the presence/absence of NaHCO_3 (Tokyo Chemical Industry Co., Ltd, Singapore) (0, 2, 4, 6, and 8 mM) by an electrochemical cell (Dongguan Sunrise Environmental Technology Co., Ltd, Guangzhou, Guangdong, China), comprising $\text{RuO}_2\text{-IrO}_2/\text{TiO}_2$ anode and TiO_2 cathode (Dongguan Zhongrui Electrode Industrial Technology Co., Ltd, Guangzhou, Guangdong, China) separated by an ion membrane (Dupont, Wilmington, DE, USA) (100 mm \times 100 mm \times 0.1 mm). The total active anodic surface was 75 cm² and the current densities used were 20, 30, 40, 50, and 60 mA/cm². The AE (adding NaHCO_3 after electrolysis) solution was prepared by adding NaHCO_3 after electrolysis of 1% (w/v) NaCl . The EW solutions with different FAC levels used in the following experiments were obtained by dilution of generated EW using DI water. Free available chlorine (FAC), pH, and oxidation-reduction potential (ORP) of the EW generated from the portable sanitising unit were determined by a chlorine test kit (Merck Pte, Ltd, Singapore), a pH meter (Thermo Scientific, Waltham, MA, USA) and an ORP meter (Metrohm Singapore Pte, Ltd, Singapore), respectively.

2.2. Bacterial strains and antimicrobial analysis in broth

L. innocua ATCC 33090 was purchased from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany) and stored at -80 °C. *L. innocua* (100 μL) was activated by transferring into a test tube containing 10 mL of sterile tryptic soy broth (TSB; Oxoid, Basingstoke, UK) and incubating at 37 °C for 24 h. After two consecutive transfers, the harvest cell pellet was collected by centrifugation (5000 \times g, 4 °C, 10 min), washed with phosphate-buffered saline (PBS, 137 mM NaCl , 2.7 mM KCl , and 10 mM phosphate buffer, pH 7.2; Vivantis Technologies Sdn. Bhd., Malaysia), and then suspended in 10 mL of PBS to obtain a working culture at a concentration of around 10⁸ CFU/mL for subsequent experiments.

For each treatment, 1 mL of bacterial suspension was mixed with 9 mL of each EW solution for 5 min. After treatment, 2 mL of neutralizing buffer (5.2 g/L; Becton, Dickinson and Comp, Sparks, MD, USA) was immediately added to stop the sanitising process. The mixtures were

serially diluted and spread on tryptic soy agar (TSA; Oxoid, UK). Bacterial colonies were counted after incubation at 37 °C for 48 h. The group treated with deionised (DI) water was used as the control group. All the experiments were performed in triplicate.

2.3. Intracellular ROS and ATP concentration detection

The oxidative-sensitive probe 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) (Sigma-Aldrich, St Louis, MO, USA) was used to investigate intracellular ROS concentrations in *L. innocua* cells (Chen, Zhang, et al., 2019). The working culture at a concentration of around 10⁸ CFU/mL obtained in Section 2.2 was pre-incubated with H_2DCFDA (200 μM final concentration) in the dark at 37 °C for 30 min. After incubation, the mixed solution was centrifuged (5000 \times g, 4 °C, 10 min), the obtained cell pellet was washed and resuspended in PBS (10 mL). One mL of bacterial suspension was mixed with 9 mL of each EW solution for several time points (0.5, 1, 2, 3, 4, and 5 min). After treatment, 2 mL of neutralising buffer was immediately added to stop the sanitising process. The cell suspension (100 μL) was then transferred to 96-well plates (Corning, Tewksbury, MA, USA). The fluorescence intensity of the collected samples was recorded using a fluorescent plate reader (Corning, Tewksbury, MA, USA) with excitation/emission wavelengths of 488/520 nm. The group treated with deionised (DI) water was used as the control group. The fluorescence intensity ratio was represented as the ratio of the fluorescence intensity in the treatment group to that in the control group, and all samples were tested in triplicate.

The intracellular ATP content was assessed according to the instructions of BacTiter-Glo microbial cell viability assay (Promega Biotec, Madison, WI, USA). *L. innocua* cells in different treated groups were harvested at an initial concentration of 8 log CFU/mL. After treatment, the reagent (100 μL) was added to 100 μL of medium-containing cells under different treatments. The mixtures were then mixed thoroughly and incubated for 5 min at room temperature. The luminescence was recorded by a multimode microplate reader (Tecan Spark 10 M, Durham, NC, USA). Control wells containing medium without cells were recorded as values for background luminescence.

2.4. Visualisation of bacterial cells by atomic force microscopy (AFM)

The cell suspensions treated in Section 2.2 were spread onto mica sheets and air-dried in a laminar flow cabinet. The morphology of the treated cells was observed using a TT atomic force microscope (AFM workshop, Signal Hill, CA, USA) that was equipped with a Sensaprobe TM190-A-15 tip (Applied Nanostructures, Mountain View, CA, USA). The images were scanned at 0.5 Hz and 512 pixels/line. Section analysis and root mean square (RMS) roughness were recorded using the offline software Gwyddion. RMS roughness was calculated from the individual areas (0.2 \times 0.2 μm^2) of the central part of the cell surface.

2.5. Inoculation on shrimp

Peeled shrimp (*Litopenaeus vannamei*) were purchased from a local supermarket, washed with running DI water and autoclaved (15 min, 121 °C), and then allowed to cool to room temperature before use. The shrimp were randomly divided into three groups (20 g), then placed into sterile petri dishes. The bacterial cells were incubated overnight to obtain the cultures (around 8 log CFU/mL) in the exponential-phase ($\text{OD}_{580\text{nm}} = 0.5\text{--}0.6$). The bacterial cultures were then diluted to an $\text{OD}_{580\text{nm}} = 0.01$ (around 6 log CFU/mL) using TSB. The TSB cultures (200 μL) were inoculated on the surface of shrimp followed by 24 h growth at 25 °C to reach a final concentration of around 8 log CFU/g (Dupre et al., 2019; Shen et al., 2019).

2.6. Antimicrobial analysis on shrimp

After appropriate inoculation, the shrimp (200 g) were immersed

into different treatment solutions (250 mL) for 10 min, followed by adding 2 mL of neutralizing buffer to stop the disinfection process. The mixtures were then homogenised for 2 min. One millilitre aliquot of each homogenised sample was serially diluted with 9 mL sterile PBS (0.1%), and 0.1 mL of the diluents was spread-plated onto TSA, followed by incubation at 37 °C for 48 h. The group treated with deionised (DI) water was used as the control group. Each experiment was conducted in triplicate.

2.7. Extraction of *L. innocua* metabolites

The *L. innocua* metabolites both in broth and on shrimp were extracted based on the method of Dupre et al. (2019) with several changes. For the *L. innocua* metabolites on inoculated shrimp after each treatment, the solutions obtained in section 2.6 were triturated with a pipette, and the debris was precipitated by centrifugation (1500×g, 4 °C, 1 min). The obtained supernatant and the cell suspensions treated as in section 2.2 were centrifuged (16,000 g, 4 °C, 10 min) to obtain the cell pellets, washed with 5 mL of PBS, and repelleted. And the prepared *L. innocua* pellets were mixed well with 1 mL of ice-cold methanol d4, immediately frozen in liquid nitrogen, and then thawed on ice and freeze-thawed twice to disrupt the cell membrane. The metabolites were subsequently extracted in the cold methanol solution under -20 °C overnight, and the extracted metabolites were obtained after centrifugation (12,000×g, 4 °C, 20 min) (Chen et al., 2020). Trimethylsilyl-propanoic acid (TSP) at a final concentration of 1 mM was added as internal chemical shift reference. The metabolites extraction was conducted in triplicate.

2.8. NMR spectroscopic analysis

All NMR tests were conducted at 25 °C with Bruker DRX-500 NMR spectrometer (Bruker, Rheinstetten, Germany) at 500.23 MHz frequency for ¹H resonance frequency, equipped with a triple inverse gradient probe. For metabolic analysis of each sample, the ¹H spectrum was acquired using a first increment of the standard NOESY pulse sequence. All the data were obtained using a spectral width of 10 ppm, and the spectrum was recorded by 128 transients and a relaxation delay of 2 s. The spectra for all samples were then processed with a line broadening factor of 1 Hz prior to Fourier transformation (Zhao et al., 2019). For the resonance assignment, the two-dimensional NMR spectra ¹H-¹³C heteronuclear single quantum correlation (HSQC) was acquired for selected samples. ¹H was collected in the F2 channel with a spectral width of 10 ppm and ¹³C was recorded in F1 channel with a spectral width of 175 ppm (Freitas et al., 2015).

2.9. Data processing

The software Topspin 3.6.0 (Bruker) was used to process the NMR data. The spectra were manually corrected for phase and baseline distortions and referenced to the chemical shift of TSP. The metabolites corresponding to the peaks were determined by 1D ¹H and 2D ¹H-¹³C NMR data referring to the Biological Magnetic Resonance Data Bank (<http://www.bmrwisc.edu/metabolomics/>) and some related references. The region containing the methanol resonance (3.31–3.35 ppm) was removed, and the normalised data were integrated over a series of 0.02 ppm integral width. The binned data were further investigated by principal component analysis (PCA) for group separation and orthogonal projections to latent structures discriminant analysis (OPLS-DA) to determine the dissimilarities among different treatments using the SIMCA software (version 13.0, Umetrics, Umeå, Sweden). Furthermore, the variable importance in projection (VIP) was analysed. The metabolites with a VIP value greater than 1 were recognised as the most influential variable in the OPLS models (Zhao et al., 2019). The enrichment pathway analysis was conducted using MetaboAnalyst 4.0 (<http://www.metaboanalyst.ca/>) with the screened metabolites and the

Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<https://www.genome.jp/kegg/pathway.html>), respectively.

2.10. Statistical analysis

All the measurements were performed in triplicate independently. Analysis of variance (ANOVA) was performed using Duncan's multiple range tests in SPSS Statistics (IBM Corp., Armonk, NY, USA) to compare the effects of different treatments against *L. innocua* in broth and on shrimp. Differences with *P* < 0.05 were considered significant.

3. Results and discussion

3.1. Effects of EW on ROS and intracellular ATP concentrations in broth

The electrolysed water produced by adding 4 mM NaHCO₃ with the current density of 50 mA/cm² was finally chosen for the following experiments, considering that the pH should not exceed 7 and FAC value should be greater than the concentration we used in the experiment (data not shown). The physical properties of the used EW are listed in Table 1. The intracellular ROS accumulation in bacteria could attack cellular components and lead to loss of physiological functions (Hao et al., 2012). Therefore, the ROS accumulation was investigated during sanitisation. There was no notable difference in ROS accumulation after the control, BE, and AE treatments with a FAC level of 4 mg/L (data not shown). The ROS accumulation treated with BE and AE at 8 mg/L FAC is shown in Fig. 1A. The result showed that both BE and AE groups led to a continuous increase in ROS accumulation. However, when the treatment time was increased to 4 min, the ROS quantity of BE group was higher than that of AE group. The EW induced oxidative stress and finally led to membrane damage (Han et al., 2016). These results were consistent with studies on the influence of acidic EW on *L. innocua* (Liu et al., 2018).

ROS generation is usually associated with many mitochondrial functions, especially ATP generation (Acton, 2004). The effect of the treated solutions on intracellular ATP concentration of *L. innocua* is shown in Fig. 1B. After treatment with AE and BE group, the luminescent value proportional to the ATP contents decreased significantly with increasing FAC level in all groups. Moreover, the intracellular ATP concentrations in BE group decreased significantly (*P* < 0.05) relative to those in the AE and control groups. Oxidative stress, related to increased intracellular ROS, could finally lead to decreased ATP levels. These results were consistent with previous research, indicating EW could enhance cell permeability, further leading to the leakage of intracellular ATP and an altered balance of cations that required ATP (Cai et al., 2019). Comparing the BE and AE treatments, the differences in ATP content and ROS might result from the antimicrobial substances produced in the presence of NaHCO₃ during electrolysis, such as peroxycarbonate and its derivatives, which acted as strong disinfectants (Fallanaj et al., 2016). Moreover, the developed sanitising unit could also generate more ·OH during electrolysis, leading to a better

Table 1
Physicochemical properties of different treatment solutions.

Treatment	FAC (mg/L)	pH	ORP (mV)
DI	0.0 ± 0.0 ^a	6.45 ± 0.20 ^{ab}	265.1 ± 27.0 ^a
BE	4.3 ± 0.2 ^b	6.28 ± 0.03 ^a	928.5 ± 13.2 ^{bc}
	8.4 ± 0.1 ^c	6.43 ± 0.06 ^{ab}	871.9 ± 26.0 ^b
AE	4.1 ± 0.3 ^b	6.50 ± 0.05 ^{ab}	854.6 ± 16.1 ^b
	8.2 ± 0.2 ^c	6.54 ± 0.10 ^b	850.7 ± 24.4 ^b

Values are expressed as the mean ± standard deviation (n = 3). Different lowercase letters within a column represent significant differences (*P* < 0.05). Note: FAC: free available chlorine, ORP: oxidation-reduction potential. DE: deionized water, BE: add NaHCO₃ before electrolysis, AE: add NaHCO₃ after electrolysis. The two lines of values for BE and AE indicated the same treatment solution with different FAC levels.

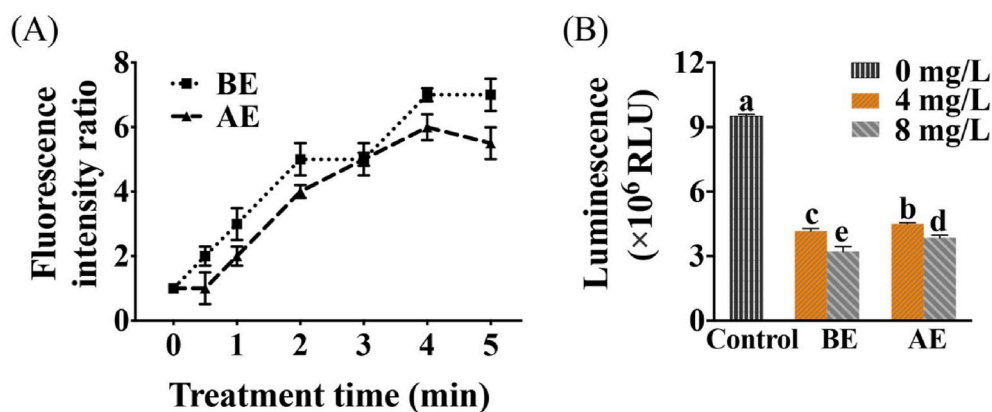


Fig. 1. Effects of different treatments on the fluorescence intensity ratio (A) and intracellular ATP concentration (B) of *L. innocua*. Note: Bars represent standard deviation ($n = 3$). Values with different lowercase letters are significantly different ($P < 0.05$). BE: add NaHCO_3 before electrolysis; AE: add NaHCO_3 after electrolysis.

antibacterial effect (Zhang, Lai, & Yang, 2018).

3.2. Effects of EW on cell morphology of *L. innocua* by AFM

AFM has been widely used to image the surface structure and to study the characteristic morphology of microorganisms. Morphological changes of *L. innocua* induced by BE and AE at 8 mg/L FAC and control cells were investigated. As shown in Fig. 2, untreated *L. innocua* cells were rod-shaped and were approximately 2.3 μm in length. Following both treatments, the cells were markedly shrunken and the cell surface became irregular. Moreover, the height, width, and RMS roughness were obtained for further analysis of the AFM images using Gwyddion software. Compared with the cell height (0.46 μm) and width (0.90 μm) of the control group, a significant decrease in these values was observed in the BE and AE group. The RMS roughness was used to evaluate microorganism surface morphology. A relatively lower RMS roughness (4.0 nm) was calculated for the control group, while the BE group showed the highest RMS value, indicating that the BE treatment led to the surface morphological changes in *L. innocua*. These results were in agreement with a previous study in which EW treatment led to wrinkled bundles and lesions of *E. coli* forming a rougher microbial surface (Liu et al.,

2017). The shrunken and more irregular cells might result from oxidative stress, altering the permeability of the cell membrane, leading to the osmotic pressure out of balance (Li et al., 2017).

3.3. In vitro and in vivo antimicrobial effects of EW on *L. innocua*

This study evaluated the antibacterial effect of two kinds of electrolysed water (BE group and AE group) on *L. innocua*. The different EWs with FAC values of 4 mg/L and 8 mg/L were chosen to study the antimicrobial effect in broth. As shown in Fig. 3A, the survival populations were significantly decreased after both BE and AE treatments. Generally, the sterilisation effect was enhanced with the increasing FAC level (Liu et al., 2017). In our study, the two treatment solutions had similar FAC, ORP, and pH levels. Interestingly, when the FAC was at 8 mg/L, the population of *L. innocua* after BE treatment (5.3 log CFU/mL) was significantly lower compared with that after AE treatment (6.0 log CFU/mL) ($P < 0.05$).

Microbial contamination of food may also lead to foodborne illness. The antimicrobial effect of EW against *L. innocua* inoculated on shrimp was also determined. The BE and AE solutions with FAC values of 30 and 60 mg/L were chosen for this experiment. The higher concentration used

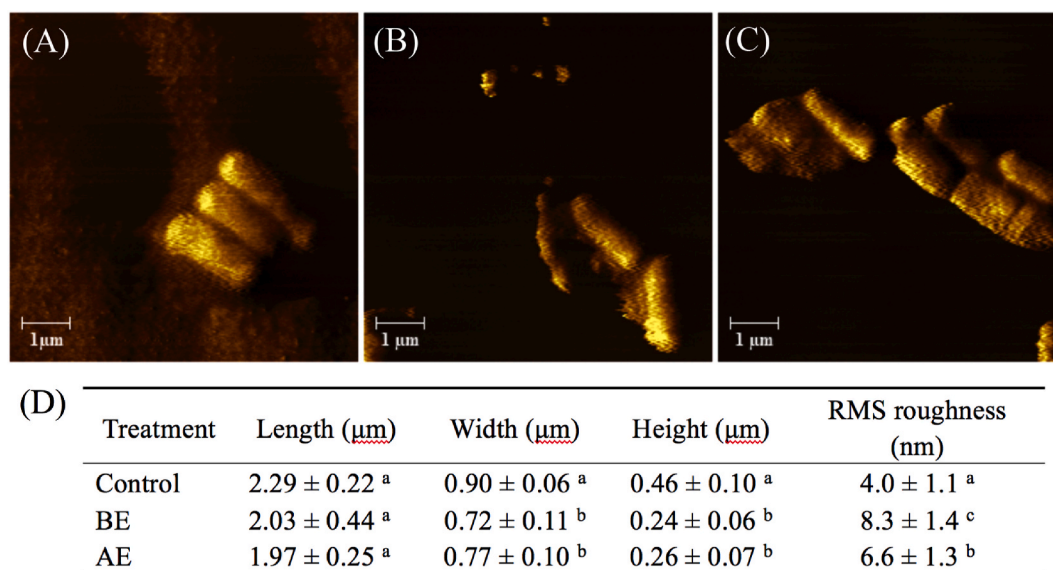


Fig. 2. Atomic force microscopy (AFM) images of *L. innocua* under different treatments (Control (A); BE (B) and AE (C)) and changes of width, length and root mean squared (RMS) roughness of *Listeria innocua* dimensions (D). Notes: Values in (D) with different lowercase letters are significantly different from each other ($P < 0.05$); BE: add NaHCO_3 before electrolysis; AE: add NaHCO_3 after electrolysis.

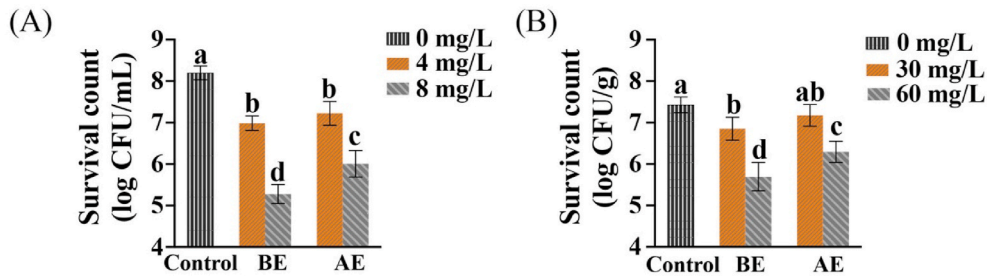


Fig. 3. Effects of different treatments on the survival count of *L. innocua* in broth (A) and on shrimp (B). Note: Bars represent standard deviation (n = 3). Values with different lowercase letters are significantly different (P < 0.05). BE: add NaHCO₃ before electrolysis; AE: add NaHCO₃ after electrolysis.

compared to that in broth was because the active agents in EW would react with the

matrix and weaken its sterilisation ability (Deborde & Gunten, 2008). The initial bacterial counts of each inoculated bacteria onto

shrimp were controlled at around 8 log CFU/g. As shown in Fig. 3B, the reductions in the bacterial count in the BE group with FAC value of 60 mg/L was 0.6 log CFU/g higher than that of AE group. The differences in the bacterial reduction might result from the extra antibacterial agents

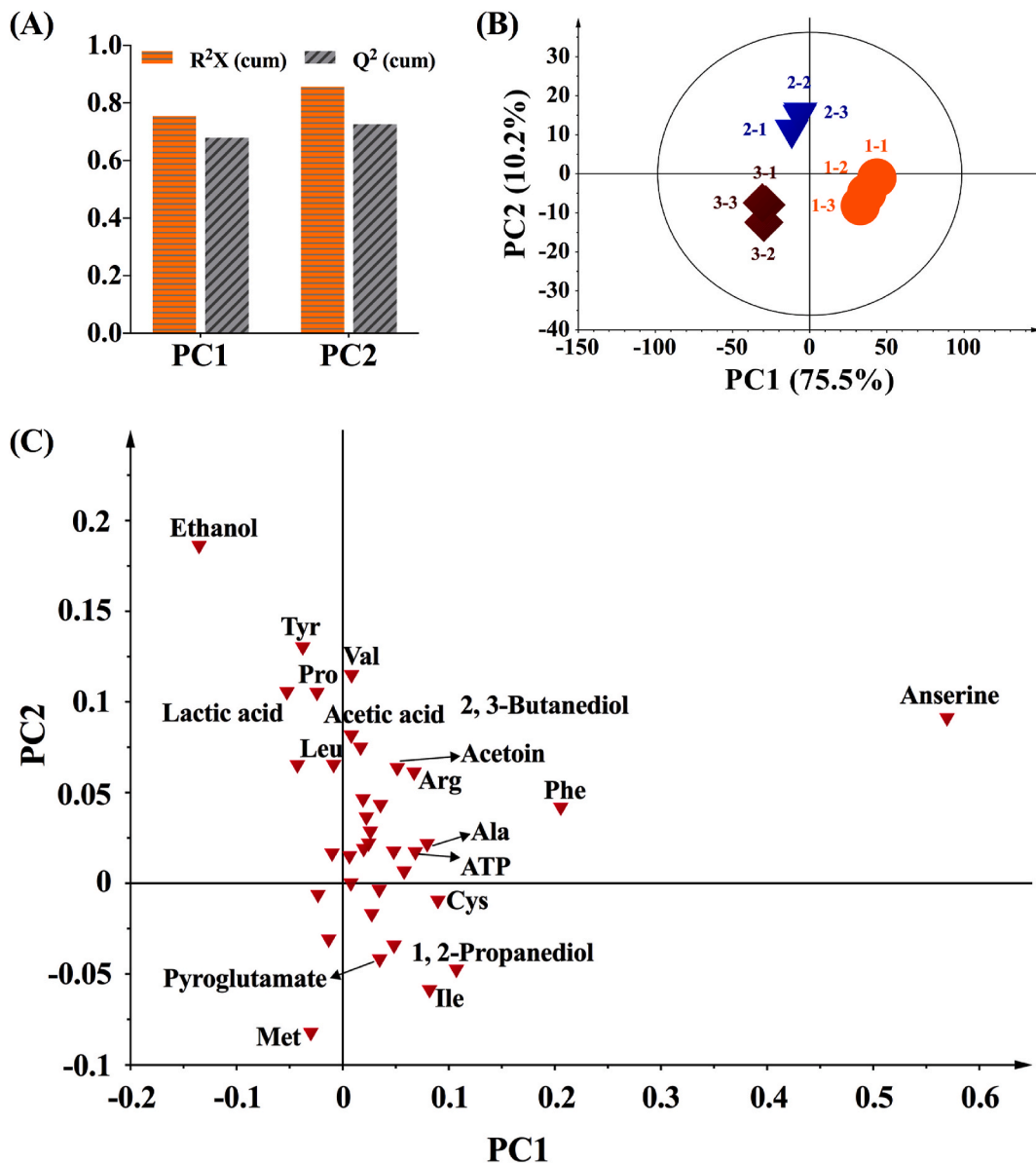


Fig. 4. Principal components analysis (PCA) for the metabolite profile of *L. innocua* in broth. The principal components explaining variances used in PCA (A); PCA score plot (B); corresponding PCA loading plot (C). Note: Group 1–3, *L. innocua* under DI water, AE, and BE treatments, respectively. DI: deionised; BE: add NaHCO₃ before electrolysis; AE: add NaHCO₃ after electrolysis. Tyr: tyrosine; Val: valine; Pro: proline; Leu: leucine; Arg: arginine; Phe: phenylalanine; Ala: alanine; ATP: adenosine triphosphate; Cys: cysteine; Ile: isoleucine; Met: methionine..

(peroxycarbonate and ·OH) presented in the BE solutions (Fallanaj et al., 2016; Zhang, Lai, & Yang, 2018) compared with that in the AE group.

3.4. Metabolic changes of *L. innocua* extracts in broth and on shrimp

NMR-based metabolomics strategy was conducted to further elucidate the mechanism of the antibacterial effects of the different treatments in broth and on shrimp. As shown in Fig. S1, the BE and AE groups had similar spectral profiles with different signal intensities. The ^1H and ^{13}C resonances were assigned to specific metabolites (Table S1) based on the previous studies (Chen et al., 2020; Lou et al., 2021; Liu et al., 2018), the 2D NMR analysis (^1H - ^{13}C HSQC), and the metabolic database. A total of 43 metabolites in broth of all treated groups were identified, including amino acids, organic acids, nucleotides, sugars, and other metabolites.

The metabolic changes caused by the different treatments were investigated using PCA, which enables the identification of subtle metabolite variations (Freitas et al., 2015; Liu et al., 2020). The model quality parameters (Fig. 4A) $R^2X = 0.86$ and $Q^2 = 0.73$, showing that the data had good reproducibility and the model had good predictability (Chen, Wu, et al., 2019). The scores plot from the PCA (Fig. 4B) indicated that more than 85% of the variables could be explained with the two principal components. And there was a well clustering between the two principal components. And there was a well clustering between the two principal components. And there was a well clustering between the two principal components, indicating that different treatments caused distinct metabolic changes.

The PCA score plot enabled the observation of the natural clustering and separation among the groups. The loading plot could provide information on dominating metabolites that contributed to the separations among different groups (Li et al., 2015). As shown in Fig. 4C, metabolites such as anserine, phenylalanine, 1,2-propanediol, ATP, and alanine had a higher loading in PC1 while there was a greater contribution of ethanol, tyrosine, proline, and methionine in PC2. These metabolites might act as potential biomarkers that are exposed to different treatments.

To further investigate the metabolite changes associated with the different treatments, coupled comparative OPLS-DA generated from the spectral data of control and AE groups (Fig. 5A1 and B1), control and BE group (Fig. 5A2 and B2), AE and BE group (Fig. 5A3 and B3) was conducted. The results were presented as cross-validated score plots (Fig. 5 left) and corresponding coefficient plots (Fig. 5 right). The R^2 and Q^2 (close to 1) indicated the good fitness and high predictabilities of the three OPLS-DA models (Zhao et al., 2019). As shown in Fig. 5A, the score plots of these models demonstrated distinct separations between groups, indicating that the metabolic changes in *L. innocua* associated with different treatments could be detected. The corresponding coefficient plots were also conducted to identify the metabolites that significantly contributed to the pairwise differentiation. These metabolites were determined by the use of VIP values. Generally, the variables with VIP >1 would be considered as having a significant effect on the discrimination (Park et al., 2019). The 36 identified metabolites without overlapping chemical shifts (except for Gln, β -D-Glucose, α -D-Glucose, G-6-P, uridine, adenine, and tryptophan) were compared pairwise. Compared with the control group, both BE and AE treatments lead to increases in ethanol, proline, leucine, and a distinct decrease in anserine, phenylalanine, threonine, succinic acid, oxoglutaric acid, and other metabolites. Notably, in the coefficient plot of BE group compared with AE group (Fig. 5B3), decreased levels of most metabolites such as anserine, phenylalanine, oxoglutaric acid, threonine, succinic acid, cysteine, alanine, tyrosine, arginine, 1,2-propanediol, ATP, valine, adenosine were observed, indicating that the BE treatment led to more metabolic changes than AE.

NMR method was also used to investigate the *in vivo* mechanism of the bactericidal effect of EW. A total of 34 metabolites were identified on shrimp, and PCA was used to further identify the variations in spectra of *L. innocua* extracts after different treatments. The quality parameter of the models ($R^2X = 0.86$ and $Q^2 = 0.76$) shown in Fig. 6A indicated good

fitness and predictability of the model. In addition, there was a distinct separation among different treatments in the PCA score plot (Fig. 6B), showing that both BE and AE treatments lead to metabolic changes to *L. innocua*. As shown in Fig. 6C, there was a greater contribution of anserine, Phe, Pro, ethanol, acetoin, and fructose in PC1 while the metabolites such as Glu, lactic acid, F-6-P, and Thr had a higher loading in PC2, which could act as potential biomarkers when *L. innocua* was exposed to different treatments on shrimp. The 27 identified metabolites without overlapping chemical shifts (except for Gln, β -D-Glucose, α -D-Glucose, G-6-P, uridine, adenine, and tryptophan) were used for the pairwise comparison to obtain the discriminant compounds for the metabolic differentiation (Chen et al., 2020). These models (R^2 and Q^2 values were close to 1) clearly differentiated the control, BE, and AE groups with good fitness along with high predictability (Fig. 7A1-A3). Compared with the control group, lower levels of most amino acids were observed after the BE treatment, including valine, phenylalanine, proline, leucine, threonine. Moreover, after BE treatment, several metabolites were observed to increase compared with the control group, such as malic acid, acetoin, glutamate, γ -aminobutyric acid (GABA), and ethanol which were also found to increase when compared with those in AE group (Fig. 7B2 and B3).

3.5. Comparison of pathway alternation of *L. innocua* between in broth and on shrimp

To further determine the mechanism of the bactericidal effect of the BE solution in broth and on shrimp, the metabolic pathways related to the metabolic response were further identified based on the screened metabolites (VIP > 1 with a fold change > 1.20 or < 0.83) (Gupta et al., 2017). Totally, thirty pathways were involved and 7 of them were considered as the significant pathways ($P < 0.05$) associated with the disinfection process in broth. These pathways were aminoacyl-tRNA biosynthesis; taurine and hypotaurine metabolism; valine, leucine, and isoleucine biosynthesis; alanine, aspartate, and glutamate metabolism; tyrosine metabolism; amino sugar and nucleotide sugar metabolism; sulphur metabolism (Fig. 8A and Table S2). Twenty pathways were involved and six metabolic pathways were significantly influenced by the BE treatment on shrimp (Fig. 8B and Table S3). They were aminoacyl-tRNA biosynthesis; valine, leucine, and isoleucine biosynthesis; arginine and proline metabolism; glutathione metabolism; carbapenem biosynthesis and alanine, aspartate, and glutamate metabolism.

The average fold changes of the screened metabolites (VIP > 1) induced by BE treatment compared with the control group in broth and on shrimp are listed in Table S4. Based on the KEGG pathway analysis, the comparison of metabolic disturbance caused by BE treatment in broth and on shrimp is presented in Fig. 8C. The most affected pathway was amino acid metabolism in both situations. Depleted levels of most amino acids were observed, suggesting that the amino acid biosynthesis was inhibited under the EW treatment. Amino acid biosynthesis was susceptible to the hostile environment (heat, osmotic stress, oxidative stress) (He et al., 2015; Wu et al., 2021). Isoleucine, valine (branched-chain amino acids, BCAAs) in *L. innocua* could provide construction blocks for protein synthesis and fatty acid synthesis associated with membrane stress. Arginine could be biosynthesised from proline in *L. innocua* and provide α -ketoglutarate, which in turn supplies carbon to the TCA cycle to generate more energy to struggle with the adverse environments (Lungu et al., 2009). While putrescine catabolism was associated with the defense against oxidative stress resulting from BE treatment, further leading to the depleted levels of putrescine (Wu et al., 2020). In addition, a lower level of anserine was observed in broth and on shrimp. It was because anserine has been proven to exhibit antioxidant activities, which was consumed by the bacterial to counteract the damage caused by oxidative stress, finally leading to the decrease of anserine (Low et al., 2018).

Most amino acids could be catabolised to pyruvate, further leading to

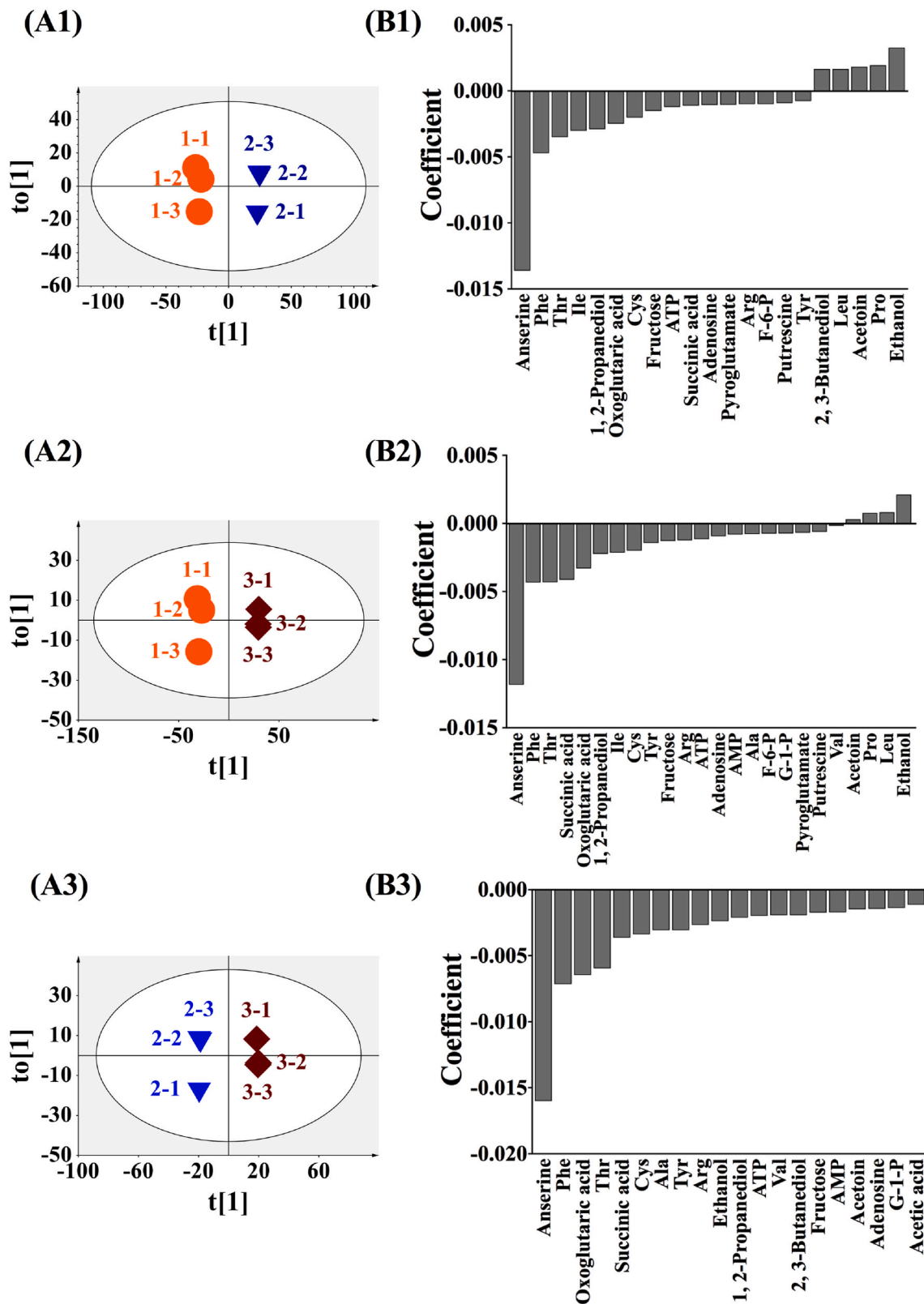


Fig. 5. Orthogonal projections to latent structures discriminant analysis (OPLS-DA) pairwise comparison between groups. OPLS-DA scores (A1) and coefficient plot (B1) of group 1–2, $R^2 = 0.98$, $Q^2 = 0.97$; OPLS-DA scores (A2) and coefficient plot (B2) of group 1–3, $R^2 = 0.99$, $Q^2 = 0.98$; OPLS-DA scores (A3) and coefficient plot (B3) of group 2–3, $R^2 = 0.99$, $Q^2 = 0.96$. Note: Groups 1–3, *L. innocua* under DI water, AE, and BE treatments, respectively. DI: deionised; BE: add NaHCO_3 before electrolysis; AE: add NaHCO_3 after electrolysis.

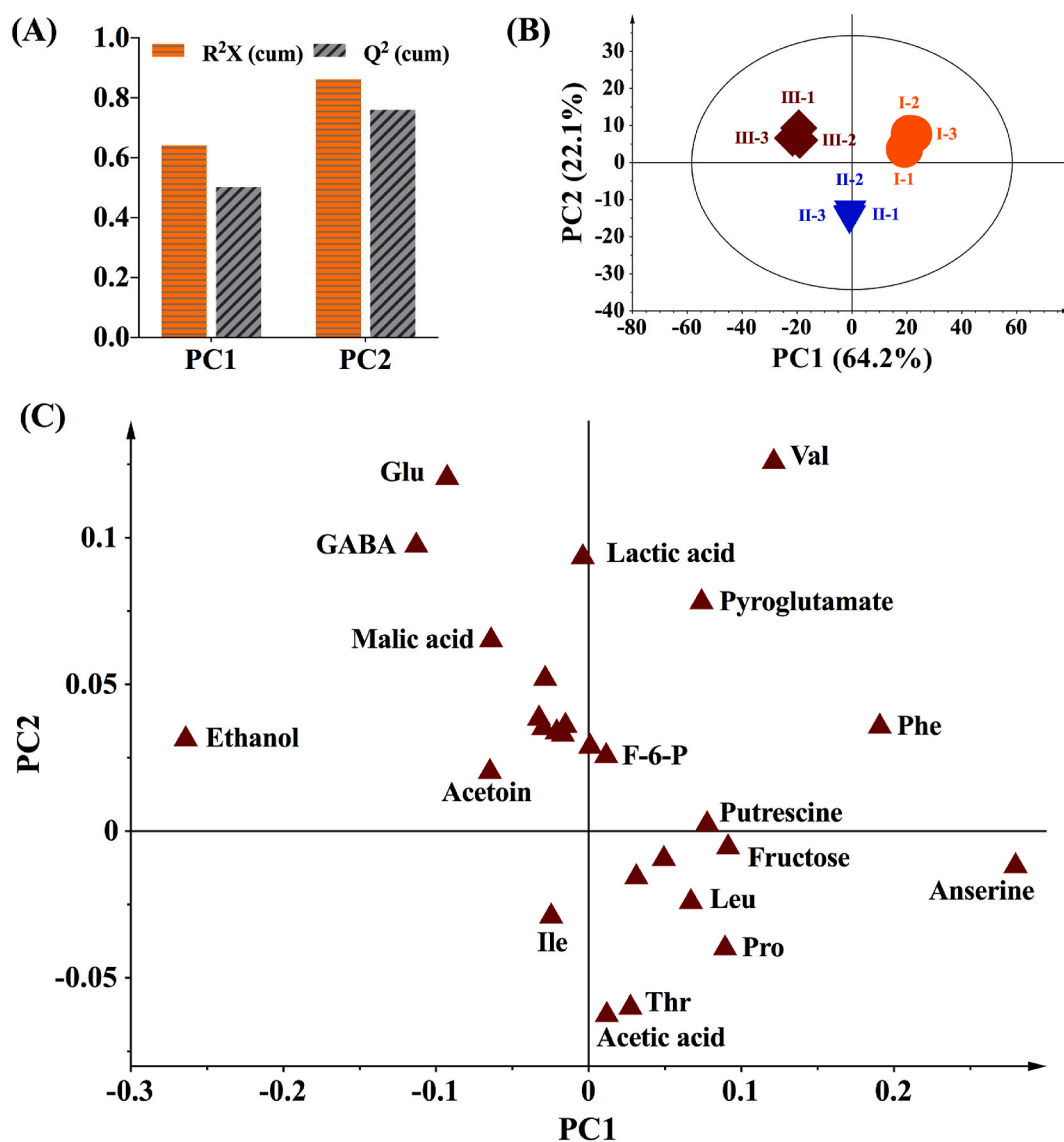


Fig. 6. Principal components analysis (PCA) for the metabolite profile of *L. innocua* on shrimp. The principal components explaining variances used in PCA (A); PCA score plot (B); corresponding PCA loading plot (C). Note: group I-III, *L. innocua* under DI water, AE, and BE treatments, respectively. DI: deionised; BE: NaHCO₃ added before electrolysis; AE: NaHCO₃ added after electrolysis. Glu: glutamate; GABA: γ -aminobutyric acid; Val: valine; Phe: phenylalanine; F-6-P: fuctose-6-phosphate; Ile: isoleucine; Leu: leucine; Pro: proline; Thr: threonine.

a higher level of ethanol both in broth and on shrimp, suggesting a possible metabolic switch to anaerobic metabolism to avoid the generation of ROS production from respiration. Similar results were also found in the *L. monocytogenes* responding to the antibiotics (Knudsen et al., 2016). However, compared with pathway changes that occurred on shrimp, more amino acid pathways were affected by the EW treatment in broth and reduction of fewer types of amino acids (cysteine, alanine, and arginine) were observed on the shrimp. It could be explained by the differences in the nutritional compositions between TSB and shrimp. Shrimp is of high protein value and could be degraded to amino acids as a carbon and energy source by *L. innocua* which in turn provide more amino acids when encountering the EW treatment.

Carbohydrate and energy metabolism (amino sugar and nucleotide sugar metabolism and purine metabolism) was found to be less affected by the BE treatment on shrimp compared with BE treatment in broth. Lower content of the metabolites such as G-1-P, F-6-P, and fructose involved in amino sugar and nucleotide sugar metabolism was only observed in BE-treated *L. innocua* in broth and metabolites such as ATP, AMP, and adenosine involved in purine metabolism were also only

found to decrease in broth. It was probable the shrimp proteins were degraded to amino acids by *L. innocua* proteases, which were utilised as a carbon and energy source. Therefore, less carbohydrate metabolism was needed to provide carbon skeletons entering TCA cycle to provide energy for *L. innocua* on shrimp (He et al., 2015). When encountering the EW stress, more energy generated from the abundant amino acids provided by the shrimp could be utilised by *L. innocua* leading to a smaller impact on energy metabolism. The results were similar to previous research demonstrating the difference in metabolomics of *S. aureus* inoculated on chicken breast or in Luria broth (Dupre et al., 2019). The succinic acid and oxoglutaric acid contents were reduced after the BE treatment indicating the weakened TCA cycle in broth. Besides, the lower content of ATP from the NMR results was consistent with the previous luminescent results. The lower level of AMP and adenosine was also recorded. Many researchers have found that ATP efflux and reduced intracellular ATP concentrations could be induced by external stress (Cai et al., 2019; Cui et al., 2018). And the weakened nucleotide metabolism might lead to the diminished ability to synthesis DNA and RNA and affect some enzymatic reactions (Horlbog et al., 2019).

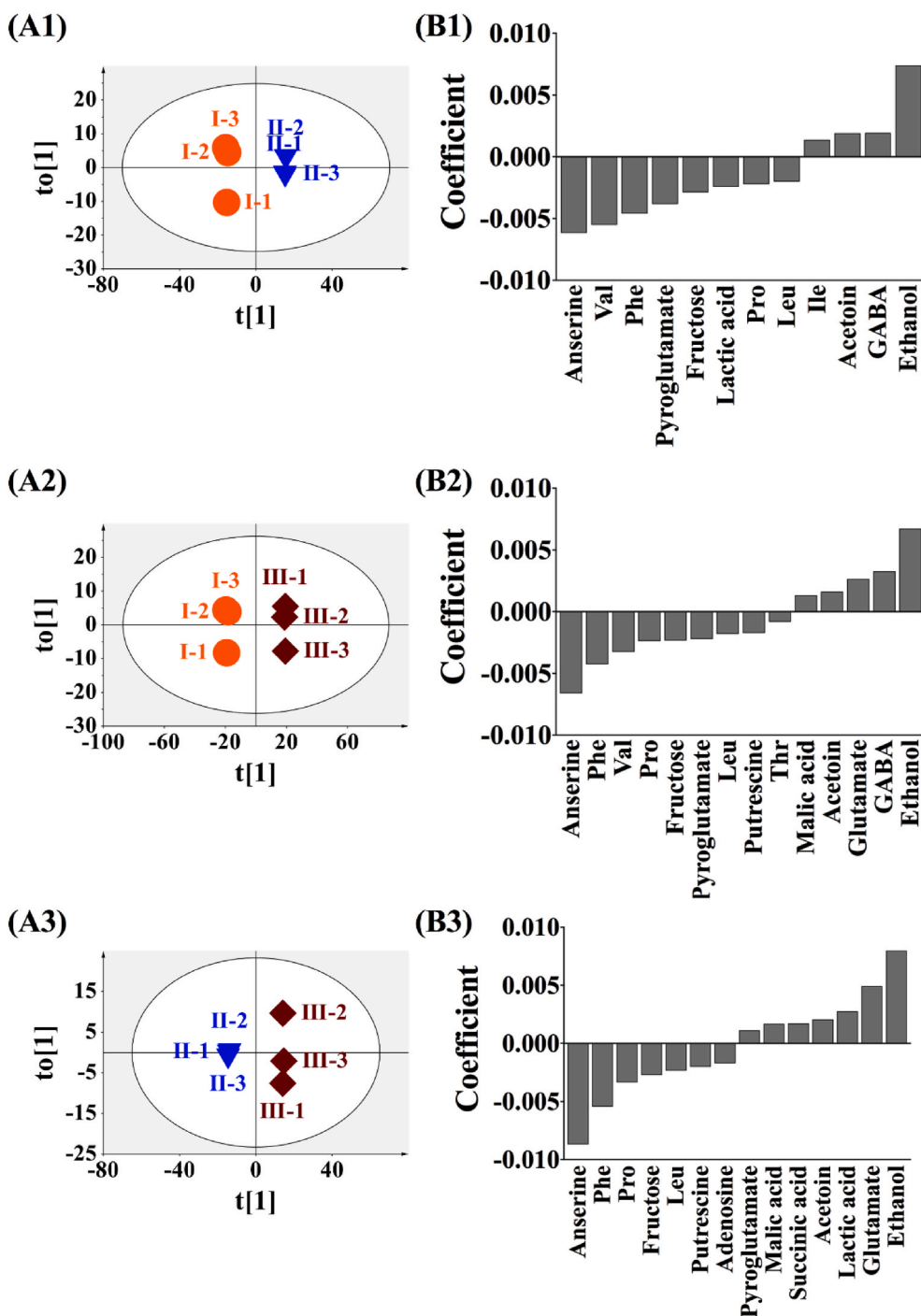


Fig. 7. Orthogonal projections to latent structures discriminant analysis (OPLS-DA) pairwise comparison between groups. OPLS-DA scores (A1) and coefficient plot (B1) of group I-II, $R^2 = 0.99$, $Q^2 = 0.98$; OPLS-DA scores (A2) and coefficient plot (B2) of group I-III, $R^2 = 0.99$, $Q^2 = 0.99$; OPLS-DA scores (A3) and coefficient plot (B3) of group II-III, $R^2 = 0.99$, $Q^2 = 0.99$. Note: group I-III, *L. innocua* under DI water, AE, and BE treatments, respectively. DI: deionised; BE: NaHCO_3 added before electrolysis; AE: NaHCO_3 added after electrolysis.

The accumulation of GABA (5.10-fold increase) and glutamate (1.66-fold increase) compared with the control group was only found in *L. innocua* on shrimp after BE treatment, which was suggested in a report that deletion of *gadD* could lead to an increase of GABA, which enhanced microbial survival (Liu et al., 2019). An elevated level of glutamate was also observed, which serves as the precursors for many stress-associated metabolites, including GABA and putrescine (Bucur et al., 2018; Zhao et al., 2020). The use of arginine as nitrogen source for bacteria relied on the GABA shunt pathway by converting from arginine to GABA via putrescine. The GABA shunt pathway could also act as an alternative route to produce succinic acid to compensate for the disrupted TCA cycle (Feehily et al., 2013). Therefore, no significant change in succinic acid level was observed on shrimp. The higher level of GABA and glutamate

could compensate for the TCA cycle on shrimp.

Overall, there were several differences when comparing the metabolic changes between *L. innocua* in TSB and on shrimp surface during the EW treatment. Compared with the metabolite changes in broth, the reduction of fewer types of amino acids (alanine and arginine) and sugars (F-6-P and G-1-P) was observed on shrimp. It could be explained by the differences in the nutritional compositions between TSB and shrimp when encountering the EW. Moreover, more metabolic changes were observed in *L. innocua* in TSB, indicating that bacteria in broth medium were more sensitive to the EW treatment than those on the shrimp surface. It was consistent with a previous study reporting that the food matrix may hinder the bactericidal activity of EW (Al-Holy & Rasco, 2015). The active free chlorine could reactive with the organic

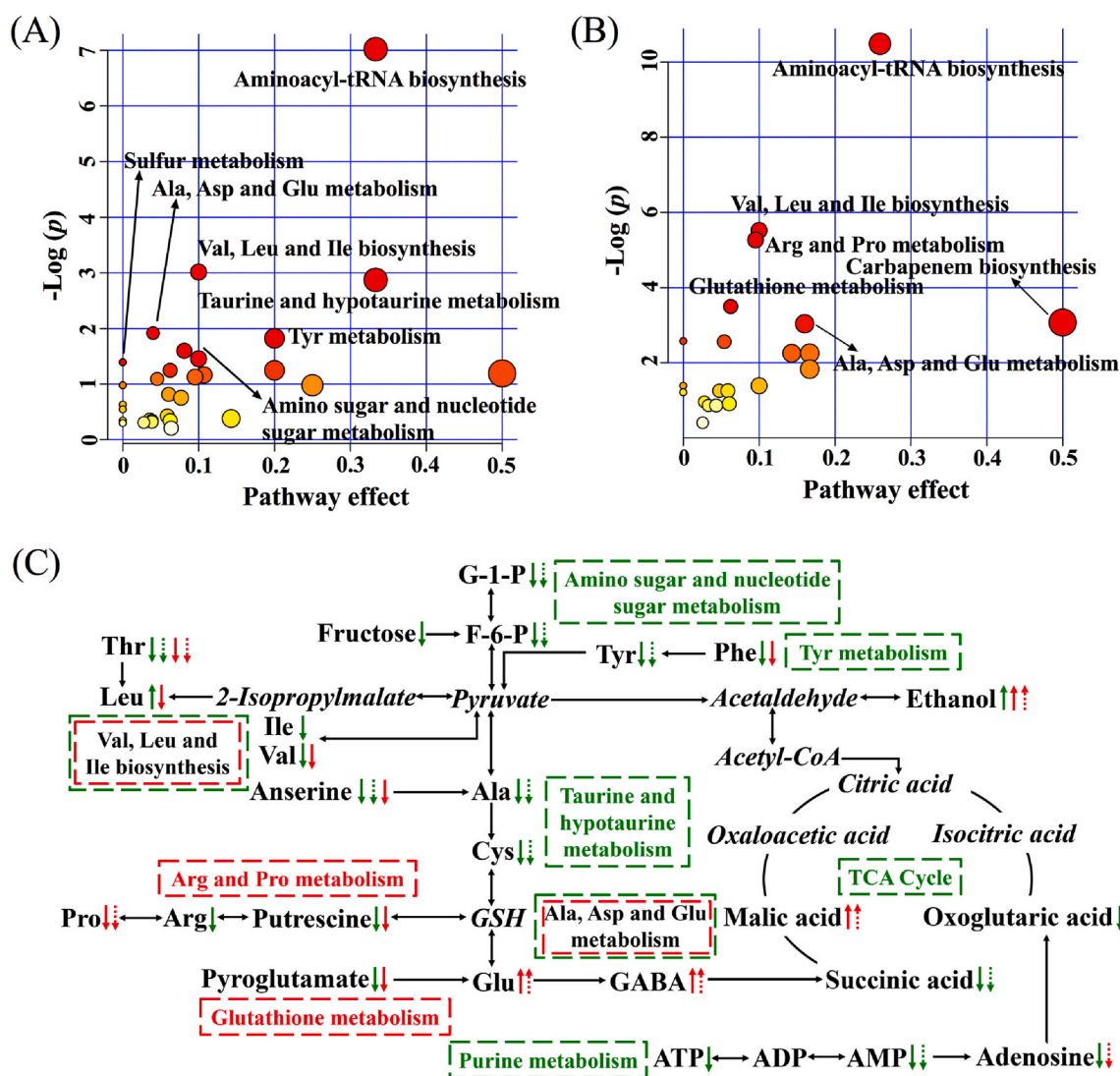


Fig. 8. Overview of the pathway analysis of *L. innocua* in broth (A) and on shrimp (B); Proposed schematic of metabolic pathway alteration under the BE treatment (C). Notes: Upward and downward arrows represent the increase and decrease of metabolite levels (variable importance in projection (VIP) > 1 with a fold change > 1.20 or < 0.83) in broth (green) and on shrimp (red), respectively. Solid arrows: metabolite changes compared with control group; dotted arrows: compared with AE group. Pathways framed in green and red indicate the alteration in the metabolic pathways that occurred only in broth or shrimp, respectively. Metabolites in italic black were not identified. BE: add NaHCO_3 before electrolysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

matter of the shrimp to generate the chlorine form, which showed less efficiency against bacteria (Deborde & Gunten, 2008). Besides, the irregular surface of the shrimp might provide shelter for the microbes (Al-Qadiri et al., 2019).

3.6. Comparison of pathway alternation of *L. innocua* between AE and BE treatment

The screened metabolites (VIP > 1) with a fold change > 1.20 or < 0.83 induced by BE treatment compared with the AE group in broth and on shrimp are also listed in Table S4 and the related pathway is shown in Fig. 8C. Compared with AE treatment, lower level of several amino acids such as proline, tyrosine, threonine, alanine, and cysteine was observed, demonstrating that more amino acid biosynthesis pathways were affected by the BE treatment, which has been reported to be very sensitive to oxidative stress (Jozefczuk et al., 2010). The elevated level of GABA and glutamate was also observed when compared with AE treatment. GABA played a role in antioxidant defense when encountering oxidative stress (Liu et al., 2017). The synthesis of GABA was

catalysed by glutamate decarboxylase from glutamate (Feehily et al., 2013). A marked increasing level of glutamate and GABA in BE treated *L. innocua* indicating that more glutamate was used for synthesis of GABA during BE stress.

The electrolysis of NaCl solution can generate many different forms of chlorine. The main form of the chlorine was HClO when the pH of the solution ranging from 5.0 to 6.5 in our research. HClO could penetrate bacterial cell membranes and produce hydroxyl radicals, which exert antibacterial effects through oxidation in key metabolic frameworks (Chhetri et al., 2019; Singh et al., 2018). Besides, the OPR value we generated was above 800 mV, which was much higher than the optimal value for microbial growth, leading to the oxidation of sulfhydryl mixtures on the cell surface and perturbing the metabolic pathways inside the cell (Liao et al., 2007). Moreover, the electrolysis of NaHCO_3 could produce peroxycarbonate and its derivatives and even $\cdot\text{OH}$ radicals (Fallanaj et al., 2016; Zhang, Lai, & Yang, 2018) which can also lead to extra oxidative stress and change the cell metabolism, leading to greater metabolic change.

4. Conclusion

The addition of NaHCO₃ before electrolysis enhanced the sanitising efficiency against *L. innocua* inoculated both in broth and on shrimp. The generated EW by the addition of NaHCO₃ before electrolysis triggered the accumulation of more ROS, leading to a lower level of intracellular ATP than adding NaHCO₃ afterward. The NMR-based metabolomics further demonstrated the EW generated with NaHCO₃ electrolysis could generate more oxidative stress leading to significant metabolic changes, mainly altering the amino acid metabolism and energy metabolism. The *in vivo* test also indicated the efficient reduction of *L. innocua* on shrimp. Moreover, because of the nutritional compositions of shrimp, there were several differences in the metabolic responses of *L. innocua* in broth and on shrimp. Fewer amino acid pathways and sugar metabolism were affected by the EW treatment on shrimp due to the degradation of protein by *L. innocua*. The accumulation of GABA and glutamate were observed on shrimp, which compensated for the disrupted TCA cycle caused by EW stress, finally showing that *L. innocua* on shrimp was more tolerant than those grown in broth culture. These findings suggest that a promising sanitising unit could be developed to control the foodborne pathogens in broth and complex food matrices and provide some guidance for seafood industry sanitisation.

CRediT authorship contribution statement

Yun He: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft. **Xue Zhao:** Data curation, Formal analysis, Investigation. **Lin Chen:** Formal analysis, Investigation, Software. **Lin Zhao:** Resources, Software. **Hongshun Yang:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodcont.2021.108134>.

References

Acton, B. M. (2004). Alterations in mitochondrial membrane potential during preimplantation stages of mouse and human embryo development. *Molecular Human Reproduction*, *10*, 23–32.

Al-Holy, M. A., & Rasco, B. A. (2015). The bactericidal activity of acidic electrolysed oxidizing water against *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on raw fish, chicken and beef surfaces. *Food Control*, *54*, 317–321.

Al-Qadiri, H. M., Smith, S., Sielaff, A. C., Govindan, B. N., Ziyaina, M., Al-Alami, N., & Rasco, B. (2019). Bactericidal activity of neutral electrolysed water against *Bacillus cereus* and *Clostridium perfringens* in broth and artificially inoculated onto the surface of selected fresh produce and polypropylene cutting boards. *Food Control*, *96*, 212–218.

Bucur, F. I., Grigore-Gurgu, L., Crauwels, P., Riedel, C. U., & Nicolau, A. I. (2018). Resistance of *Listeria monocytogenes* to stress conditions encountered in food and food processing environments. *Frontiers in Microbiology*, *9*, 2700.

Cai, L. L., Hu, H. J., Lu, Q., Wang, H. H., Xu, X. L., Zhou, G. H., Kang, Z. L., & Ma, H. J. (2019). Morphophysiological responses of detached and adhered biofilms of *Pseudomonas fluorescens* to acidic electrolyzed water. *Food Microbiology*, *82*, 89–98.

Chen, L., Wu, J., Li, Z., Liu, Q., Zhao, X., & Yang, H. (2019). Metabolomic analysis of energy regulated germination and sprouting of organic mung bean (*Vigna radiata*) using NMR spectroscopy. *Food Chemistry*, *286*, 87–97.

Chen, L., Zhang, H., Liu, Q., Pang, X., Zhao, X., & Yang, H. (2019). Sanitising 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 characterisation of eight *Escherichia coli* strains including “Big Six” and acidic responses of selected strains revealed by NMR spectroscopy. *Food Microbiology*, *88*, Article 103399.

Chhetri, V. S., Janes, M. E., King, J. M., Doerler, W., & Adhikari, A. (2019). Effect of residual chlorine and organic acids on survival and attachment of *Escherichia coli* O157: H7 and *Listeria monocytogenes* on spinach leaves during storage. *LWT- Food Science and Technology*, *105*, 298–305.

Costa, A., Lourenco, A., Civera, T., & Brito, L. (2018). *Listeria innocua* and *Listeria monocytogenes* strains from dairy plants behave similarly in biofilm sanitiser testing. *LWT-Food Science and Technology*, *92*, 477–483.

Cui, H., Zhang, C., Li, C., & Lin, L. (2018). Antimicrobial mechanism of clove oil on *Listeria monocytogenes*. *Food Control*, *94*, 140–146.

Deborde, M., & Gunten, U. (2008). Reactions of chlorine with inorganic and organic compounds during water treatment—kinetics and mechanisms: A critical review. *Water Research*, *42*, 13–51.

Dupre, J. M., Johnson, W. L., Ulanov, A. V., Li, Z., Wilkinson, B. J., & Gustafson, J. E. (2019). Transcriptional profiling and metabolomics analysis of *Staphylococcus aureus* grown on autoclaved chicken breast. *Food Microbiology*, *82*, 46–52.

Fallanaj, F., Ippolito, A., Ligorio, A., Garganese, F., Zavanella, C., & Sanzani, S. M. (2016). Electrolysed sodium bicarbonate inhibits *Penicillium digitatum* and induces defence responses against green mould in citrus fruit. *Postharvest Biology and Technology*, *115*, 18–29.

Feehily, C., O’Byrne, C. P., & Karatzas, K. A. G. (2013). Functional γ -aminobutyrate shunt in *Listeria monocytogenes*: Role in acid tolerance and succinate biosynthesis. *Applied and Environmental Microbiology*, *79*(1), 74–80.

Freitas, D. D. S., Carlos, E. F., Gil, M. C. S., Vieira, L. G. E., & Alcantara, G. B. (2015). NMR-based metabolomics analysis of Huanglongbing-asymptomatic and symptomatic citrus trees. *Journal of Agricultural and Food Chemistry*, *63*, 7582–7588.

Gupta, S., Seydel, K., Miranda-Roman, M. A., Feintuch, C. M., Saidi, A., Kim, R. S., Birbeck, G. L., Taylor, T., & Daily, J. P. (2017). Extensive alterations of blood metabolites in pediatric cerebral malaria. *PLoS One*, *12*(4), Article e0175686.

Han, X., Wang, Z., Wang, X., Zheng, X., Ma, J., & Wu, Z. (2016). Microbial responses to membrane cleaning using sodium hypochlorite in membrane bioreactors: Cell integrity, key enzymes and intracellular reactive oxygen species. *Water Research*, *88*, 293–300.

Hao, J., Qiu, S., Li, H., Chen, T., Liu, H., & Li, L. (2012). Roles of hydroxyl radicals in electrolysed oxidizing water (EOW) for the inactivation of *Escherichia coli*. *International Journal of Food Microbiology*, *155*, 99–104.

He, L., Deng, Q. L., Chen, M. T., Wu, Q. P., & Lu, Y. J. (2015). Proteomics analysis of *Listeria monocytogenes* ATCC 19115 in response to simultaneous triple stresses. *Archives of Microbiology*, *197*, 833–841.

Horlboog, J. A., Stevens, M. J. A., Stephan, R., & Guldemann, C. (2019). Global transcriptional response of three highly acid-tolerant field strains of *Listeria monocytogenes* to HCl stress. *Microorganisms*, *7*, 455–464.

Jozefczuk, S., Klie, S., Catchpole, G., Szymanski, J., Cuadros, A., Steinhäuser, D., Selbig, J., & Willmitzer, L. (2010). Metabolomic and transcriptomic stress response of *Escherichia coli*. *Molecular Systems Biology*, *6*(1), 364.

Knudsen, G. M., Fromberg, A., Ng, Y., & Gram, L. (2016). Sublethal concentrations of antibiotics cause shift to anaerobic metabolism in *Listeria monocytogenes* and induce phenotypes linked to antibiotic tolerance. *Frontiers in Microbiology*, *7*, 1091.

Liao, L. B., Chen, W. M., & Xiao, X. M. (2007). The generation and inactivation mechanism of oxidation–reduction potential of electrolysed oxidizing water. *Journal of Food Engineering*, *78*, 1326–1332.

Li, J., Ding, T., Liao, X., Chen, S., Ye, X., & Liu, D. (2017). Synergistic effects of ultrasound and slightly acidic electrolyzed water against *Staphylococcus aureus* evaluated by flow cytometry and electron microscopy. *Ultrasonics Sonochemistry*, *38*, 711–719.

Li, A. P., Li, Z. Y., Sun, H. F., Li, K., Qin, X. M., & Du, G. H. (2015). Comparison of two different astragali radix by a ¹H NMR-based metabolomic approach. *Journal of Proteome Research*, *14*, 2005–2016.

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.

Liu, Y., Orsi, H., Gaballa, A., Wiedmann, M., Boor, K., & Oropesa, V. (2019). Systematic review of the *Listeria monocytogenes* σ^B regulon supports a role in stress response, virulence and metabolism. *Future Microbiology*, *14*, 801–828.

Liu, Q., Wu, J., Lim, Z. Y., Aggarwal, A., Yang, H., & Wang, S. (2017). Evaluation of the metabolic response of *Escherichia coli* to electrolysed water by ¹H NMR spectroscopy. *LWT-Food Science and Technology*, *79*, 428–436.

Liu, Q., Wu, J. E., Lim, Z. Y., Lai, S., Lee, N., & Yang, H. (2018). Metabolite profiling of *Listeria innocua* for unravelling the inactivation mechanism of electrolysed water by

- nuclear magnetic resonance spectroscopy. *International Journal of Food Microbiology*, 271, 24–32.
- 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.
- Low, Y. M., Chong, C. W., Yap, I. K. S., Chai, L. C., Clarke, S. C., Ponnampalavanar, S., & Teh, C. S. J. (2018). Elucidating the survival and response of carbapenem resistant *Klebsiella pneumoniae* after exposure to imipenem at sub-lethal concentrations. *Pathogens and Global Health*, 112(7), 378–386.
- Lungu, B., Ricke, S. C., & Johnson, M. G. (2009). Growth, survival, proliferation and pathogenesis of *Listeria monocytogenes* under low oxygen or anaerobic conditions: A review. *Anaerobe*, 15, 7–17.
- Olaimat, A. N., Al-Holy, M. A., Shahbaz, H. M., Al-Nabulsi, A. A., Abu Ghoush, M. H., Osaili, T. M., Ayyash, M. M., & Holley, R. A. (2018). Emergence of antibiotic resistance in *Listeria monocytogenes* isolated from food products: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, 17, 1277–1292.
- Park, S. E., Seo, S. H., Kim, E. J., Byun, S., Na, C. S., & Son, H. S. (2019). Changes of microbial community and metabolite in kimchi inoculated with different microbial community starters. *Food Chemistry*, 274, 558–565.
- Rahman, S. M. E., Khan, I., & Oh, D. H. (2016). Electrolyzed water as a novel sanitizer in the food industry: Current trends and future perspectives. *Comprehensive Reviews in Food Science and Food Safety*, 15(3), 471–490.
- Shen, C., Lemonakis, L., Etienne, X., Li, K., Jiang, W., & Adler, J. M. (2019). Evaluation of commercial antimicrobials against stress-adapted *Campylobacter jejuni* on broiler wings by using immersion and electrostatic spray and an economic feasibility analysis. *Food Control*, 103, 161–166.
- Simon, A., Fujioka, T., Price, W. E., & Nghiem, L. D. (2014). Sodium hydroxide production from sodium carbonate and bicarbonate solutions using membrane electrolysis: A feasibility study. *Separation and Purification Technology*, 127, 70–76.
- Singh, P., Hung, Y. C., & Qi, H. (2018). Efficacy of peracetic acid in inactivating foodborne pathogens on fresh produce surface. *Journal of Food Science*, 83(2), 432–439.
- Wu, Z., Cheng, Y., Yang, Y., Gao, Y., Sun, X., Wang, L., Sun, Q., Zhang, J., & Xu, X. (2020). In vitro and in vivo anti-*Listeria* effect of succinoglycan riclin through regulating MAPK/IL-6 axis and metabolic profiling. *International Journal of Biological Macromolecules*, 150, 802–813.
- Wu, J., Zhao, L., Lai, S., & Yang, H. (2021). NMR-based metabolomic investigation of antimicrobial mechanism of electrolysed water combined with moderate heat treatment against *Listeria monocytogenes* on salmon. *Food Control*, 125, Article 107974.
- Youssef, K., Sanzani, S. M., Ligorio, A., Ippolito, A., & Terry, L. A. (2014). Sodium carbonate and bicarbonate treatments induce resistance to postharvest green mould on citrus fruit. *Postharvest Biology and Technology*, 87, 61–69.
- Zhang, J., Lai, S., & Yang, H. (2018). Physicochemical and antibacterial effects of sodium bicarbonate and brine water on the electrolysed water generated by a portable sanitising unit. *LWT-Food Science and Technology*, 98, 524–532.
- Zhang, J., Yang, H., & Chan, J. Z. Y. (2018). Development of portable flow-through electrochemical sanitising unit to generate near neutral electrolysed water. *Journal of Food Science*, 83(3), 780–790.
- Zhao, X., Chen, L., Wu, J. E., 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, Article 108494.
- Zhao, L., Li, S., & Yang, H. (2021). Recent advances on research of electrolyzed water and its applications. *Current Opinion in Food Science*. <https://doi.org/10.1016/j.cofs.2021.03.004>
- Zhao, X., Wu, J., Chen, L., & Yang, H. (2019). Effect of vacuum impregnated fish gelatin and grape seed extract on metabolite profiles of tilapia (*Oreochromis niloticus*) fillets during storage. *Food Chemistry*, 293, 418–428.