



Antimicrobial activity of syringic acid against *Cronobacter sakazakii* and its effect on cell membrane



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ABSTRACT

Syringic acid (SA) has been reported to exhibit antibacterial ability against various microorganisms, but little work has been done on its effect on *Cronobacter sakazakii*. In this study, minimum inhibitory concentrations (MICs) of SA against various *C. sakazakii* strains were determined. Moreover, changes in intracellular ATP concentration, intracellular pH (pH_{in}), membrane potential and membrane integrity were measured to evaluate the influence of SA on cell membrane. Finally, field emission scanning electron microscope (FESEM) was used to assess the morphological changes of bacterial cells caused by SA. It was shown that the MICs of SA against all tested *C. sakazakii* strains were 5 mg/mL. SA retarded bacterial growth, and caused cell membrane dysfunction, which was evidenced by intracellular ATP concentration decrease, pH_{in} reduction, cell membrane hyperpolarization and changes in cellular morphology. These findings indicated that SA has potential to be developed as a natural preservative to control *C. sakazakii* in foods associated with this pathogen and prevent related infections.

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1. Introduction

Cronobacter sakazakii is an opportunistic pathogen that has been implicated in bacteremia, necrotizing enterocolitis, and neonatal meningitis (Iversen, Waddington, On, & Forsythe, 2004). The bacterium has been isolated from a range of food sources including dairy-based foods, dried meats, milk and fruit powders, vegetables, and rice etc. (Beuchat et al., 2009). In particular, powdered infant formula (PIF) has been epidemiologically linked with many of *C. sakazakii* infections reported (Yan et al., 2012). Infants with a low birth weight (<2500 g) and premature (<37 weeks) infants are most susceptible to infections, and the reported fatality rates for *C. sakazakii* infections in these two groups are 50–80% (Healy et al., 2010). In addition to neonates, *C. sakazakii* can also cause infections in immuno-compromised adults and the elderly (Yan et al., 2012).

To control the level of *C. sakazakii* in PIF or other related foods, various intervention technologies have been developed or explored, such as simultaneous near-infrared radiant heating, UV radiation, microwave processing and electron beam irradiation (Ha & Kang, 2014). However, these methods may cause a loss of

sensitive nutrients from the formula and have limitations because of high capital costs for installation (Ha & Kang, 2014; Harouna et al., 2015). Chemical preservatives have also been commonly used to prevent microbial contamination in various foods, but their application in food is not favored by consumers due to their synthetic nature and possible negative effect on human health (Kashani, Nikzad, Mobaseri, & Hoseini, 2012). There is currently an increased trend in food industry to use natural antimicrobials, which gain increased consumer acceptance (Joshi, Howell, & D'Souza, 2014). Several plant extracts or phytochemicals have been reported to exhibit inhibitory effect on *C. sakazakii*, including blueberry proanthocyanidins, commercial blueberry juice, trans-cinnamaldehyde, caprylic acid and citric acid (Amalaradjou & Venkitanarayanan, 2011; Frankova et al., 2014).

Syringic acid (4-hydroxy-3, 5-dimethoxybenzoic acid, $\text{C}_9\text{H}_{10}\text{O}_5$, SA) is a hydroxybenzoic acid of phenolic compound (Fig. 1) (Campos et al., 2009). It has been found in *Lentinula edodes* (shiitake), cereal grains, *Herba dendrobii* (Shihu in Chinese), *Radix Isatidis* (Banlangen in Chinese) (Kong, Zhao, Shan, Xiao, & Guo, 2008) and the leaves of *Alpinia calcarata* Roscoe (Muthukumaran, Srinivasan, Venkatesan, Ramachandran, & Muruganathan, 2013). Previous studies have demonstrated that SA could suppress hepatic fibrosis in chronic liver injury (Itoh et al., 2010) and exhibit antidiabetic activity in experimental diabetes mice models (Muthukumaran et al., 2013). SA has also been reported to show

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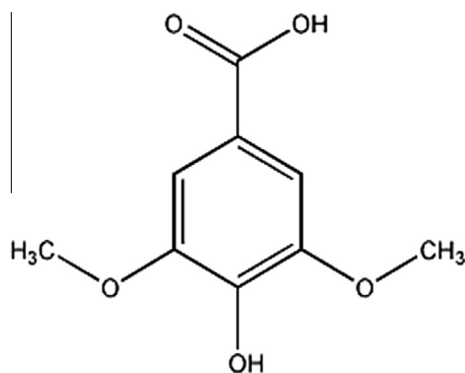


Fig. 1. Chemical structure of syringic acid (SA).

antibacterial activity against various microorganisms. Zaldivar and Ingram (1999) demonstrated that SA inhibited growth and ethanol production in *Escherichia coli* LY01 by collapsing ion gradients and increasing internal anion concentrations. In addition, SA could inactivate *Oenococcus oeni*, causing a noticeable decrease of viable cells after 90 min treatment (Campos, Couto, & Hogg, 2003).

Although antimicrobial activity of SA has been reported for certain microorganisms, its antimicrobial activity against *C. sakazakii* has been rarely reported and its effects on the pathogen's cell membrane remains unclear. In this study, minimum inhibitory concentrations (MICs) of SA against several *C. sakazakii* strains from clinical and food source were determined. Moreover, changes in cell membrane potential, intracellular ATP concentration, membrane integrity and intracellular pH (pH_{in}) were analyzed to explore possible mechanism of action. Finally, morphological changes of cell membrane were assessed by using field emission scanning electron microscope (FESEM).

2. Materials and methods

2.1. Reagents

SA (CAS: 530-57-4, HPLC purity $\geq 98\%$) was purchased from the Chengdu Must Bio-technology Co., Ltd (Chengdu, Sichuan, China). SA was dissolved in water containing 2% anhydrous ethanol (v/v) and filter-sterilized before use to minimize oxidation of the compound. All other chemicals were of analytical grade.

2.2. Bacterial strains and culture conditions

C. sakazakii strains ATCC 29544, ATCC 29004, ATCC 12868 and ATCC BAA-894 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Five other *C. sakazakii* strains were taken from our laboratory collection, which were isolated from various infant formula or powdered infant rice cereal in China. A loopful of each strain was inoculated into 30 mL of tryptone soya broth (TSB) and incubated for 18 h at 37 °C.

2.3. MIC determinations

MIC of SA was determined by agar dilution method as described by the European Committee with modification (European Committee for Antimicrobial Susceptibility Testing of the European Society of Clinical & Infectious, 2000). Ampicillin was used as reference antibiotic. Melted tryptic soy agar (TSA) was aseptically transferred into sterile 24-well plates containing either SA or ampicillin. The content (a final volume 500 μL) of each well was gently mixed. The final concentrations of SA were 0, 0.3125,

0.625, 1.25, 2.5, 5, and 10 mg/mL, whereas that of ampicillin was 0.1 mg/mL. After hardening, the agar in each well was spotted with 2 μL (approximately 10^4 CFU) of the tested bacterium. The spots were left to dry and then plates were incubated at 37 °C for 24 h. The lowest concentration of SA that results no visible growth of test organisms was determined as MIC.

2.4. Growth curves

The growth curves were constructed by the method described by Qiu et al. (2010) with some modifications. Briefly, *C. sakazakii* strain ATCC 29544 was grown to an OD_{600} value of 0.2 (approximately 1.5×10^8 CFU/mL) in TSB. Then 125 μL of the culture was transferred into each well on 96-well microtiter plates. SA was dissolved in 2% anhydrous ethanol (v/v) and added to the cultures to obtain final concentrations of 1/64MIC, 1/32MIC, 1/16MIC, 1/8MIC, 1/4MIC, 1/2MIC, MIC and 2MIC, and TSB containing 2% anhydrous ethanol (v/v) was used as a negative control. Bacteria were further cultured at 37 °C, and cell growth was monitored by enzyme micro-plate reader at 600 nm at 1 h intervals, using a multimode plate reader (Tecan, Infinite[™] M200 PRO, Männedorf, Switzerland).

2.5. Measurement of intracellular ATP concentrations

The intracellular ATP concentration of *C. sakazakii* strain ATCC 29544 was analyzed according to the method described by Sanchez, Garcia, and Heredia (2010), with some modifications. The overnight culture of *C. sakazakii* strain ATCC 29544 was harvested by centrifugation (5000g, 5 min). Then, the supernatant was removed and cells were washed three times with 0.1 mol/L of phosphate-buffered saline (PBS, pH 7.0). Cells were resuspended in PBS to achieve an OD_{600} of 0.5 (approximately 4×10^8 CFU/mL), and 2 mL of cell solution was taken into Eppendorf tubes. SA was then added to each tube to achieve final concentrations of 0 (control), MIC, and 2MIC, respectively. And the samples were incubated at 37 °C for 30 min. ATP were extracted by ultrasound on ice. Then the samples were centrifuged for 5 min at 5000g, the supernatant was taken and stored on ice to prevent ATP loss. ATP was measured by using an ATP assay kit according to manual instructions (Beyotime Bioengineering Institute, Shanghai, China).

2.6. Membrane potential determinations

The method described by Sanchez et al. (2010) was followed with minor modifications. Cells were grown in 30 mL of TSB at 37 °C to an OD_{600} of 0.5. Cells were then harvested by centrifugation (5000g, 5 min) and washed twice with PBS (pH 7.0). Next, 125 μL of cell suspensions were placed in black, opaque 96-well microtiter plates (Nunc, Copenhagen, Denmark) for 30 min at 37 °C. Then, 1 μM of the membrane potential-sensitive fluorescent probe bis-(1,3-dibutylbarbituric acid) trimethine oxonol (DiBAC₄ (3); Molecular Probes, Sigma, Louis, USA) was added, which is an anionic, fluorescent membrane potential dye that has been used as an indicator of changes in membrane potential. The mixture was then incubated for 30 min at 37 °C. SA was added to achieved final concentrations of 0, MIC, and 2MIC. After 5 min, fluorescence was measured at the excitation and emission wavelengths of 492 and 515 nm, using a fluorescence microplate reader (Tecan, Infinite[™] M200 PRO, Männedorf, Switzerland). The excitation and emission slit widths were 3 and 5 nm, respectively. Background fluorescence resulting from the medium was determined and the results corrected.

2.7. pH_{in} measurements

Intracellular pH was determined according to a modified method of Breeuwer, Drocourt, Rombouts, and Abee (1996). Two hundred and fifty microliter of overnight cultures of *C. sakazakii* strain ATCC 29544 was transferred into TSB (30 mL) and incubated at 37 °C for 8 h. Cells were then harvested by centrifugation (5000g, 10 min) and washed twice with 50 mmol/L HEPES buffer (containing 5 mmol/L EDTA, pH 8). The cell pellet was re-suspended in 20 mL of this buffer. Then, 3.0 µmol/L of the probe, carboxyfluorescein diacetate succinimidyl ester (cFDA-SE; Molecular Probes, Sigma, Louis, USA) was added and the mixture was incubated for 20 min at 37 °C. Cells were washed once with 50 mmol/L potassium phosphate buffer with 10 mmol/L MgCl₂ (pH 7.0), and re-suspended in 10 mL of the same buffer. To eliminate non-conjugated cFDA-SE, glucose (10 mmol/L) was added and the cells were incubated for an additional 30 min at 37 °C. Lastly, cells were washed twice as described before, then re-suspended in 50 mmol/L potassium phosphate buffer, and stored on ice.

An aliquot (2 mL) of cell suspension labeled by fluorescence was treated with SA at three different concentrations (0, MIC and 2MIC) in black, opaque 96-well microtiter plates (Nunc, Copenhagen, Denmark). After treatment for 20 min, fluorescence intensities were measured under two excitation wavelengths (440 nm and 490 nm). The emission was collected at 520 nm, where excitation and emission slit widths were 9 and 20 nm, respectively. Intracellular pH was determined as the ratio of the fluorescence signal at the pH-sensitive wavelength (490 nm) and that at the pH-insensitive wavelength (440 nm). All of the measurements were made by a microplate reader (Tecan, Infinite™ M200 PRO, Männedorf, Switzerland). The system was maintained at 25 °C during the assay. The fluorescence of the cell-free controls was measured and deducted from fluorescence of samples.

Calibration curves were constructed for cFDA-SE loaded cells with different pHs buffers. Buffers consisted of glycine (50 mmol/L), citric acid (50 mmol/L), Na₂HPO₄·2H₂O (50 mmol/L), and KCl (50 mmol/L). pH was adjusted with either NaOH or HCl to various values (3, 4, 5, 6, 7, 8, 9, and 10). After pH_{in} and pH_{out} were equilibrated by addition of valinomycin (10 µmol/L) and nigericin (10 µmol/L), the fluorescence intensity was measured at 25 °C.

2.8. Assessing the percent of bacterial membrane integrity

In this study, the LIVE/DEAD® BacLight™ Bacterial Viability Kit (Molecular Probes, Eugene, OR) was utilized to assess the percent of the membrane damaged according to a modified method of Alakomi, Mattö, Virkajarvi, and Saarela (2005). The overnight culture of *C. sakazakii* strain ATCC 29544 was harvested by centrifugation (10,000g, 15 min). Then, the supernatant was removed and the pellet was re-suspended in 2 mL of 0.85% NaCl. To obtain viable and non-viable cells, 1 mL of the suspension was added to each of two 50 mL centrifuge tubes containing either 20 mL of 0.85% NaCl (for live bacteria) or 20 mL of 70% isopropyl alcohol (for killed bacteria). After incubation at room temperature for 1 h (mixing every 15 min), both samples were pelleted by centrifugation twice at 10,000g for 10 min. The optical density was adjusted to an OD₆₀₀ of 0.5 and five viable cells' proportions (0%, 10%, 50%, 90% and 100%) were made to obtain standard samples. A 2× working stain solution was prepared by mixing equal volumes (6 µL) of SYTO 9 and PI and adding the mixture into 2 mL of filter-sterilized water.

Cells treated with SA (0, MIC, 2MIC at 37 °C, 15 min) were centrifuged (11,000g, 1 min) and re-suspended in 0.85% NaCl. One hundred microliter of standard samples and cell suspension were pipetted in three parallels into black, opaque 96-well microtiter plates (Nunc, Copenhagen, Denmark). Aliquots of 100 µL of the

2× staining solution were added to each well and mixed thoroughly. The plate was then incubated at 25 °C in the dark for 15 min and the fluorescence was measured with a fluorescence microplate reader (Tecan, Infinite™ M200 PRO, Männedorf, Switzerland). The excitation/emission maxima for the dyes are 485/542 nm for SYTO 9 and 485/610 nm for PI. Suspension with 2% anhydrous ethanol in the absence of SA served as control.

2.9. FESEM analysis

FESEM observation was performed as described previously with some modifications (Li, Wang, Xu, Zhang, & Xia, 2014). Cells (OD₆₀₀ = 0.5) were treated with SA at 0, MIC and 2MIC. After incubation at 37 °C for 4 h, cells were harvested by centrifugation for 10 min at 5000g and washed twice with PBS (pH 7.0). Then, cells were re-suspended in water containing 2.5% glutaraldehyde and kept at −4 °C for 12 h. After centrifugation, the cells were dehydrated in water-alcohol solutions at various alcohol concentrations (30%, 50%, 70%, 80%, 90% and 100%). The samples were then fixed on FESEM support, and sputter-coated with gold under vacuum, followed by microscopic examination using a field emission scanning electron microscope (S-4800, Hitachi, Tokyo, Japan).

2.10. Statistical analysis

All experiments were performed in triplicate, and each biological replicate included two technical replicates. Statistical analyses were performed using SPSS software (version 19.0; SPSS, Inc., Chicago, IL). The data were presented as the mean values ± SD (*n* = 3) and differences between means were tested by Student's *t*-test. Differences are considered significant at *P* ≤ 0.01.

3. Results

3.1. MIC of SA on *C. sakazakii* strains

SA showed moderate inhibitory effects against nine tested *C. sakazakii* strains (Table 1). The MICs of SA against ATCC 29544, ATCC 12868, ATCC BAA-894, ATCC 29004 and other tested strains were 5 mg/mL. *C. sakazakii* ATCC 29544 was used for further studies.

3.2. Growth curves

When grown in media with SA at MIC concentration, the growth rate of ATCC 29544 was significantly decreased but not completely inhibited, after 4, 8 and 12 h of SA treatment, OD₆₀₀ values were 32.9%, 32.7% and 37.7% of the control culture, respectively. Moreover, concentrations ranging from the 1/2MIC to 1/8MIC exhibited slight inhibition against *C. sakazakii* ATCC 29544 (Fig. 2).

Table 1
Minimum inhibitory concentrations of SA against different *C. sakazakii* strains.

Strains	MIC (mg/mL)
ATCC 29544	5
ATCC BAA-894	5
ATCC 29004	5
ATCC 12868	5
18-8	5
18-13	5
18-7	5
12-2	5
14-15	5

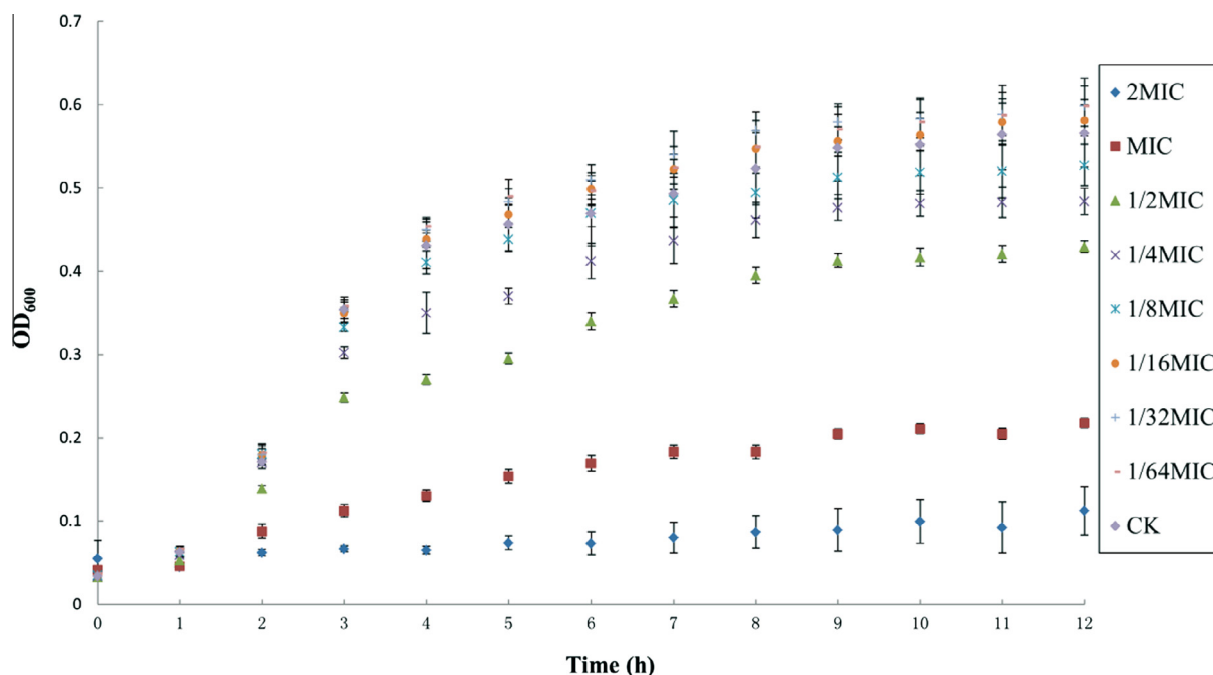


Fig. 2. Growth curves for *C. sakazakii* ATCC 29544 cultured in TSB with SA at different concentrations. Each value represents the average of six independent measurements. Bars represent the standard deviation.

3.3. Intracellular ATP concentrations

Data were analyzed to assess the effects of SA on intracellular ATP concentrations (Fig. 3-A). Significant reduction ($P \leq 0.01$) of ATP concentration occurred when *C. sakazakii* ATCC 29544 was treated with SA at MIC and 2MIC comparing the control, while no significant difference ($P > 0.05$) was found between cells treated at two different concentrations of SA.

3.4. Membrane potential

The change of the membrane potential of *C. sakazakii* strain ATCC 29544 was investigated by using a membrane potential sensitive fluorochrome, DiBAC₄ (3). Cells treated with SA displayed cell membrane hyperpolarization, as evidenced by a noticeable decrease in fluorescence (negative values) (Fig. 3B). In addition, more decrease in membrane potential was observed when concentration of SA increased from MIC to 2MIC.

3.5. pH_{in}

A significant change in pH_{in} was observed after the addition of SA (Fig. 3C). The pH_{in} of normal *C. sakazakii* ATCC 29544 was 6.10 ± 0.10 . SA at MIC caused a significant ($P \leq 0.01$) decrease in pH_{in} from 6.10 ± 0.10 to 4.65 ± 0.19 . pH_{in} decreased ($P \leq 0.01$) from 6.10 ± 0.10 to 3.58 ± 0.14 when cells were treated with SA at 2MIC. There was a significant ($P \leq 0.01$) difference in pH_{in} by increasing the concentration of SA from MIC to 2MIC.

3.6. Fluorimetric detection of cell membrane injury

There was a good linearity between the green fluorescent intensity and the percent of viable bacteria ($R^2 = 0.99$). Membrane-damaged cells (<4%) in controls were negligible. SA at MIC caused a 39% reduction of cell fluorescence, while SA at 2MIC caused a 54% decrease.

3.7. FESEM observation

FESEM demonstrated that cell membrane changes occurred after treatment with SA. *C. sakazakii* cells exposed to SA had a more shrinkage surface (Fig. 4B and C) compared than the normal smooth cell surface (Fig. 4A). Moreover, cells treated with SA at 2MIC showed surface collapse (Fig. 4C). The percent of damaged cells and degree of damage increased with the increase of SA concentration.

4. Discussion

This study describes antimicrobial activity and mode of action of SA against *C. sakazakii* strains. Our results showed that SA demonstrated a modest inhibitory effect against *C. sakazakii* strains compared with thymol (0.2 mg/mL) and eugenol (1.0 mg/mL) (Frankova et al., 2014). Growth curves showed that SA retarded the growth of *C. sakazakii*, and higher concentrations of SA resulted in a longer lag phase and a lower specific growth rate in exponential phase.

ATP is used for many cell functions including transport of substances across cell membranes. It could be a potential parameter for understanding the mode of action of antimicrobial agents (Bajpai, Sharma, & Baek, 2013). In prokaryotes, the cell membrane is not only important for cell energy conversion, but also for processing nutrients, synthesizing structural macromolecules and secretion of enzymes (Bajpai, Sharma, & Baek, 2014). Our results showed that the intracellular ATP concentration of *C. sakazakii* ATCC 29544 decreased rapidly when cells were exposed to SA, showing a disruption of cell viability. A similar finding has been reported by Ultee, Kets, and Smid (1999), they found that carvacrol (2 mmol/L) significantly depleted the intracellular ATP pool to values close to 0 within 7 min while no proportional increase of the extracellular ATP pool was observed. They also showed that depletion of the internal ATP pool was associated with a change of the membrane potential. Fitzgerald et al. (2004) reported that membrane perturbation, decrease in respiration and loss of K^+ and H^+

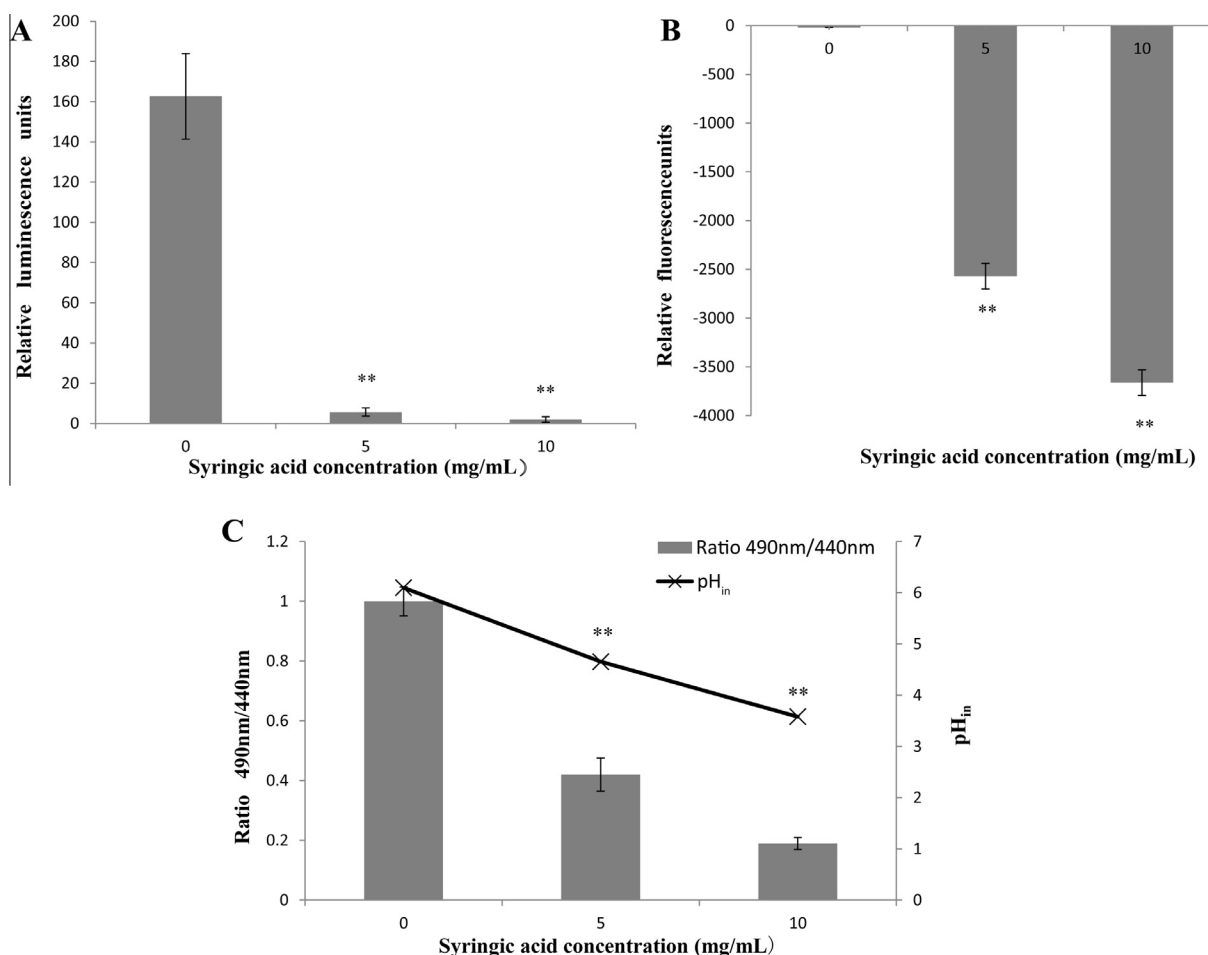


Fig. 3. (A) Effects of SA on intracellular ATP in *C. sakazakii* ATCC 29544, which was represented by relative luminescence. (B) Effects of SA on the membrane potentials of *C. sakazakii* ATCC 29544, which was represented by relative fluorescence. (C) Effects of SA on the intracellular pH (indicated by lines) of *C. sakazakii* ATCC 29544, which was calculated from the ratio of fluorescence emission intensities at 490 and 440 nm (indicated by bars). Values represent the means of independent triplicate measurements. Bars represent the standard deviation ($n = 3$). $^{**}P \leq 0.01$.

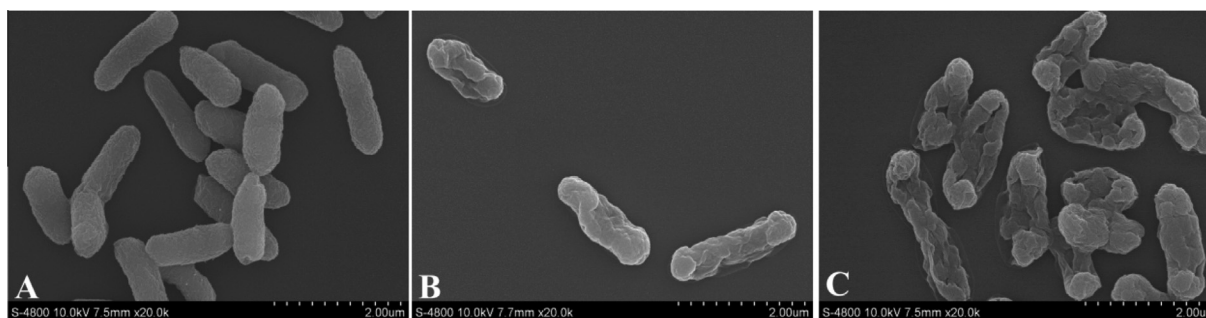


Fig. 4. Scanning electron micrographs of *C. sakazakii* ATCC 29544 untreated for 4 h (A), treated with SA at MIC for 4 h (B), and treated with SA at 2MIC for 4 h (C).

ion gradients could cause detrimental effect on cell energy generation. It was reported that exposure of *Bacillus cereus* cells to some monoterpene alcohols resulted in decreased level of intracellular ATP and disproportionately increased level of extracellular ATP (Burt, 2004). Reduction in intracellular ATP may be due to decreased rate of ATP synthesis and increased rate of ATP hydrolysis inside the cells, and the loss of inorganic phosphate through the highly compromised permeable cell membrane (Burt, 2004).

The pH_{in} is critical for the control of many cellular processes, such as DNA transcription, protein synthesis, and enzyme activity (Breeuwer et al., 1996). The change of pH_{in} can be a perfect

indicator of damage in bacterial cell. Cells with an intact membrane could maintain their internal pH via ion channels and pumps in the presence of a moderate change of outside pH (Fitzgerald et al., 2004). In this study, the decrease of pH_{in} was showed to correlate with the increase of SA concentration. Similarly, the addition of 0.025% (vol/vol) Spanish oregano, 0.025% (vol/vol) Chinese cinnamon, and 0.05% (vol/vol) savory essential oils caused a significant decrease ($P \leq 0.05$) of the pH_{in} of *E. coli* O157:H7 and the pH_{in} decreased from 7.25 to 6.68, 5.16, and 5.94, respectively (Oussalah, Cailliet, & Lacroix, 2006). Sanchez et al. (2010) reported that all the plant extracts used in their study induced changes in

the pH_{in} of *Vibrio cholerae*, indicating that membrane damage had occurred. Reduction of pH_{in} in *Staphylococcus aureus* has been observed when cells were treated with oregano essential oil, thymol, or carvacrol (Lambert, Skandamis, Coote, & Nychas, 2001).

In this study, SA caused membrane hyperpolarization as a result of a decrease of the membrane potential. Hyperpolarization has been reported as an important type of membrane damage (Yuroff, Sabat, & Hickey, 2003). Previous studies showed that hyperpolarization occurs primarily due to a pH change (from acidic to neutral) or increased movement of ions, specifically K^+ , which diffuses out of the cell membrane through K^+ channels and affects cellular homeostasis (Bot & Prodan, 2009; Sanchez et al., 2010). Ultee et al. (1999) observed that low concentrations of carvacrol (<0.01 mmol/L) cause a decrease of the membrane potential, and the membrane becomes more permeable for protons. Bennik, Vanloo, Brasseur, Gorris, and Smid (1998) reported that bactericidal effect of mundticin against *Listeria monocytogenes* was related to a rapid loss of the membrane potential, which indicates the dissipation of ionic gradients as a result of pore-formation.

The cell membrane is an active structure that acts as a barrier between the cytoplasm and the extracellular medium. It is essential to maintain optimal internal conditions for metabolism and energy transduction (Sanchez et al., 2010). Phenolic compounds have been shown to cause leakage of cell constituents (Johnston, Hanlon, Denyer, & Lambert, 2003). These compounds may diffuse through the cytoplasmic membrane and increasing membrane permeability. Following their influx, there is usually a leakage of bacterial cell constituents including proteins, nucleic acids, and inorganic ions such as potassium or phosphate (Cuvelier, Richard, & Berset, 1992). Many studies have suggested that phenolic compounds, such as vanillin, primarily target the cytoplasmic membrane via the accumulation of hydrophobic phenolic groups (hydroxyl group) in the lipid bilayer. This process could disrupt lipid protein interactions, alter the membrane structure, function, and permeability, accelerate the leakage of cell contents, disrupt the proton-motive force and electron influx, and finally destroy cell integrity (Char, Guerrero, & Alzamora, 2010). It is assumed that antimicrobial activity of SA may in part originate from its special chemical structure (methoxy and phenolic hydroxyl group specially) and its lipophilic character.

In previous studies, SA has been demonstrated to affect cell membrane permeability of lactic acid bacteria from wine by inducing ion leakages and proton influx (Campos et al., 2009). In the present study, although leakage of intracellular constituents was not evident from FESEM images, membrane damage could be evidenced by decrease of intracellular ATP concentration, reduction of pH_{in} and cell membrane hyperpolarization. Moreover, LIVE/DEAD assay and FESEM both confirmed damage in cell membrane. These findings suggest that SA showed antibacterial activity against *C. sakazakii* and exerted its mode of action on membrane permeability. SA could be a promising compound for inactivating *C. sakazakii* in various types of foods. However, further studies investigating its inhibitory activities in foods and effects on the organoleptic properties of foods are required prior to commercial application.

Conflict of interest

The authors declare that there are no conflicts of interest.

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