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Inactivation of *Escherichia coli* and *Staphyloccocus aureus* in Litchi Juice by Dimethyl Dicarbonate (DMDC) Combined With Nisin

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Abstract

Inactivation of Gram-negative *Escherichia coli* and Gram-positive *Staphyloccocus aureus* in litchi juice by DMDC combined with nisin was individually investigated. A 1.66 log cycles reduction of *E. coli* and 2.03 log cycles reduction of *S. aureus* in litchi juice (pH 4.5) added without nisin was achieved as exposed to 150 mg/l DMDC at 30 °C for 1 h, and the inactivation rate of *E. coli* and *S. aureus* during initial 1 h was far greater than during the remaining 5 h. As exposed to 150 mg/l DMDC at 30 °C for 1 h, the inactivation of *E. coli* and *S. aureus* in the litchi juice showed a trend toward increase with increasing of nisin addition level in the range from 0 to 200 IU/ml. Moreover, DMDC and nisin exhibited a synergistic effect on the inactivation of *E. coli* and *S. aureus* in litchi juice, and the inactivation of *E. coli* and *S. aureus* in the litchi juice also depends on the temperature of litchi juice, pH value of litchi juice and DMDC concentration when treated with DMDC and nisin. In addition, *E. coli* showed higher resistance to nisin as comparing with *S. aureus*. After *E. coli* and *S. aureus* in the litchi juice of pH 4.0 were individually treated with 150 mg/l DMDC combined with 200 IU/ml nisin at 30 °C for 1 h, a complete inactivation of *S. aureus* (6.59 log cycles) was achieved, but only 3.52 log cycles reduction of *E. coli* was observed.

Keywords: dimethyl dicarbonate (dmdc), nisin, litchi juice, inactivation, *E. coli*, *Staphyloccocus aureus*

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is a non-climacteric subtropical fruit with high commercial value for sweet and juicy flesh and attractive bright red pericarp. Apart from being consumed freshly, litchi fruit is also processed into juice, canned litchi and dried fruits. Litchi juice is enriched with sugar, minerals, vitamin, and various antioxidants and widely appreciated flavor, and is able to compete in the market, either as litchi juice or as mixtures with other juice (Wu et al., 2007; Zeng et al., 2008; Saxena et al., 2011). Thermal processing technologies used for inactivating the microorganism and enzymes in fruit juices are generally at 70-12 °C for 30-120 s and it seriously destroys the quality of litchi juice resulting from the loss of nutritional components and changes of color, flavor and texture due to heat sensitivity of litchi juice (Guo et al., 2011; Li et al., 2012). Besides the quality of juice, economic cost is another reason for industry to avoid thermal processing. Therefore, novel non-thermal techniques need to be evaluated for effective control of microorganism in litchi juice while not affecting product quality.

DMDC (dimethyl dicarbonate), a dicarbonic acid ester, is a colorless, transparent liquid with a fruity aroma. In 1988, dimethyl dicarbonate (DMDC) was approved for use as an inhibitor of yeasts in wine and ready-to-drink tea beverages and was in 1996 for use in sport drinks and fruit or juice sparklers at 0.025% (m/v). In addition, as of 2000, there is an effective notification for the use of DMDC as a microbial control agent in noncarbonated juice beverages containing up to and including 100% juice with the maximum limit of 250 mg/l (USFDA, 2001). DMDC presently is approved for use as a direct food additive to be used as a microbial control agent in certain beverages in which the microbial population has been reduced to 500 microorganisms per milliliter or less by current good manufacturing practices.

Nisin is a heat-stable antimicrobial peptide produced by certain strains of *Lactococcus lactis* subsp. lactis (De Vuyst, 1994), and it is the only bacteriocin recognized as safe for the food industry by the World Health Organization (Cao-Hoang et al., 2010). Nisin exhibits antimicrobial activity toward a wide range of

Gram-positive bacteria (Brewer et al., 2002; Cabo et al., 2009; Pinto et al., 2011). In normal circumstances, nisin does not significantly inhibit yeasts, molds, or Gram-negative bacteria (Pinto et al., 2011). The effectiveness of nisin depends on growth and exposure conditions, such as temperature and pH, and nisin is more active at lower pH values (pH < 4.0), whereas the influence of temperature on its effectiveness is controversial (De Arauz et al., 2009; Antolinos et al., 2011).

There is a growing need to develop new alternative processing technologies that could be applied in combination with biological antimicrobials (Williams et al., 2005; Taylor et al., 2007; Li et al., 2012). However, the efficacy of DMDC treatments in combination with nisin for the reduction of bacterial pathogens in fresh juices, especially litchi juice has not been reported. Litchi juice is a low-acid food with an approximate pH of 4.5-5.0, so it has a higher risk of bacterial contamination than more acidic foods, such as apple juice and orange juice. Considering the consumer demand for minimally-processed food products and the side effects of thermal pasteurization, DMDC treatments may be a good treatment option for litchi juice. The purpose of this work was to investigate the efficacy of DMDC combined with nisin to disinfect litchi juices respectively inoculated with *E. coli* and *S. aureus*, surrogates for the foodborne pathogens, and to evaluate the synergistic effect of DMDC and nisin on the pathogens inactivation.

2. Material and methods

2.1 Litchi Juice Preparation

Litchi fruit (*Litchi chinensis* Sonn.) cv. Huaizhi at 95% maturation was harvested from a commercial orchard in Guangzhou, China. The litchi fruits were peeled, destoned and juiced with a juice extractor (Midea Co., Guangdong, China). The juice was then centrifuged at 5000×g for 5 min at ambient temperature (approx. 25 °C), and the pH value of litchi juice was 4.5. In this experiment, various pH value of litchi juice (3.5, 4.0, 4.5, 5.0, and 6.0) was obtained by adjusting the pH of litchi juice using 20% (w/v) citric acid or 15% (w/v) NaOH solution. After Ultra-high temperature sterilization (108 °C /10 s), different pH value of litchi juice were immediately placed into sterilized aluminum-coated polyethylene bags and stored at 2 °C until use.

2.2 Bacterial Cultures

Escherichia coli ATCC 8739 and *Staphylococcus aureus* ATCC 6538 were obtained from Guangdong culture collection center, China. Stock *E. coli* ATCC 8739 and *S. aureus* ATCC 6538 cultures were maintained in 15% (w/v) glycerol at -80 °C. Nutrient Broth (Guangzhou HuanKai Microbiological Technology Co. Ltd., China) were inoculated with the two strains and incubated at 37 °C on a rotary shaker (200 rpm). After being incubated to stationary phase, the cultures of the two strains were respectively inoculated into aseptically litchi juice for further inactivation study.

2.3 Preparation of Nisin and DMDC Solution

Two grams of commercial nisin powder (10^6 IU/g, Zhejiang Silver-Elephant Bio-engineering Co., Zhejiang, China) was dissolved in 100 ml of 0.05 M citric acid solution, and then were serially diluted into different concentration of nisin stock solution using 0.05 M citric acid solution. Nisin stock solution was sterilized by immersed in boiling water (100 °C) for 5 min. Dimethyl dicarbonate (sigma-aldrich Co. LLC., USA) were also diluted serially into different concentration of DMDC stock solution using 100% ethyl alcohol before being added to litchi juices.

2.4 DMDC and Nisin Inactivation Treatment of Bacteria in Litchi Juice

The initial number of *E. coli* and *S. aureus* ranged from 3.5×10^6 and 4.0×10^6 in the litchi juice, and each strain was individually treated by DMDC and nisin. The prepared litchi juice (98.8 ml) was filled into a 300 ml aseptically screw cap glass bottles, and then preheated to the experimental temperature (30 °C, 40 °C, and 45 °C) in a water bath (Shanghai YiHeng instruments Co., Ltd., China). When appropriate, 100 μl DMDC stock solution also was added into litchi juice sample to reach the final concentrations of 0, 50, 100, 150, 200 and 250 mg/l after 100 μl culture of *E. coli* or *S. aureus*, and 1 ml different concentration of nisin stock solution was mixed into litchi juice sample, and immediately mixed vigorously by hand. The final level of nisin in litchi juice sample was 0, 25, 50, 100, 150 and 200 IU/ml. The sterilized 0.05 M citric acid was being as the blank of nisin, and 100% ethyl alcohol was being as the blank of DMDC. After DMDC and nisin treatments, bottles were immediately taken out from water bath, and then kept in an ice bath until survivor enumerations

2.5 Enumeration of Survivors

Immediately following DMDC and nisin treatment, bacterial survivors were 10-fold serially diluted in sterile 0.85% (w/w) NaCl aqueous, and 1.0 ml of each dilution was poured plating with 25 ml of Nutrient agar

(Guangzhou HuanKai microbiological technology Co. Ltd., China). Following 48 h aerobic incubation at 37 °C, the colonies were counted. Log N/N_0 was calculated to determine the inactivation effect, where N_0 was the number of initial microorganisms in juice sample and N was the corresponding viable number of microorganisms after DMDC and nisin treatment.

2.6 Statistical Analysis

Each reported value is a mean of three replicate experiments. Analysis of variance (ANOVA) was carried out using software SPSS Statistics 19.0 (IBM Co., USA). The significance level was set at 0.05.

3. Results

3.1 Effect of Nisin Level on Inactivation of E. coli and S. aureus in Litchi Juice Exposed to DMDC

The inactivation curves of *E. coli* and *S. aureus* in litchi juice of pH 4.5 when individually treated by 150 mg/l DMDC at 30 °C were depicted in Figure 1. The inactivation rate of *E. coli* and *S. aureus* during initial 1 h was far greater than during the remaining 5 h (Figure 1), and 1.66 log cycles reduction of *E. coli* and 2.03 log cycles reduction of *S. aureus* in litchi juice was achieved as individually treated by 150 mg/l DMDC at 30 °C for 1 h.

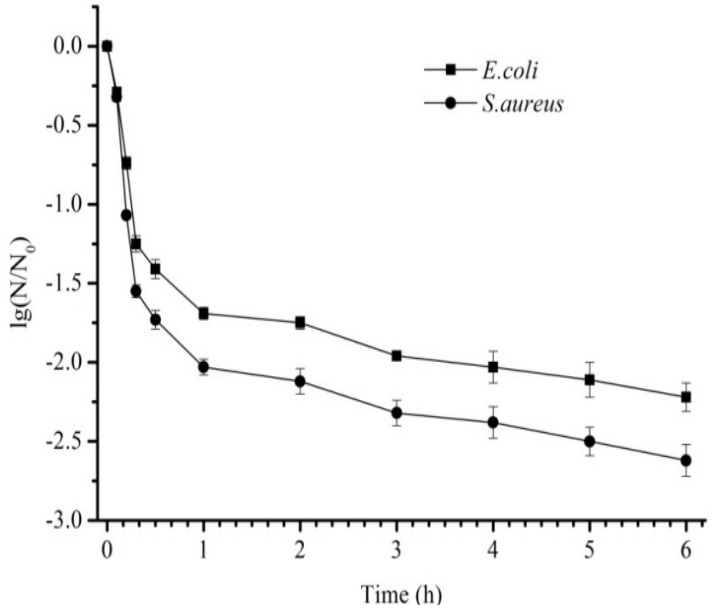

Figure 1. Inactivation of *E. coli* and *S. aureus* in litchi juice of pH 4.5 during treatment of 150 mg/l DMDC at 30 °C

Figure 2 showed the reduction of *E. coli* and *S. aureus* in litchi juice (pH 4.5) with different nisin level as exposed to 150 mg/l DMDC for 1 h at 30 °C. No significant increase ($p > 0.05$) on reduction of *E. coli* was observed as the nisin level in litchi juice sample was below 50 IU/ml, but a linear increase of *E. coli* reduction was observed as the nisin level increased in the range from 50 to 200 IU/ml (Figure 2). However, low level of nisin addition could significantly increase ($p < 0.05$) the *S. aureus* reduction in litchi juice of pH 4.5 as exposed to 150 mg/l DMDC for 1 h at 30 °C, and the *S. aureus* reduction also showed a linear increase as the nisin addition level increased in the range from 0 to 150 IU/ml (Figure 2). Moreover, the *S. aureus* reduction in litchi juice sample with 200 IU/ml of nisin show no significant increase ($p > 0.05$) as compared with the litchi juice with 150 IU/ml of nisin (Figure 2).

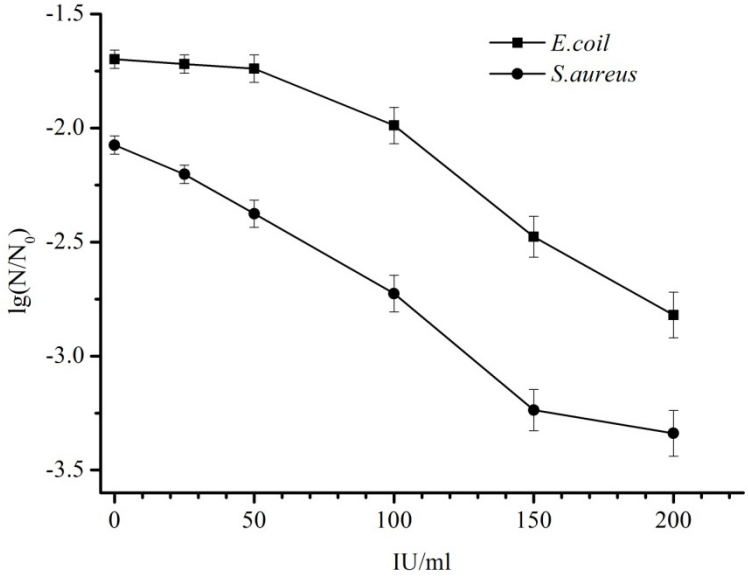

Figure 2. Change of reduction of *E. coli* and *S. aureus* in litchi juice (pH 4.5) with various nisin level as exposed to 150 mg/l DMDC for 1 h at 30 °C

3.2 Effect of Temperature on Inactivation of E. coli and S. aureus in Litchi Juice Exposed to DMDC and Nisin

Table 1 presented the reduction of *E. coli* and *S. aureus* in litchi juice (pH 4.5) as treated with DMDC (150 mg/l) combined with nisin (200 IU/ml) for 1 h at different temperature. The reduction of *E. coli* in litchi juice individually exposed to 150 mg/l DMDC for 1 h at 40 °C was only 0.2 log cycles higher as comparing with 30 °C, while the increase of *E. coli* reduction at 45 °C was 4.11 log cycles higher as comparing with 30 °C (Table 1). As for the inactivation of *S. aureus* in litchi juice of pH 4.5 individually exposed to 150 mg/l DMDC for 1 h at different temperature, 2.81 log cycles higher reduction was observed at 40 °C as compared with 30 °C, and a complete inactivation of *S. aureus* was achieved at 45 °C (Table 1), with a reduction of 6.59 log cycles.

Nisin also showed a significant inactivation to *E. coli* and *S. aureus* ($p < 0.05$) in litchi juice, and the reduction of *E. coli* and *S. aureus* in litchi juice increased with increasing temperature as individually exposed to 200 IU/ml nisin for 1 h at 30, 40 and 45 °C (Table 1). Moreover, *E. coli* have a higher resistance to nisin than *S. aureus*. For example, a reduction of 3.13 log cycles was obtained for *S. aureus* in litchi juice, while a reduction of 1.31 log cycles was obtained for *E.coli* in litchi juice as individually exposed to 200 IU/ml nisin for 1 h at 40 °C.

Table 1. Inactivation [Lg (N/N_0)] of *E. coli* and *S. aureus* in litchi juice of pH 4.5 as treated with DMDC (150 mg/ml) combined with nisin (200 IU/ml) for 1 h at 30, 40, and 45 °C. No DMDC and nisin was added in untreated sample of litchi juice

Strain	Temperature	various DMDC and nisin combinations			
	(°C)	DMDC	Nisin	DMDC+Nisin	Untreated
E.coli	30	-1.70±0.09	-0.29±0.06	-2.82±0.11	0.02±0.01
	40	-1.89±0.11	-1.31±0.07	-3.86±0.12	0.11±0.03
	45	-5.81±0.16	-2.03±0.11	<-6.59[a]	-0.14±0.04
S. aureus	30	-2.07±0.08	-0.41±0.05	-3.34±0.09	0.01±0.002
	40	-4.88±0.1	-3.13±0.08	<-6.59[a]	0.04±0.01
	45	-6.4±0.2	-3.39±0.14	<-6.59[a]	-0.17±0.03

[a] No colonies were obtained by plating undiluted 1 ml litchi juice.

Nisin and DMDC exhibited a synergistic effect on the inactivation of *E. coli* and *S. aureus* in litchi juice of pH 4.5 as exposed to 150 mg/ml DMDC and 200 IU/ml nisin for 1 h at 30, 40 and 45 °C (Table 1). For example, the

reduction of *E. coli* in litchi juice was 1.70 and 0.29 log cycles as individually exposed to 150 mg/l DMDC and 200 IU/ml nisin for 1 h at 30 °C, respectively, while a reduction of 2.82 log cycle was obtained when *E. coli* in litchi juice of pH 4.5 was exposed to a combination of 150 mg/l DMDC and 200 IU/ml nisin for 1 h at 30 °C. Moreover, 0.86 log cycles higher reduction of *S. aureus* also was observed in litchi juice exposed to a combination of 150 mg/l DMDC and 200 IU/ml nisin for 1 h at 30 °C as comparing with the sum of log cycles reduction of *S. aureus* in litchi juice of pH 4.5 individually exposed to 150 mg/l DMDC and 200 IU/ml nisin for 1 h at 30 °C. In addition, growth of *E. coli* and *S. aureus* were observed in litchi juice of pH 4.5 without DMDC and nisn (Untreatment) after 1h at 30 and 40 °C, and lower reduction of *E. coli* and *S. aureus* occurred in litchi juice of pH 4.5 without DMDC and nisn after 1 h at 45 °C (Table 1).

3.3 Effect of pH on Inactivation of E. coli and S. aureus in Litchi Juice Exposed to DMDC and Nisin

Table 2 showed the reduction of *E. coli* and *S. aureus* in litchi juice of pH 3.5, 4.0, 4.5, 5.0 and 6.0 as treated with DMDC (150 mg/l) combined with nisin (200 IU/ml) for 1 h at 30 °C. At pH ranging from 3.5 to 6.0, the reduction of *E. coli* in litchi juice showed a slight trend toward increase with declining the pH value as separately exposed to 200 IU/ml nisin for 1 h at 30 °C, and the maximal reduction of approximately 0.38 log cycles was reached at pH 3.5 (Table 2). Similarly, lower than 0.5 log cycles reduction of *S. aureus* in litchi juice was observed at the pH ranging from 4.5 to 6.0 as separately exposed to 200 IU/ml nisin for 1 h at 30 °C, but a reduction of 2.81 log cycles and 3.88 log cycles was achieve in litchi juice at pH 4.0 and pH 3.5, respectively (Table 2).

As for the combinations of DMDC and DMDC + nisin, the inactivation of *E. coli* and *S. aureus* in the litchi juice increased with declining pH value at the pH ranging from 4.0 to 6.0 as separately treated with DMDC and DMDC+nisin for 1 h at 30 °C (Table 2). And a lower inactivation of *E. coli* and *S. aureus* was observed in the litchi juice of pH 3.5 as comparing with the litchi juice of pH 4.0 separately treated with DMDC and DMDC + nisin for 1 h at 30 °C (Table 2). After exposed to DMDC (150 mg/l) combined with nisin (200 IU/ml) for 1 h at 30 °C, nisin and DMDC exhibited a synergistic effect on the inactivation of *S. aureus* in litchi juice at the pH ranging from 3.5 to 6.0, and a synergistic effect of DMDC and nisin on the inactivation of *E.coli* in litchi juice at the pH ranging from 4.0 to 5.0 also was observed (Table 2), but no significnat synergistic effect ($p > 0.05$) of DMDC and nisin on the inactivation of *E.coli* in litchi juice of pH 4.0 and 3.5 was found (Table 2).

Table 2. Inactivation rate of *E. coli* and *S. aureus* in litchi juice of pH 3.5, 4.0, 4.5, 5.0 and 6.0 when treated with DMDC (150 mg/l) combined with nisin (200 IU/ml) for 1 h at 30 °C. No DMDC and nisin was added in untreated sample of litchi juice

Strain	pH vaule	various DMDC and nisin combinations			
		DMDC	Nisin	DMDC+Nisin	Untreated
E.coli	3.5	-1.77±0.08	-0.38±0.02	-1.89±0.11	-0.34±0.03
	4.0	-2.46±0.12	-0.34±0.01	-3.52±0.12	-0.18±0.001
	4.5	-1.7±0.06	-0.29±0.03	-2.82±0.07	0.02±0.005
	5.0	-1.53±0.08	-0.24±0.01	-2.31±0.08	0.03±0.002
	6.0	-0.34±0.02	-0.19±0.01	-0.34±0.03	0.88±0.08
S. aureus	3.5	-1.13±0.07	-3.88±0.15	<-6.59[a]	-0.18±0.01
	4.0	-3.04±0.11	-3.06±0.03	<-6.59[a]	-0.01±0.005
	4.5	-2.07±0.12	-0.4±0.04	-3.34±0.15	0.01±0.003
	5.0	-1.47±0.06	-0.32±0.01	-2.82±0.09	0.02±0.01
	6.0	-0.31±0.01	-0.23±0.02	-0.54±0.02	0.14±0.02

[a] After the citric acid in juice sample treated with DMDC and nisin was neutralized with 0.1 M aseptically $NaHCO_3$ solution, no colonies were obtained by plating 1 ml undiluted litchi juice.

After 1 h at 30 °C, a growth of *E. coli* and *S. aureus* in the litchi juice of pH 4.5, 5.0 and 6.0 were observed, and the growth rate of *E. coli* and *S. aureus* was fast as pH value raised. However, a slight inactivation of *E. coli* and *S. aureus* in the litchi juice of pH 3.5 and 4.0 were found (Table 2).

3.4 Effect of DMDC Concentration on Inactivation of E. coli and S. aureus in Litchi Juice Exposed to DMDC and Nisin

Effect of DMDC concentration on inactivation of *E. coli* and *S. aureus* in litchi juice of pH 4.5 exposed to DMDC or combined with nisin (200 IU/ml) for 1 h at 30 °C was illustrated in Figure 3. The reduction of *E. coli* and *S. aureus* in litchi juice showed a trend toward increase with increasing of DMDC concentration, and the inactivation of *E. coli* and *S. aureus* in litchi juice was lower as the DMDC concentration was below 150 mg/l (Figure 3).

At the DMDC ranging from 50 to 250 mg/l, nisin and DMDC exhibited a synergistic effect on the inactivation of *S. aureus* in litchi juice as exposed to DMDC+nisin (200 IU/ml) for 1 h at 30 °C (Figure 3). However, no significnat synergistic effect ($p > 0.05$) of DMDC and nisin on the inactivation of *E.coli* in litchi juice was found as exposed to DMDC + nisin (200 IU/ml) of 50 and 250 mg/l DMDC for 1 h at 30 °C, but nisin and DMDC also exhibited a synergistic effect on the inactivation of *S. aureus* in litchi juice at the DMDC ranging from 100 to 200 mg/l (Figure 3).

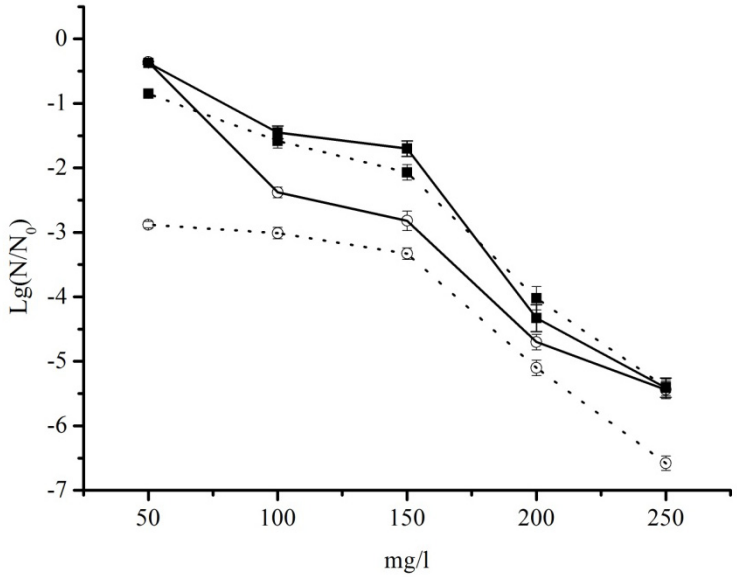

Figure 3. Change of reduction of *E. coli* (solid line) and *S. aureus* (dot line) in litchi juice (pH 4.5) with and without 200 IU/ml nisin as exposed to various concentration of DMDC for 1 h at 30 °C. (○) is the litchi juice sample with 200 IU/ml nisin, and (■) is the litchi juice sample without 200 IU/ml nisin

4. Discussion

DMDC is a sterilant recommended for use in soft drinks, wine, beer and juice products. Researchers had found protein modification, through reaction of nucleophilic groups, such as imidazoles, amines, or thiols, can rapidly occur with the dicarbonate of DMDC, so the inactivation of microorganisms by DMDC seems strongly related to the inactivation of the enzymes of microorganisms, and microorganisms can be rapidly killed after addition of DMDC (Osterman-Golkar et al., 1974; Golden et al., 2005). In addition, DMDC was also hydrolyzes into carbon dioxide and methanol almost immediately after addition to beverages. The hydrolysis of DMDC depends on temperature, the higher the temperature the more rapid the rate. The kinetics of hydrolysis of DMDC had been reported, and half-life of DMDC in water was 60 min at 4 °C, 15 min at 25 °C, 10 min at 30 °C, and 3 min at 45 °C (Golden et al., 2005). Generally, the inactivation rate of microorganisms will declined with the hydrolysis of DMDC during DMDC treatment. In this study, the inactivation rate of *E. coli* and *S. aureus* in litchi juice of pH 4.5 was far greater during initial 1 h than during the remaining 5 h as individually treated by 150 mg/l DMDC at 30 °C (Figure 1), and the inactivation curves could describe by fast-to-slow two stage kinetic equations.

Despite the more rapid hydrolysis at higher temperatures, increase of temperatures also enhanced the reaction effectiveness of enzymes from microorganisms with DMDC. Splittstoesser and Wilkinson (1973) reported that increasing the temperature from 20 °C to 40 °C against *Saccharomyces cerevisiae* and *Lactobacillus plantarum*

resulted in a 100-fold increase in effectiveness. Fisher and Golden (1998) reported that *E. coli* O157:H7 survived well in unpasteurized apple cider over 18 days of storage at 4 °C. However, they observed that *E. coli* survived for up to 3 days, 9 days, and 2 days in cider containing 250 mg/l of DMDC at 4 °C, 10 °C, and 25 °C, respectively. Our result also found increase of temperature of litchi juice can significantly enhanced the inactivation rate of *E. coli* and *S. aureus* in the litchi juice exposed to DMDC (Table 1).

In this study, the inactivation of *E. coli* and *S. aureus* in the litchi juice increased with declining pH value at the pH ranging from 4.0 to 6.0 as exposed to 150mg/l DMDC for 1 h at 30 °C (Table 2). After exposed to 150 mg/l DMDC for 1 h at 30 °C, a lower inactivation of *E. coli* and *S. aureus* was observed in the litchi juice of pH 3.5 as comparing with the litchi juice of pH 4.0 (Table 2). Researchers found the reaction of microorganism protein with DMDC was dependent on the degree of ionization of the R–NH group (protein imidazole and amine). The degree of ionization of the R–NH group decreased with increasing of pH value at the pH range 4.0 to 6.0, whereas the ammonia becomes ionized at the lower pH (below pH 4.0), the reaction of microorganism protein with DMDC is lessened (Golden et al., 2005). Overall, pH 4.0 was the optimum pH value for maximum inactivation of microorganism by DMDC.

Nisin acts on energized vegetative cells (and membrane vesicles) by inserting into the membrane, forming pores, and dissipating the proton motive force. This inhibits uptake of amino acids and promotes rapid efflux of small metabolites, ions, or cytoplasmic solutes such as amino acids and nucleotides (Abee, 1995). The pore formation theory of nisin on the inactivation of bacteria is widely accepted. The theory asserts that nisin forms pores that could disrupt the proton motive force and the pH equilibrium, causing the leakage of ions and hydrolysis of ATP and leading to cell death (De Arauz et al., 2009). The outer membrane of Gram-negative bacteria effectively excludes nisin from making contact and interacting with the cytoplasmic membrane (Kordel et al., 1989). Any treatment, such as chelating agent, sublethal heat, hydrostatic pressure, or freezing, or organic acids, which disrupts the outer membrane, may render Gram-negative cells sensitive to nisin (De Arauz et al., 2009). In this study, increasing the temperature from 30 °C to 40°C resulted in a 1000-fold increase in effectiveness of Gram-positive *S. aureus* inactivation in litchi juice of pH 4.5, and also resulted in a 10-fold increase in effectiveness of Gram-negative *E. coli* inactivation in litchi juice of pH 4.5 as exposed to 200 IU/ml nisin for 1 h (Table 1).

The solubility and stability of nisin solutions decrease as the pH increases. Rayman et al. (1981) showed that the effectiveness of nisin decreased with increasing pH, and Scannel et al. (1997) reported similar results. In this study, decline the pH from 4.5 to 4.0 resulted in a 1000-fold increase in effectiveness of Gram-positive S. aureus inactivation in litchi juice as exposed to 200 IU/ml nisin for 1 h (Table 2). As for Gram-negative *E. coli* in litchi juice, declining the pH from 4.5 to 4.0 resulted in only 0.19 log cycles increase as exposed to 200 IU/ml nisin for 1 h at 30 °C. Outer membrane of Gram-negative *E. coli* in litchi juice may be not disrupted at 30 °C which resulted in the resistance of nisin at lower pH.

In view of pore formation theory of nisin on the inactivation of bacteria, we inferred nisin formed pores at the cell membrane, allowing DMDC to penetrate the cell membrane more easily and also declining the internal pH by disrupting cell's pH equilibrium, and which increased the reaction effectiveness of intracellular enzymes from bacteria with DMDC. Increase of reaction effectiveness of intracellular enzymes from bacteria with DMDC resulted in a faster inactivation of bacteria. Overall, a synergetic inactivation of DMDC and nisin to the bacteria of litchi juice was showed.

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