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Changes in Quality Attributes During Storage of Litchi Juice Treated With Dimethyl Dicarbonate (DMDC) and Nisin

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Abstract

The aim of this work was to evaluate the change in the quality of litchi juice treated by DMDC combined with Nisin during storage of 4 °C. Results found that addition of 250 mg/L of DMDC combined with 100 IU/mL of Nisin can ensure the microbiological safe of litchi juice during storage at 4 °C. Compare with heat treatment (95°C, 1 min), the treatment of DMDC combined with Nisin can retain a more value of sensory attributes, but a more loss in the content of total phenolics, ascorbic acid, and antioxidant capacity was observed during storage at 4 °C because of the ineffectiveness of DMDC and Nisin to the oxidase of litchi juice. Moreover, no significant change ($P > 0.05$) was observed in the value of $L*$, $a*$, $b*$, and ΔE in the heat-treated litchi juice, and yet the litchi juice treated by DMDC and Nisin gradually turned into light red at the end of storage because of the oxidation of phenolics by residual POD in the litchi juice, which resulted in a significant changes ($P < 0.05$) in the value of $L*$, $a*$, $b*$, and ΔE in the litchi juice. This study would provide technical support for commercial application of DMDC combined with Nisin in litchi juice processing.

Keywords: litchi juice, Dimethyl Dicarbonate (DMDC), nisin, quality, storage

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 thus it is able to compete in the market of fruit juices (Wu et al., 2007; Zeng et al., 2008; Saxena et al., 2011). But litchi juice is a low-acid food with an approximate pH of 4.5-5.0, so it has a higher risk of microbial contamination than more acidic foods, such as apple juice and orange juice. Some yeast and lactic acid bacteria grow quickly in litchi juice, which can cause spoilage and produce undesirable organoleptic changes in products (Li et al., 2012). Therefore, litchi juices should undergo some type of preservation to ensure product quality and safety.

Thermal processing is the most straightforward means to inactivate the microorganism and enzymes in fruit juices and which are generally at 70-121 °C for 30-120 s (Li et al., 2012). But many juice producers, especially small seasonal operators, are unable to use thermal processing for economic reasons or opposed to thermal processing because of perceived undesirable effects on product quality and acceptability. Therefore, low-temperature alternatives are being sought: particular attention has been given to the use of antimicrobials (Cao et al., 2012; Yu et al., 2013).

DMDC (dimethyl dicarbonate), a dicarbonic acid ester, is a powerful antimicrobial agent due to its potential high reaction capacity with nucleophilic groups of enzymes from microorganisms, such as imidazoles, amines, or thiols, and which results in the rapid inactivation of microorganisms (Golden et al., 2005). In 1988, dimethyl dicarbonate (DMDC) (Velcorin) was first approved for use as an inhibitor of yeasts in wine and ready to drink tea beverages; and subsequently in 1996, approved as a yeast inhibitor in spirit drinks and in carbonated or non-carbonated, non-flavored beverages containing added electrolytes, juice sparklers, dilute beverages containing juice, fruit flavor, or both, with juice content not to exceed 50%, at a concentration of 250 mg/L (FDA,

1996; Golden et al., 2005). 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 (FDA, 2000). 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 (De Arauz et al., 2009; Li et al., 2012). Nisin exhibits antimicrobial activity toward a wide range of Gram-positive bacteria in juices (Li et al., 2012; Zhao et al., 2013).

The efficiency of DMDC against microorganism depends on the strain, initial cell concentration, temperature, pH, and kinds of juice beverages (Fisher et al., 1998; Williams et al., 2005). Some bacteria in juice are very resistant to DMDC, especially *Leuconostoc mesenteroides*. So individual addition of 250 mg/L DMDC to juice cannot completely kill and control some bacteria of juice, especially the low-acid juice (Yu et al., 2013a). Nisin had been evaluated for its efficacy in enhancing the inactivation of DMDC to bacteria of litchi juice in our previous study, and results showed that the treatment of DMDC combined with nisin offers a useful alternative to conventional heat treatment for controlling microbial growth and significantly extending the shelf-life of litchi juice (Yu et al., 2013a).

The aim of this work was to compare the effects of DMDC combined with nisin, and thermal pasteurization (95 °C /1 min) on the quality attributes of litchi juice during storage of 4 °C. This study would provide technical support for commercial application of DMDC combined with Nisin in litchi juice processing.

2. Materials and methods

2.1 Litchi Juice Preparation

Litchi fruit (cv. Fei zi xiao) at 95% maturation was harvested from a commercial orchard in Guangzhou, China. The peeled and destined litchi fruits were processed with a pulper (Midea Co., Guangdong), and passed through filter cloth (100 mesh). And then the pulps were centrifuged at 3000 × g for 5 min, and the supernatant was collected as fresh litchi juice.

2.2 Preparation of Nisin Solution

Nisin stock solution (20 000 IU/ml) was prepared by dissolving commercial nisin powder (10^6 IU/g, Zhejiang Silver-Elephant Bio-engineering Co., Zhejiang, China) in a 0.05 M of citric acid solution. Nisin stock solution was sterilized by immersed in boiling water (100 °C) for 5 min, and stored in the refrigerator of 4 °C after cooling (no more than 6 days).

2.3 Thermal Pasteurization of Litchi Juice

Fresh litchi juice was thermally processed (95 °C, 1 min) in a tubular heat exchanger (Shanghai pilotech Equipment Co., Ltd., China). After heating, the juice was manually filled under hygienic conditions into aseptic PET bottles, and immediately cooled down to 15 ± 2 °C by a cooled water bath.

2.4 DMDC and Nisin Treatment of Litchi Juice

After fresh litchi juice was cooled to 4 ± 1 °C, 100 IU/mL of Nisin and 250 mg/L of DMDC was added to the fresh litchi juice, immediately mixed vigorously, and then the litchi juice above was divided into aseptic PET bottles under hygienic conditions.

2.5 Storage and Sampling

The PET bottles were stored in the refrigerator of 4 ± 2 °C, and taken out at a regular interval for further analysis.

2.6 Microbial Analysis

Each sample was serially diluted with sterile 0.85% NaCl solution, and then the dilution was used for microbial enumeration by pour plate methods. The viable cells of total aerobic bacteria were enumerated on the Plate Count Agar (PCA, Guangzhou HuanKai Microbiological Technology Co. Ltd., China), and the incubation was performed at 37 °C for 2 days. The Rose Bengal Chloramphenicol Agar (Guangzhou HuanKai Microbiological Technology Co. Ltd., Guangdong, China) was used for detecting the viable cells of yeast and molds, and the incubation was performed at 30 °C for 3 days. The total viable cells of lactic acid bacteria were detected using MRS agar (Guangzhou HuanKai Microbiological Technology Co. Ltd.). The MRS agar plates were incubated at 30 °C for 2 to 3 days. Each test was performed in duplicate and results were expressed as colony-forming units (CFU) per milliliter. Rose bengal agar (Guangzhou HuanKai Microbiological Technology Co. Ltd., China) was used for counting the molds and yeasts after incubating at 28 °C for 72-120 h.

2.7 Determination of pH, Titratable Acidity and Total Soluble Solids (TSS)

TSS were determined with a refractometer (Model RP-101, Atago Co. Ltd, Tokyo, Japan) at 20 °C and expressed in degree Brix (°Brix). The pH was determined using a pH meter (Metrohm744, Netherland) with a glasselectrode. Titratable acidity values were obtained by titrating 10 mL of juice with 0.1 mol/L NaOH to pH 8.1, expressing the results as g of citric acid per 100 mL.

2.8 Determination of Polyphenol Oxidase (PPO), and Peroxidase (POD) Activity

The activity of PPO was assayed by a spectrophotometric method (Yingsanga et al., 2008) with some modifications. Catechol was chosen as the substrate. The reaction mixture contained 20 µl sample juice and 2.5 ml substrate solution (10 mM catechol in 50 mM phosphate buffer, pH 7.0). The absorbance of the mixture was measured at 420 nm for 3 min at 1 min intervals at 30 °C immediately after incubation in a spectrophotometer (UV-2450, Shimadzu Co., Japan) with a peltier thermostatic cell holder.

The activity of POD was also assayed by a spectrophotometric method (Yingsanga et al., 2008). The reaction mixture contained 50 µl of sample juice and 2.95 ml of substrate solution (0.1 mL 4.0% guaiacol solution, dissolved with 50% ethanol; 0.1 mL 0.46% H_2O_2; 2.75 mL 0.1M phosphate buffer, pH 5.5). The absorbance of the mixture was measured at 470 nm for 5 min at 1 min intervals at 30 °C immediately after incubation in a spectrophotometer (UV-2450, Shimadzu Co., Japan) with a peltier thermostatic cell holder.

One unit of PPO or POD activity was defined as a change in absorbance of 0.001 OD/min of each microlitre of litchi juice. The relative activities of PPO or POD were obtained with the following formula:

$$\text{Residual activity} = \frac{\text{Specific activity of PPO or POD in the treated litchi juice}}{\text{Specific activity of PPO or POD in fresh litchi juice}} \times 100\%$$

2.9 Determination of Ascorbic Acid, Total Polyphenols, and Antioxidant Capacity

Ascorbic acid was determined by HPLC method using an Agilent system. The litchi juice (1 mL) was mixed with 1 mL of metaphosphoric acid (6%, v/v) aqueous solution, centrifuged at 10000g (5 min), and then the supernatant was used for further HPLC analysis (Hernandez, Lobo, & Gonzalez, 2006). Ascorbic acid was separated on an Agilent ZORBAX SB-Aq (4.6 × 250 mm) column using 0.02 mol/L $(NH_4)_2HPO_4$ aqueous solution (pH 2.7) as the mobile phase at a flow rate 1 mL/min and 30 °C. Its content was detected using DAD detector at 254 nm and reported using external standards (L-ascorbic acid).

Total polyphenols were determined using the Folin-Ciocalteu method with some modifications (Singleton et al., 1965). In a 30 mL of test tube, 1mL of litchi juice diluents (litchi juices diluted 20-50 folds with distilled water), and 2 mL of Folin-Ciocalteu reagent was added and mixed. After exactly 1 min, 2 ml of sodium carbonate (10 g/100ml) was added and mixed, and allowed to stand at room temperature for 1 h. The absorbance was read at 760 nm by a spectrophotometer (UV-1800, Shimadzu, Japan), and the total polyphenol concentration was calculated from a calibration curve (R^2=0.999), using pyrogallic acid as standard (0-50 mg/L).

The antioxidant capacity of litchi juice sample was evaluated by oxygen radical absorbance capacity (ORAC). ORAC assay was performed according to Ou et al. (2001) using the Infinite M200 microplate reader (Tecan Group Ltd., Switzerland). The 80µL of freshly prepared sodium fluorescein solution (1.25 µM/L in 75 mM/L of phosphate buffer, pH 7.4) and 20 µL litchi juice diluted with water was added into microplate wells for 5 min. Then, 100 µL of freshly prepared 2, 2'-Azobis (2-methylpropionamidine) dihydrochloride (140 mM/L in 75 mM/L phosphate buffer, pH 7.4) was also added in wells. Fluorescence was collected at 485 nm on excitation at 520 nm on emission, taking measurements every 150 s for 1.5 h at 37 °C. The standard curve was linear between 100 and 500 µM/L Trolox. The result was expressed as mM Trolox equivalent (TE)/L.

2.10 Determination of Browning Degree and Color Analysis

The browning degree (BD) of litchi juice was analyzed using a spectrophotometric method (Roig et al., 1999). Juice was centrifuged with a refrigerated Centrifuge (GL-166-A, Shanghai Anting Scientific Equipment Factory, Shanghai, China) at 10000 rpm/min at 4 °C for 5 min, then passed through a 0.45µm cellulose nitrate membrane (Beijing Bomex Co., Beijing, China). The BD was determined by measuring the absorbance value at 420 nm using a spectrophotometer (UV-726 Shimadzu, Shanghai, China) at room temperature.

Color assessment was conducted using a color measurement spectrophotometer (HunterLab ColorQuest XE, Hunter Associates Laboratory, Inc., Virginia, USA) in the reflectance (transmission) mode. Color was expressed as L^* (lightness; 0=black, 100=white), a^*(-a^*=greenness, +a^*=redness) and b^* (-b^*=blueness, +b^*=yellowness). Three measurements were performed, and the results were averaged. These values were then used to calculate the total color difference (ΔE) (Yu et al., 2013b). The calculated equations were as follows, where L^*_0, a^*_0 and

b^*_0 are the control values for fresh litchi juice samples:

$$\Delta E = \sqrt{(L^*-L_0^*)^2 + (a^*-a_0^*)^2 + (b^*-b_0^*)^2}$$

2.11 Sensory Analysis

Sensory analysis of litchi juice was performed by a semi-trained panel composed by 15 panelists (12 and 39 year of age, equal distribution between male and female), using a 9-point hedonic scale for scoring odor, sweetness, acidity, color and overall acceptability (9 like very much, 1 dislike very much, and 5 as rejection point) (YeH et al., 1998; Walkling-Ribeiro et al., 2009; Lim 2011)

2.12 Statistical Analysis

For all kinds of samples, two different batches were considered and analyzed separately. All experiments were conducted in triplicate. Analysis of one-way ANOVA was accomplished with the software SPSS Statistics 19.0 (IBM Co., USA). Duncan's multiple range tests were used to determine statistically significant differences of variables at 95% confidence.

3. Results and Discussion

3.1 Changes in the Indigenous Microorganism of Litchi Juice Treated With DMDC and Nisin During Storage

After added 250 mg/L of DMDC and 100 IU/mL of Nisin to the fresh litchi juice at 4 °C, the counts of total aerobic bacteria, lactic acid bacteria, yeast and mold in litchi juice linearly declined with increase of time during initial 6 h, and the total aerobic bacteria, lactic acid bacteria, yeast and mold in litchi juice were not detected after 12 h and during 3-month storage, indicating that the juices were microbiologically safe in this study (Table 1). This study focused on the changes in quality of the juices during storage at 4 °C, and the analyses concerning the changes were presented as the following. Moreover, the litchi juice processed by the thermal pasteurization (95 °C, 1 min), which was equivalent effects on the inactivation of microorganisms with the litchi juice treated with 250 mg/L DMDC and 100 IU/mL of Nisin, was as the contrast for quality analysis.

Table 1. Changes of microbial counts (Lg CFU/mL) in the litchi juice treated with 250 mg/L of DMDC combined with 100 IU/mL of Nisin, and thermal pasteurization (95 °C, 1 min) during storage at 4 °C

	Heat-treated			DMDC and Nisin treated		
	Total aerobic bacteria	Lactic acid bacteria	Mold/Yeast	Total aerobic bacteria	Lactic acid bacteria	Mold/Yeast
0 d	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
1 d	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
15 d	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
30 d	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
45 d	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
60 d	0.61±0.04	N.D.	N.D.	0.31±0.01	N.D.	N.D.
90 d	0.70±0.06	N.D.	N.D.	0.48±0.05	N.D.	N.D.

N.D.: Colony counts below the detection limit (1 CFU/mL).

3.2 Changes of pH, Titratable Acidity and Total Soluble Solids

The pH, titratable acidity and total soluble solids of fresh litchi juice were 4.48 ± 0.08, 0.28 ± 0.03 g of citric acid per 100 mL, and 17.22 ± 0.24 °Brix, respectively. Compared with untreated fresh litchi juice, no significant changes ($P > 0.05$) of pH, titratable acidity and total soluble solids were observed as treated by DMDC combined with Nisin, and heat (95 °C, 1 min). And also, during storage of 90 d at 4 °C, the pH, titratable acidity and total soluble solids did not show significant changes ($P > 0.05$) in the litchi juice treated by DMDC combined with Nisin, and heat (95 °C, 1 min), which may related to the inhibition of growth of indigenous microorganism in the litchi juice.

3.3 Changes of PPO and POD Activity, Color and Browning Degree

The POD activity of fresh litchi juice was 2206.6 IU/mL. No PPO activity in the fresh litchi juice was detected which was similar to those described in the literature (Yang et al., 2010; Xu et al., 2014). The POD can be efficiently inactivated by the heat treatment (95 °C, 1 min), and thus no POD activity was detected in the heat-treated litchi juice during storage of 90 d at 4 °C. However, 85.6% of POD activity was residual after 12 h as added 250 mg/L of DMDC and 200 IU/mL of Nisin to the fresh litchi juice at 4 °C, indicating the DMDC and Nisin cannot inactivate the POD (Figure 1). The POD activity of litchi juice treated by DMDC and Nisin did not showed significant decrease ($P > 0.05$) during initial 30 d at 4 °C, and yet a fast decline of POD activity was observed at the end of storage (Figure 1), which may be attributed to the damage of native structure of POD protein (Davies & Truscott, 2001; Müller et al., 2014).

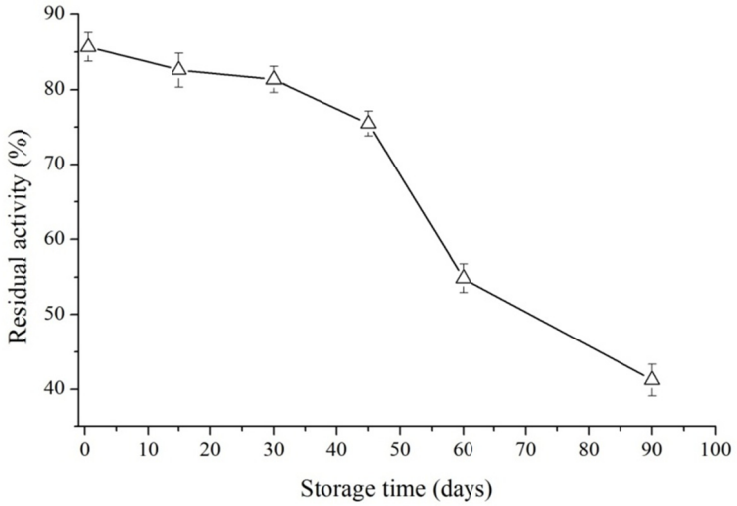

Figure 1. Changes of residual relative activity of POD in the litchi juice treated by DMDC combined with Nisin during storage at 4 °C

Table 2. Changes of color parameters in litchi juice treated by DMDC combined with Nisin, and heat during storage at 4 °C

Storage time	*L**	*a**	*b**	ΔE	BD
Heat-treated litchi jucie					
0 d	81.08±0.27 [a]	-2.32±0.58 [a]	5.32±0.21 [c]	3.82±0.29 [b]	0.12±0.01 [b]
15 d	81.66±0.24 [a]	-2.60±0.56 [a]	5.63±0.57 [c]	4.23±0.19 [b]	0.12±0.01 [b]
30 d	81.54±0.14 [b]	-3.04±0.59 [a]	4.88±0.47 [c]	4.74±0.14 [c]	0.15±0.02 [b]
45 d	81.19±0.23 [b]	-3.40±0.51 [a]	4.78±0.36 [c]	4.99±0.20 [c]	0.17±0.01 [bc]
60 d	81.04±0.10 [b]	-3.38±0.48 [a]	4.72±0.15 [c]	5.06±0.53 [c]	0.18±0.02 [c]
90 d	80.93±0.12 [c]	-4.05±0.52 [a]	9.32±0.51 [d]	8.85±0.35 [e]	0.20±0.01 [e]
Litchi juice treated by DMDC combined with Nisin					
0.5 d	81.95±1.42 [b]	-2.49±0.31 [a]	1.64±0.01 [a]	2.92±0.01 [a]	0.09±0.01 [a]
15 d	83.77±1.84 [b]	-1.61±0.26 [b]	2.75±0.04 [b]	2.38±0.05 [a]	0.10±0.01 [a]
30 d	77.73±1.44 [d]	-1.36±0.22 [b]	3.09±0.03 [b]	7.23±0.12 [d]	0.13±0.01 [b]
45 d	76.72±1.04 [d]	0.01±0.16 [c]	4.14±0.26 [c]	8.86±0.19 [e]	0.17±0.02 [bc]
60 d	76.09±1.25 [d]	0.62±0.34 [d]	5.58±0.35 [c]	10.10±0.26 [f]	0.17±0.01 [bc]
90 d	76.98±1.42 [d]	1.31±0.26 [e]	10.25±0.52 [d]	12.28±0.61 [g]	0.19±0.02 [d]

[a, b, c, d, e, f, g] Different letters represented a significant difference within the same column ($P < 0.05$).

Table 2 presented the changes in color parameters of litchi juice treated by DMDC combined with Nisin, and heat during storage at 4 °C. No significant change ($P > 0.05$) was observed in $L*$, $a*$, $b*$, and ΔE value after 0.5 d as added DMDC and Nisin to fresh litchi juice (Table 1). During further storage, the $a*$, $b*$, and ΔE value showed a tendency to increase, and yet a tendency to decrease was observed in the $L*$ value (Table 1). And the litchi juice treated by DMDC and Nisin gradually turned into light red at the end of storage. The changes in the color of litchi juice treated by DMDC combined with Nisin may be due to the enzymatic browning of residual POD in the litchi juice (Zhang & Quantick, 1997). POD can oxidize phenols to quinones that in turn polymerize to form brown pigments (Yingsanga et al., 2008).

No significant changes in the L^* and a^* value were observed (Table 1), and yet the b^* values of litchi juice showed slight increase because the caramelization reaction during heat treatment (Zhang et al., 2013). Unlike the juice treated by DMDC combined with Nisin, the value of $L*$, $a*$, $b*$, and ΔE in the heated-treated litchi juice did not showed significant changes during storage of the initial 60 d (Table 1), which may be attributed to the inactivation of POD by heat. And also the litchi juice treated by heat did not gradually turned into light red at the end of storage.

The (browning degree) BD value only showed a slow tendency to increase (Table 1). The increase of BD value during storage mainly was resulted in the Maillard reaction taking place between alphaamino groups and reducing sugars (Liu et al., 2012). The reaction rate of Maillard reaction can be slowed down by the low temperature. In this study, the storage temperature was 4 °C, which can well inhibit the increase of BD value.

3.4 Changes of Ascorbic Acid

The fresh litchi juice initially contains a high ascorbic acid, reaching 230.16 mg/L. A 94.9% loss of ascorbic acid were observed after 12 h as added 250 mg/L of DMDC and 100 IU/mL of Nisin to fresh litchi juice at 4 °C. During further storage at 4 °C, no ascorbic acid was detected in the litchi juices treated by DMDC and Nisin. Some studies reported that ascorbic acid barely reactive with DMDC or Nisin, and thus the loss of ascorbic acid in the litchi juice added with DMDC and Nisin could be due to the oxidation of ascorbic acid oxidase (Golden et al., 2005; Leong & Oey, 2012). Generally, the oxidation of ascorbic acid in the fresh litchi juice can be inhibited by the growth of indigenous microorganism because of the depletion of dissolved oxygen of litchi juice by the indigenous microorganism. As adding DMDC and Nisin to fresh litchi juice, the indigenous microorganisms showed rapid inactivation. The inactivation of indigenous microorganisms would decrease the depletion of dissolved oxygen, resulting in the more ascorbic acid was oxidized (Leong & Oey, 2012).

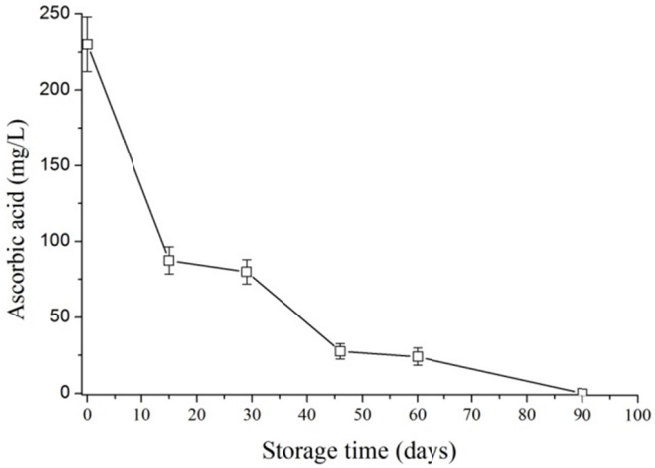

Figure 2. Changes in the contents of ascorbic acid in the heat-treated litchi juice during storage at 4 °C

The heat treatment can retain ascorbic acid of litchi juice well, and thus only 3.4% loss of ascorbic acid was observed in litchi juice after treated by heat at 95 °C for 1 min (Figure 2). During further storage at 4 °C, the contents of ascorbic acid in heat litchi juice showed a fast-to-slow tendency, and no ascorbic acid was detected in the heat-treated litchi juices after 90 d (Figure 2). Earlier studies had showed that ascorbic acid can degrade aerobically and anaerobically during storage, at the rates depending on storage conditions, packaging and the processing method employed (Kennedy et al., 1992; Kabasakalis et al., 2000). In the initial stage of storage,

residual oxygen and ascorbic acid oxidase in heat treated litchi juice could accelerate the aerobic degradation of ascorbic acid, and when the oxygen was totally depleted, the anaerobic degradation of ascorbic acid occurred, whereas it has been reported that the aerobic degradation rate constants were 100 to 1000 times higher than the anaerobic degradation rate constants (Kabasakalis et al., 2000).

3.5 Changes of Total Phenolics, and Antioxidant Capacity

The content of total phenolics in fresh litchi juice reached 561.66 mg/L. The content of total phenolics in the litchi juice showed a 19.9% of reduction after heat treatment at 95 °C for 1 min, which could be caused by thermal degradation. The degradation of phenolic compounds during thermal processing had been observed in other studies (Klopotek et al., 2005). And a 22.9% of reduction in the total phenolics was also observed after 12 h as added 250 mg/L of DMDC and 200 IU/mL of Nisin to litchi juice at 4 °C. Similarly with the decrease of ascorbic acid, the inactivation of indigenous microorganisms by DMDC and Nisin would decrease the depletion of dissolved oxygen, resulting in the more phenolics was oxidized by POD (Zhang et al., 1997; Yingsangaa et al., 2008).

During further storage at 4 °C, a tendency to decrease in the contents of total phenolics was in the heat-treated litchi juice, and the litchi juice added with DMDC and Nisin (Figure 3). And at the end of storage at 4 °C, the rate of decrease in total phenolics in the litchi juice added with DMDC and Nisin was faster than heat-treated litchi juice. It is thought that loss of phenolics was probably due to oxidation as well as reciprocal condensation of phenolic compounds (Trewhella & Grint, 1988; Castañeda-Ovando et al., 2009). Condensation reactions of phenolic compounds naturally occurred in juices during storage, and condensation products were unstable and further degrade to colorless compounds (Es-Safi et al., 2003; Turkyilmaz & Ozkan 2014). In addition, the residual POD in the litchi juice added with DMDC and Nisin can accelerate the oxidation of phenolic compounds, which will resulted in the enhancement of enzymatic browning in the litchi juice (Yingsangaa et al., 2008).

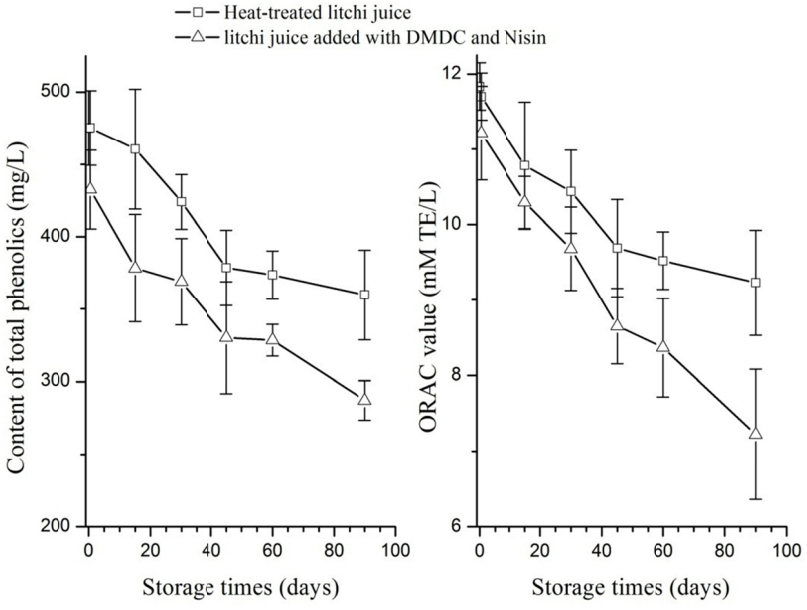

Figure 3. Changes in the content of total phenolics and antioxidant capacity (ORAC value) in the heat-treated litchi juices, and the litchi juice treated by DMDC combined with Nisin during storage at 4 °C

The ORAC value of fresh litchi juice was 14.87 mM TE/L. The ORAC value showed a 20.4% reduction in litchi juice after heat treatment, and 24.6% of reduction was also observed after 12 h as added 250 mg/L of DMDC and 200 IU/mL of Nisin to litchi juice at 4 °C. During further storage at 4 °C, the ORAC value showed a tendency to decrease in the heat-treated litchi juice, and the litchi juice added with DMDC and Nisin, and the data trends for antioxidant capacity (ORAC value) and total phenolics content of both litchi juice seem to be positively associated (Figure 3), indicating that total phenols made greater contribution to the antioxidant

capacity of litchi juices. Previous studies have demonstrated that phenolic compounds are responsible for antioxidant capacities in fruits, and the fruits with higher phenolic contents generally show stronger antioxidant capacities (Kalt et al., 1999; Da Silva et al., 2007).

3.6 Changes of Sensory Attributes

Comparing with the fresh litchi juice, no significant differences ($P > 0.05$) were detected by the panelists in the litchi juice in terms of odour, acidity, sweetness, and overall acceptability as added with 250 mg/L of DMDC and 200 IU/mL of Nisin to the litchi juice at 4 °C for 12 h (Figure 4). During further storage of 90 d at 4 °C, no significant changes ($P > 0.05$) in the scores of odour, acidity, and sweetness were observed in the litchi juice treated with DMDC combined with Nisin, and yet the scores of overall acceptability showed a reduction of 1 scores at the end of storage because of the changes of color. Moreover, a greater number of panelists sensed slightly cooked or flavor and rough feel in heat-treated litchi juice, resulting in the decreased of scores in odour and overall acceptability (Figure 4). During further storage of 90 d at 4 °C, no significant changes ($P > 0.05$) in the scores of odour, acidity, sweetness and overall acceptability were observed in the heat-treated litchi juice.

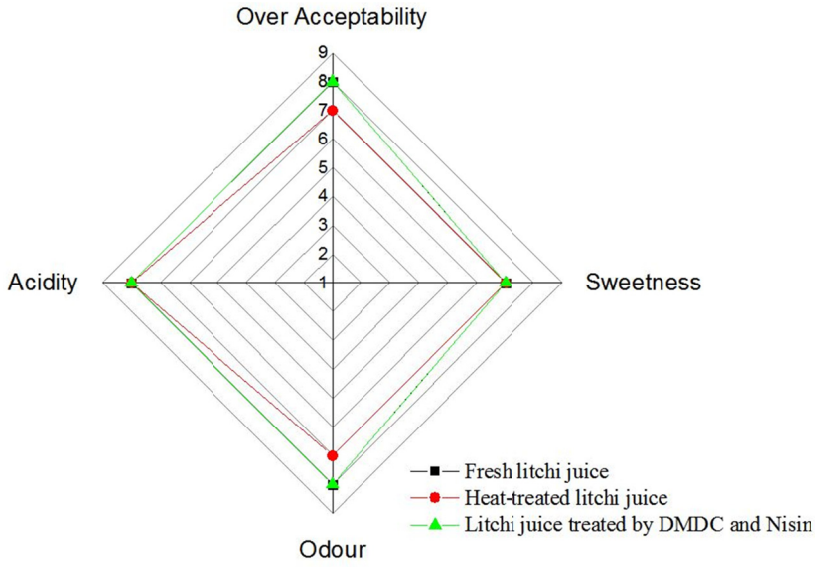

Figure 4. Sensory evaluation of odour, acidity, sweetness, and overall acceptability of litchi juice samples

4. Conclusion

Addition of DMDC combined with Nisin can ensure the microbiological safe of litchi juice during storage at 4 °C. Compare with heat treatment (95 °C, 1 min), which was equivalent effects on the inactivation of microorganisms with the litchi juice treated with 250 mg/L DMDC and 100 IU/mL of Nisin, the treatment of DMDC combined with Nisin can retain a more value of sensory attributes, but a more loss in the content of total phenolics, ascorbic acid, and antioxidant capacity was observed during storage at 4 °C because of the ineffectiveness of DMDC and Nisin to the oxidase of litchi juice. Moreover, no significant changes were observed in the value of $L*$, $a*$, $b*$, and ΔE in the heat-treated litchi juice, and yet the litchi juice treated by DMDC and Nisin gradually turned into light red at the end of storage because of the oxidation of phenolics by residual POD in the litchi juice, which resulted in a significant changes ($P < 0.05$) in the value of $L*$, $a*$, $b*$, and ΔE in the litchi juice. According to the results discussed above, it can be concluded that DMDC combined with Nisin treatment offers a useful alternative to conventional heat for controlling microbial growth and significantly extending the shelf-life of litchi juice as only considering the inactivation and inhibition of indigenous microorganism, but some treatments, which can inhibit the activity of residual oxidase in the litchi juice treated by DMDC combined with Nisin, will need be applied for well retaining the quality of litchi juice during storage at 4 °C.

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