Changes in microorganism, enzyme, aroma of hami melon (Cucumis melo L.) juice treated with dense phase carbon dioxide and stored at 4 °C

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A R T I C L E  I N F O

Keywords:
- Hami melon (Cucumis melo L.) juice
- Dense phase carbon dioxide (DP-CO2)
- Microorganism
- Enzyme
- Color
- Aroma compounds

A B S T R A C T

The effects of dense phase carbon dioxide (DP-CO2) treatment of 8, 15, 22, 30 and 35 MPa for 5 min, 15 min, 30 min, 45 min, 60 min at 35 °C, 45 °C, 55 °C, 65 °C on microorganism, enzyme, and aroma compounds in hami melon juice during storage at 4 °C for 4-weeks were investigated. Meanwhile, the color, browning degree, and Vitamin C were also studied. The DP-CO2 treatment had significant effects on inactivation of microorganism and enzyme. It was indicated that higher pressure caused more inactivation of microbial total count and enzyme activity. When it reached 35 Mpa, 55 °C, 60 min, the microorganism was totally inactivated. The least residual activity of polyphenol oxidase (PPO), peroxidase (POD), and lipoygenase (LOX) was 25.26%, 38.46 and 0.02% at 35MP, respectively. The restoration of PPO, POD and LOX residual activity after DP-CO2 treatment was also observed, which was dependent on the pressure level. The aroma compounds were less affected after being treated with DP-CO2, and the flavor of the melon juice was close to the fresh juice after storage at 4 °C for 4 weeks and did not produce cook off-odor. The changes of lightness L and browning degree A during storage were well fitted to a first-order kinetic model. The Vitamin C concentration decreased by DP-CO2 processing, but this loss was lower than of the untreated sample.

1. Introduction

Hami melon, or cantaloupe (Cucumis melo var. reticulatus. Hami melon) is a classical fruit produced in Xinjiang Uigur Autonomous Region, PR China, and it is highly appreciated for its nutritional quality and special flavor. Processed cloudy melon juice could be a best way to raise the merchandise rate of cantaloupe and to prolong its industrial chain (Ma, 2004; Ma et al., 2007). However, the flesh of melon is heat sensitive, the sensitive nutrients, color and aromatic profile will be spoiled greatly or off-odour when it was produced with high temperature treatment. It was reported that the total esters decreased 20%, and 6-carbon, 9-carbon alcohol and aldehyde contents decreased significantly after thermal treatment. Meanwhile, new aroma compounds, such as dimethyl disulfide (0.56%), dimethyltrisulfide (0.09%), 2-methylpyperazine (0.04%) and N-ethyl-methylthiamine (0.21%) were produced. The flavor of high temperature-treated melon juice had a cooked off-odor and no green flavor from the sensory (Ma, 2004). So it is important to find an innovative food process to inactivate the enzyme and microorganism and protect the nutrient and unique flavor.

Dense phase carbon dioxide processing (DPDC or DP-CO2), a collective term for liquid CO2 and supercritical CO2 or high pressurized carbon dioxide (HPCD), it is a non-thermal alternative pasteurization for fresh-squeezed juice, and it is attracting much interest in the food industry. Dagan, and Balaban (2006) described that HPCD retained the fresh-like sensory, nutritional, and physical properties of many liquid foods by avoiding thermal effects of traditional pasteurization. Studies with orange juice showed that HPCD treatment could improve some physical and nutritional quality attributes such as cloud formation and stability, color, and ascorbic acid retention (Arreola et al., 1991; Kincal, 2000; Kincal et al., 2006). Dagan, and Balaban (2006) observed that aroma and flavor of HPCD-treated beer was not significantly different from fresh beer, but beer haze was significantly reduced by HPCD. Many reports have shown that SCCO2 had significant lethal effect on microorganisms in liquid foods (Ballestra, and Cuq, 1998; Ballestra, Silva, and Cuq, 1996;
Corwin, and Shellhammer, 2002; Erkmen, and Karaman, 2001; Gui et al., 2007; Hong, and Pyun, 2001; Park, Lee, and Park, 2002; Shimoda et al., 1998, 2001). Meanwhile, recent reports have dealt with the influence of SCCO₂ on enzymes.

 Peroxidase (POD, EC 1.11.1.7), polyphenol oxidase (PPO, EC 1.14.18.1) and lipoxigenase (LOX, EC 1.13.11.12 ) are the important enzymes in many fruits and vegetables. Their residual activities were detrimental to the quality of processed products of fruits and vegetables resulting in effects such as browning, off flavor and loss of vitamins. Therefore, the inactivation of POD, PPO and LOX in the processing of fruits and vegetables is a major quality indicator of processed fruits and vegetables.

Most of the reports were focused on the inactivation of microorganisms and enzymes in pure solution and liquid food (Liao, Zhang, Hu, Liao, and Wu, 2008; Gui et al., 2006). So far, there is limited data available about inactivation of microorganisms and enzymes and flavor changes in melon juice. Therefore, the aim of this study was to investigate the effects of DP-CO₂ treatment with different pressure level on microorganism, enzyme, and to analyze the changes of microorganism, enzyme, and flavor of DP-CO₂ processed melon juice during storage at 4 °C.

2. Materials and methods

2.1. Materials

The melon samples studied were Xiangfei Mi cantaloupe (Cucumis melo var. reticulatus. Hami melon), a variety planted in Xinjiang Uigur Autonomous Region, PR China. All melon samples were fully ripe without any quality deterioration or decay.

Phosphoric acid and catechol and other chemicals in the investigation were of analytical grade, and they were purchased from Xiangsheng Chemicals Co. (Xinjiang, PR China). The purity of CO₂ was 99.9%, which was purchased from Urumqi Analytical Apparatus Co., (Xinjiang, China). The authentic standards of volatiles (ethyl acetate, ethyl propanoate, ethyl butyrate, ethyl-2-methyl butyrate, hexanal, (Z)-6-nonenal, nonanal, (Z)-nonel-6-ol, (E, Z)-3,6-nonadien, 2-nonenal, (Z)-nonel-3-ol, (E, Z)-3-hexan, 2-nonenal, (Z)-nonel-6-ol, nonanol ) were used as authentic compounds to identify the compounds.

2.2. Preparation of cantaloupe juice

Each melon was washed and sanitized with bleach solution (100 mg /kg) in a bail for 15 min, and then rinsed with tap water. Melons were then manually peeled. The pedicel, calyx sections, seeds and their circumambient section were removed. Finally, the melons were cut into pieces and squeezed into juice. Then the hami melon juice was filtered through a 4-layer cheese cloth and stored at −18 °C in darkness until use. The initial pH of the hami melon juice was 6.36. Samples were thawed at ambient temperature before use. All treated samples and control samples were stored at 4 °C for 4-weeks after treatment.

2.3. Dense phase carbon dioxide (DP-CO₂) treatment system and treatment of melon juice

DP-CO₂ treatment was performed with a system (Hua’an Company, Jiangsu, China) (Fig. 1). The system consisted of 500 mL stainless steel pressure. The minimum pressure (8 MPa) was chosen slightly above the critical pressure for CO₂ (7.36 MPa) and the maximum pressure (35 MPa) was slightly less than the operating limit of the equipment (50 MPa).

For each experiment, 50 mL of Hami melon juice was placed in a 200 mL Plastic Tube (Beijing Bomex Co, Beijing, PR China) without the cap and then placed in the DP-CO₂ vessel which had been preheated to the experimental temperature 35 °C, 45 °C, 55 °C, 65 °C and then exposed to 8, 15, 22, 30,35 MPa pressure for 5–10 min until the pressure reached the experimental level. At the end of DP-CO₂ treatment, the vessel was slowly depressurized over a period of 15 min. After treatment, the melon juice was removed and immediately cooled in an icebox. Ten consecutive experimental runs were performed on the condition above to produce the total volume of 500 mL needed for aroma and chemical analyses. Unpressurized samples were used as control. Following equilibration to ambient temperature, the residual activity of PPO, POD, and LOX, microorganism, color, and aroma compounds of hami melon juice were determined. Experiments and measurements were triplicate.

2.4. Microbial count

The dominant micro-organisms in the melon juice were isolated and cultivated with media (10 g glucose, 3 g beef extract, 5 g sodium chloride, 5 g peptone, 15 g agar and 1000 mL water) adjusted into pH 7.2, and sterilized (121.8 °C, 30 min) according to the standard methods (Zhou, 1997). The plate count method stipulated by GB4789.2-94 was used to calculate the amount of bacteria. The above plate count method is the same as the one adopted by other researchers in microbiological analysis (Song et al., 2006).

2.5. PPO, POD and LOX activity measurement

For the assay, 0.4 ml of the sample was mixed with 1.3 ml 0.05 mol/L sodium phosphate buffer (pH=6.8) and 1.3 ml 0.02 mol/L catechol. PPO activity was determined by measuring absorbance of the mixture at 420 nm, using a UVmini-1240 spectrophotometer (Shimadzu, Tokyo Japan ) at 32 °C. One unit of PPO activity was defined as the change in absorbance at 420 nm/min and per milliliter of Cantaloupe juice. The residual activity of PPO and POD was calculated as the activity after treatment divided by the activity before the treatment. The residual activity of PPO was obtained with the following equation.

\[
PPO\ residual\ activity = \frac{\text{Specific\ activity\ of\ PPO\ treated\ with} \ DP-CO_2\ treatment}{\text{Specific\ activity\ of\ PPO\ before} \ DP-CO_2\ treatment} \times 100\%
\]

POD activity was assayed by the method proposed by Kochba (Koch, Meier, Elben, and Stusarenko, 1992) with some modifications. The substrate was a mixture of 3 ml 30% hydrogen peroxide and 1.9 ml liquid guaiacol, made up to 300 ml with 0.2 mol/L sodium phosphate buffer (pH=6.0). For the assay, 0.2 ml of the sample was mixed with 3.0 ml substrate solution, and the absorbance of the mixture was measured at 470 nm with a Shimadzu UVmini-1240 spectrophotometer (Tokyo, Japan). One unit of POD activity was

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**Fig. 1.** Schematic diagram of DP-CO₂ processing equipment.
defined as the change in absorbance at 470 nm/min and per milliliter of cantaloupe juice. The residual activity of POD was obtained with the following equation.

\[
\text{POD residual activity} = \frac{\text{Specific activity of POD treated with DP} - \text{CO}_2 \text{ treatment}}{\text{Specific activity of POD before DP} - \text{CO}_2 \text{ treatment}} \times 100\%
\]

LOX activity was determined spectrophotometrically at 234 nm as proposed by Denys, Van-Loey, and Hendrickx (2000) with some modifications, by measuring the formation of conjugated dienes at ambient temperature (25 ± 1 °C) using a UV-T6 spectrophotometer (Xinshiji, Shanghai, China). Reaction substrate was prepared using sodium linoleate as follows: 60 μl linoleic acid and 120 μl Tween 20 was added into 4 mL of O2 free water. After vibrating, 30 μl 10 mol/L NaOH was added to clear the solution. Finally, the solution was made up with distilled water to a total volume of 25 mL, and stored at 4 °C in a refrigerator until being used. 20 μg LOX was dissolved in 1 mL pH 9.0 borate buffer to a concentration of 20 ng/mL. LOX activity was determined by adding 0.2 mL enzyme sample to 0.2 mL substrate solution and 2.6 mL borate buffer. Product formation was recorded by measuring the accumulation of the conjugated diene at 234 nm every 20 s for 3 min. A unit of enzyme was defined as the amount which produced a change in O.D. All samples were measured in triplicates and the means were reported. The residual activity of LOX was obtained with the following equation.

\[
\text{LOX residual activity} = \frac{\text{Specific activity of LOX treated with DP} - \text{CO}_2 \text{ treatment}}{\text{Specific activity of LOX before DP} - \text{CO}_2 \text{ treatment}} \times 100\%
\]

2.6. Isolation of volatile compounds using solid-phase microextraction (SPME)

Melon juice (8 mL) was quickly transferred into a 15-mL headspace flask containing 2.2 g NaCl, in order to minimize the loss of volatile components and avoid browning. The volatiles were sampled by manual headspace solid phase microextraction at 40 °C while stirring. The fibre (100 mL PDMS, Supelco) was pierced into the injection port of the GC/MS after 30 min of sampling, and then desorbed at 250 °C for 10 min. Each analytical sample was measured in triplicate.

2.7. Gas chromatography/mass spectrometry condition

A Hewlett-Packard 6890 GC/MS with a flame ionization detector (J&W Scientific inc., Germany) was used, with the injector and detector maintained at 250 and 270 °C, respectively. The column dimensions were 0.32 mm i.d. x 30 m x 0.5 μm film thickness (Hewlett-Packard). The carrier gas (He) had a flow rate of 40 mL/min. The temperature program was: isothermal at 40 °C for 2 min, increase to 75 °C at 4 °C/min, increase to 80 °C at 1 °C/min, increase to 250 °C at 12 °C/min, and then hold for 8 min. The analysis was done in triplicate. Compounds were identified by spectral matching against the NIST98 library of standard compounds. When available, MS identifications were confirmed by comparing GC retention times with authentic compounds.

2.8. Color assessment

Color assessment was conducted at 25 °C using a Color Difference Meter (WSC-S, Shanghai exact science apparatus Co, PR China) in the reflectance mode. Hunter \( L, a, \) and \( b \) values of samples were measured and the total color difference \( \Delta E \) was calculated as:

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

where \( L \): lightness of treated sample at time \( t; \) \( L_0 \): lightness of control sample at 0 day; \( a \): redness of treated sample at time \( t; \) \( a_0 \): redness of control sample at 0 day; \( b \): yellowness of treated sample at time \( t; \) and \( b_0 \): yellowness of control sample at 0 day.

2.9. Measurement of browning degree in melon juice

The browning degree of hami melon juice was evaluated using a spectrophotometric method described by Roig et al. (Roig, Bello, Rivera, and Kennedy, 1999). Hami melon juice was centrifuged in a Refrigerated Centrifuge (Neofuge15R, shanghai Lishen science apparatus Co, PR China). The browning degree was determined by measuring the \( A \) (absorbance at 420 nm) value using a UVmini-1240 spectrophotometer (Shimadzu, Tokyo Japan ) at ambient temperature (25 ± 1 °C) with a 1 cm path length cell.

2.10. Kinetic model

\( L \) value of hami melon juice during storage time was fitted to the first-order models:

\[
\frac{L_t}{L_0} = \exp(-k_t t) \quad (1)
\]

where \( L_t \) and \( L_0 \) are \( L \) and \( A \) value of samples at time \( t; \) \( k_t \) and \( k_0 \) are the initial \( L \) and \( A \) value of control sample at 0 day; \( t \) was the storage time, \( k_t \) and \( K_a \) are the first-order rate constants.

2.11. Measurement of Vitamin C in melon juice

It was analyzed by a titrimetric method by using 2, 6-dichloroindophenol (AOAC, 1975).

2.12. Data analysis

Analysis of variance (ANOVA) was carried out by using the software Microcal Origin 7.5 (Microcal Software, Inc., Northampton, USA). ANOVA tests were performed to determine the significance at 95% confidence.

3. Results and discussion

3.1. Inactivation of microorganism in melon juice after DP-CO\(_2\) treatment and stored at 4 °C

The composition of microorganisms in the melon juice was relatively complex, and different species of microorganisms had different levels of resistance. The exact means of microbial inactivation by DP-CO\(_2\) are not clear. Studies show that several mechanisms may be involved (Daniels, Krishnamurti, and Rizvi, 1985; Fraser, 1951; Ishikawa, Shimoda, Shiratsuchi et al., 1995a; Lin, Chan et al., 1991; Nakamura, Enomoto, Fukushima et al., 1994; Spilimbergo, and Bertucco, 2003). DP-CO\(_2\) may inactivate microorganisms by (a) pH lowering effect (Meyssami, Balaban, and Texeira, 1992). (b) Physical disruption of cells (Fraser, 1951). (c) Modification of cell membrane and extraction of cellular components (Kamihira, Taniguchi, and Kobayashi, 1987). (d) Inhibitory effect of molecular CO\(_2\) and bicarbonate ion (Ishikawa et al., 1995). The effect of temperature (35–65 °C) and treatment time (5–60 min) on inactivation of microorganisms was investigated. At 35 Mpa, 35–65 °C, 5–60 min, the total microbial count decreased significantly (Table 1). It meant higher pressure caused more inactivation of microbial count and the microorganism. It was perhaps that the higher pressure, the more
After DP-CO₂ pasteurization (8, 15, 22, 30, 35 Mpa), the total microbial count in melon juice exhibited an increase tendency with the increasing storage time. The microbial count in untreated melon juice was 5.11-log cycle. The microbial count was zero-log cycle at 35 Mpa, butyl acetate and ethyl-2-methyl butyrate) after DP-CO₂ treatment and stored at 4 °C was also observed (Table 2). After 4 week of storage, the residual activity of PPO was 23.59%, PPO was 35.05%, LOX was 0%. It meant that DP-CO₂ treatment can totally inactivate the LOX activity.

3.3. Aroma compounds change in melon juice after DP-CO₂ treatment and stored at 4 °C

The volatile components of muskmelon have been analyzed by a number of authors (Beaulieu, and Grimm, 2001), and approximately 240 compounds have been identified. Over half of these compounds are esters, of which some contain sulfur. Most of the remaining compounds are aldehydes and alcohols (Dimtritios, Stephen Elmore, and Donald, 2006). Esters, alcohols and aldehydes containing a nine-carbon straight chain have been shown to be important in muskmelon aroma (Buttery et al., 1982; Kemp, Knavel, and Stoltz, 1971; Kemp, Knavel, and Stoltz, 1972; Kemp, Knavel, Stoltz, and Lundin, 1974; Kemp, Stoltz, and Knavel, 1972). There are few reports in the literature regarding the effects of DP-CO₂ treatment on Hami melon aroma.

Forty-five volatile compounds were analyzed by SPME/GC/MS. The main compounds were indicated in Table 3. There was no change in ester composition (ethyl acetate, ethyl propanoate, ethyl butyrate, butyl acetate and ethyl-2-methyl butyrate) after DP-CO₂ treatment. There were slight changes in alcohols and aldehydes, such as

3.2. Inactivation and reactivation of PPO, POD and LOX activity in melon juice after DP-CO₂ treatment and stored at 4 °C

The inactivation of enzymes subjected to DP-CO₂ treatment is of interest to prevent the formation of off-flavor or enzymatic browning due to the residual activity of indigenous enzymes in food. Enzyme inactivation by DP-CO₂ could be due to many causes such as pH lowering, conformational changes of the enzyme, and inhibitory effect of molecular CO₂ on enzyme activity. (Balaban et al., 1991; Gui et al., 2007). In this study, the inactivation of PPO, POD, and LOX residual activity on hami melon subject to DP-CO₂ treatment for 4-week at 4 °C was shown in Table 1. After DP-CO₂ treatment, the loss of PPO, POD and LOX activity in melon juice were significant (P<0.05) as compared to control sample at least residual activity of PPO was 25.26%, POD was 38.46%, LOX was 0.02% at 35 Mpa. Meanwhile, the restoration of PPO, POD and LOX residual activity in melon juice after DP-CO₂ treatment and stored at 4 °C was 71.23% for melon juice.

Table 1
The effects of temperature and time on inactivation of microorganisms and enzymes and the change of I value under DP-CO₂ treatment. 35 Mpa, 55 °C, 60 min.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>5 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPO activity (%)</td>
<td>35 Mpa</td>
<td>38.46</td>
<td>37.46</td>
<td>36.59</td>
<td>35.77</td>
</tr>
<tr>
<td>30 Mpa</td>
<td>47.26</td>
<td>46.72</td>
<td>46.51</td>
<td>46.19</td>
<td>45.63</td>
</tr>
<tr>
<td>22 Mpa</td>
<td>58.37</td>
<td>58.42</td>
<td>58.23</td>
<td>58.16</td>
<td>58.06</td>
</tr>
<tr>
<td>15 Mpa</td>
<td>71.57</td>
<td>71.62</td>
<td>71.77</td>
<td>71.43</td>
<td>71.10</td>
</tr>
<tr>
<td>8 Mpa</td>
<td>93.31</td>
<td>93.42</td>
<td>93.58</td>
<td>92.1</td>
<td>91.03</td>
</tr>
<tr>
<td>0 Mpa</td>
<td>100</td>
<td>81.46</td>
<td>71.30</td>
<td>69.43</td>
<td>62.38</td>
</tr>
<tr>
<td>POD activity (%)</td>
<td>35 Mpa</td>
<td>25.26</td>
<td>27.92</td>
<td>29.03</td>
<td>27.24</td>
</tr>
<tr>
<td>30 Mpa</td>
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<td>40.09</td>
<td>41.71</td>
<td>40.19</td>
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<td>49.46</td>
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<td>58.37</td>
<td>56.93</td>
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</tr>
<tr>
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<td>55.29</td>
<td>62.91</td>
<td>67.78</td>
<td>62.22</td>
<td>60.41</td>
</tr>
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<td>81.46</td>
<td>71.30</td>
<td>69.43</td>
<td>62.38</td>
</tr>
<tr>
<td>LOX activity (%)</td>
<td>35 Mpa</td>
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<td>3.53</td>
<td>3.49</td>
<td>3.39</td>
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</tr>
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<td>81.46</td>
<td>71.30</td>
<td>69.43</td>
<td>62.38</td>
</tr>
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</table>

Table 2
Effect of DP-CO₂ treatment (8–35 Mpa, 55 °C, 60 min) and storage on the activity of PPO, POD and LOX in hami melon juice.

<table>
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<tr>
<th>Microorganism</th>
<th>0 week</th>
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<th>2 week</th>
<th>3 week</th>
<th>4 week</th>
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<td>71.30</td>
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<tr>
<td>8 Mpa</td>
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<td>47.62</td>
<td>51.32</td>
<td>49.46</td>
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<tr>
<td>30 Mpa</td>
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<td>40.09</td>
<td>41.71</td>
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<td>38.66</td>
</tr>
<tr>
<td>35 Mpa</td>
<td>25.26</td>
<td>27.92</td>
<td>29.03</td>
<td>27.24</td>
<td>23.59</td>
</tr>
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<td>46.51</td>
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<tr>
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<td>36.59</td>
<td>35.77</td>
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<td>LOX activity (%)</td>
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<td>80.77</td>
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</tr>
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<td>8 Mpa</td>
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<td>5.01</td>
<td>4.93</td>
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</tr>
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</table>

Fig. 2. Effect of DP-CO₂ treatment and storage on the microorganism in hami melon juice.
samples subjected to DP-CO₂ treatment and heat was smaller than the control sample. For the DP-CO₂ treated samples, the color of control sample was remarkable. Meanwhile, the kinetic rate of DP-CO₂ treated samples were greater than 0.8464, indicating a good linear fit. The kinetic rate of samples subjected to DP-CO₂ treatment were significantly reduced compared with the control sample. Moreover, the kinetic rate of DP-CO₂ treatment with higher pressures resulted in lower kinetic rate. The kinetic rate of DP-CO₂ treatment increased from 8 MPa to 35 MPa.

The effects of DP-CO₂ treatment control sample on browning degree of hami melon juice during storage at 4 °C were investigated. DP-CO₂ treatment resulted in a significant reduction of the browning degree of hami melon juice during storage as compared to control sample (p<0.05). It indicated that DP-CO₂ treatment could effectively prevent the browning of hami melon juice. The A value change in hami melon juice was fitted to a first-order kinetic model (Fig. 4) subjected to DP-CO₂ treatment during storage. This model proved to be adequate with determination coefficients (R²) of 0.9504 to 0.9534 in Table 4. The kinetic rate of the browning degree, which were computed by Eq. (2) and displayed, decreased significantly as the pressure increased during DP-CO₂ treatment. The kinetic rate of the browning degree was less in DP-CO₂ treated samples than in control sample. The kinetic rate of samples subjected to DP-CO₂ treatment during storage. This model proved to be adequate with determination coefficients (R²) of 0.9504 to 0.9534 in Table 4. The kinetic rate of the browning degree, which were computed by Eq. (2) and displayed, decreased significantly as the pressure increased during DP-CO₂ treatment. The kinetic rate of the browning degree in hami melon samples subjected to DP-CO₂ treatment were significantly reduced as the pressure increased during DP-CO₂ treatment. The kinetic rate of the browning degree in hami melon samples subjected to DP-CO₂ treatment were significantly reduced as the pressure increased during DP-CO₂ treatment.

3.4. Color and browning degree change in melon juice after DP-CO₂ treatment and stored at 4 °C

To describe the total color of melon juice, the combination of parameters L, a, and b, were determined in terms of total color difference (∆E). The plot between the total color difference of hami melon juice and storage time is shown in Fig. 3. The increase of color ∆E of control sample was remarkable. Meanwhile, the ∆E increase of samples subjected to DP-CO₂ treatment and heat was smaller compared with control sample. For the DP-CO₂ treated samples, ∆E could be reduced by enhancing the pressure level. The minimum ∆E of hami melon juice for 4-week storage was 0.77 after DP-CO₂ treatment at 35 MPa.

The L value was shown in Table 1. At the same treated-time, there was less in L value. At the same temperature, with time increasing, there was less reduction in L value. The change of hami melon juice was followed first-order model (Eq. (1)). The kinetic rate of DP-CO₂ treatment sample was greater than 0.8464, indicating a good linear fit. The kinetic rate of samples subjected to DP-CO₂ treatment were significantly reduced compared with the control sample. Moreover, the kinetic rate of DP-CO₂ treatment with higher pressures resulted in lower kinetic rate. The kinetic rate of DP-CO₂ treatment increased from 8 MPa to 35 MPa.

The effects of DP-CO₂ treatment control sample on browning degree of hami melon juice during storage at 4 °C were investigated. DP-CO₂ treatment resulted in a significant reduction of the browning degree of hami melon juice during storage as compared to control sample (p<0.05). It indicated that DP-CO₂ treatment could effectively prevent the browning of hami melon juice. The A value change in hami melon juice was fitted to a first-order kinetic model (Fig. 4) subjected to DP-CO₂ treatment during storage. This model proved to be adequate with determination coefficients (R²) of 0.9504 to 0.9534 in Table 4. The kinetic rate of the browning degree, which were computed by Eq. (2) and displayed, decreased significantly as the pressure increased during DP-CO₂ treatment. The kinetic rate of the browning degree was less in DP-CO₂ treated samples than in control sample. The kinetic rate of samples subjected to DP-CO₂ treatment during storage. This model proved to be adequate with determination coefficients (R²) of 0.9504 to 0.9534 in Table 4. The kinetic rate of the browning degree, which were computed by Eq. (2) and displayed, decreased significantly as the pressure increased during DP-CO₂ treatment. The kinetic rate of the browning degree in hami melon samples subjected to DP-CO₂ treatment were significantly reduced as the pressure increased during DP-CO₂ treatment.

Vitamin C is an important nutrient in fruit juice. The content following the DP-CO₂ pasteurization varied (Fig. 5). Ascorbic acid reduced both in control sample and in DP-CO₂-treated sample during storage at 4 °C for 4-week. After DP-CO₂ processing at the selected range (35 MPa, 55 °C, 60 min), no significant difference (p>0.05) in ascorbic acid content of the melon juice was detected if compared...
with that of the fresh juice (the amount of ascorbic acid in control: 25.5 mg/100 mL and 24.2 mg/100 mL in the juice treated with DP-CO2). It can be observed in Fig. 5. When stored at 4 °C for 4-week, the control sample caused a considerable losses of ascorbic acid 99.6% in fresh melon juice. Meanwhile, the ascorbic acid content in DP-CO2-treated melon juice was 0.5 mg/100 mL, and it was 6.4 times than it in fresh melon juice (0.078 mg/100 mL). Perhaps it was because CO2 can lower pH when dissolved in the aqueous part of foods, ascorbic acid has higher stability at low pH and oxidizes easily when oxygen is present in the environment. Further studies should be carried on the effect of DP-CO2 on vitamins in fruit juices.

4. Conclusion

DP-CO2, a non-thermal preservation technique topasteurize melon juice, proved to efficiently preserve some quality attributes, including the color, browning degree, and most of the main aroma compounds in the melon juice after being treated with DP-CO2. During storage at 4 °C for 4-week, it caused minimal losses in some quality of the melon juice, such as, color and browning degree, ascorbic acid content and the aroma compounds which was responsible for the fresh melon juice flavor. DP-CO2 can be considered as a promising non-thermal alternative pasteurization to preserved fresh-squeezed melon juice through microorganism and enzyme inactivation of microbe destruction and make a good measure of both quality and process stability during storage. Specific investigations are needed to determine the parameters required to optimize the quality and nutrient of melon juice.

![Fig. 5. Effect of DP-CO2 treatment and storage on the Vc content in hami melon juice.](image)

### Table 4

Kinetic constants of the first-order model fitted to L (lightness) and A (absorbance at 420 nm) values of hami melon juice during storage after DP-CO2 treatment.

<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th>Kinetic rates (week^-1)</th>
<th>Determination coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( K_L \times 10^{-2} )</td>
<td>( R^2 )</td>
</tr>
<tr>
<td>0</td>
<td>1.02</td>
<td>0.932</td>
</tr>
<tr>
<td>8</td>
<td>1.54</td>
<td>0.846</td>
</tr>
<tr>
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<td>1.14</td>
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<tr>
<td>22</td>
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</tr>
<tr>
<td>30</td>
<td>1.03</td>
<td>0.896</td>
</tr>
<tr>
<td>35</td>
<td>1.25</td>
<td>0.985</td>
</tr>
</tbody>
</table>

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**References**


