Effect of dense phase carbon dioxide treatment on physicochemical and textural properties of pickled carrot

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ABSTRACT
Dense phase carbon dioxide (DPCD), as a new type of non-thermal technologies, has exhibited a remarkable effect on sterilization to improve the quality of many fruits and vegetables, but little known on the pickled carrot. In this study, the effect of DPCD treatment on physicochemical, textural properties and microbiological quality of pickled carrot treatment was investigated. Our results showed that the optimum condition of DPCD treatment was 30 min under 20 Mpa. Compared with thermal treatment, the DPCD treatment significantly improved the storage quality of the pickled carrot at hardness, color, and β-carotene content. Moreover, DPCD treatment increased the total pectin content and reduced the water-soluble pectin content of the pickled carrot without changing the pectin structures and cell-wall composition. Taken together, our study showed that DPCD is a comprehensive technique to improve the quality of the carrot in pickles processing.

1. Introduction
Pickled carrot is one of the important forms of the traditional fermented products in China. It presents a crispy texture and unique flavor due to the lactic acid content of the fermented product (Acosta, Vermeylen, Noel, & Padilla-Zakour, 2015). Pickled carrot contains lactic acid bacteria and their metabolic products. The microbial count is one of the most important factors effecting the quality of pickled carrot. (Bao, Fan, Hu, Liao, & Chen, 2016). Generally, 4 log10 CFU/g of lactic acid bacteria can maintain the quality of pickles. Less lactic acid bacteria may lead to incomplete fermentation, while, too much of them may cause excessive acidification. The total acid content can be used to investigate the fermentation degree of products, which is mainly related to the number of lactic acid bacteria. Structural parameters such as hardness directly affect the crispy taste of the pickled carrot (Llorca et al., 2001). Furthermore, cell wall and intercellular layer structure are the key determinants of structural properties. Cell wall is mainly composed of cellulose, lignin polymer and pectic substances. Pectin content and property have an important influence on cellular structure, which will relate to the hardness and brittleness of pickled carrot (Zhang et al., 2012).

Pickled carrot has a short shelf life if it is not sterilized. Thermal sterilization and preservation (potassium sorbate and sodium benzoate, etc.) can prolong the shelf life. However, thermal sterilization will not only kill the beneficial lactic acid bacteria but also resulted in texture softening and other bad effects (Awuah, Ramaswamy, & Economides, 2007). DPCD treatment prolongs the new type of non-thermal technologies. DPCD treatment prolongs the shelf life by using CO₂ molecular effect plus high pressure to

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reduce the total number of microorganisms (Galvanin et al., 2014; Ortuno, Martinez Pastor, Mulet, & Benedito, 2012, 2013). The CO₂ pressures can range from 7.0 to 40.0 MPa, which is lower than ultra-high pressure sterilization technology (Ferrentino & Spilimbergo, 2017). This treatment can effectively maintain product quality without the inclusion of water inside the product, and promote the transformation of β-carotene in the cells compared to thermal sterilization. DPCD is an acidic environment sterilization technology, which will keep a proper amount of lactic acid bacteria in pickled carrot to maintain the special flavor. Theoretically, DPCD treatment is more suitable for pickles processing than other non-thermal sterilization treatment. However, the effects of DPCD treatment on pickled carrot quality are not well known, although DPCD effectively maintained many fruits and vegetables’ quality such as, fresh-cut carrot, feijoa Puree and so on (Bi, Wu, Zhang, Xu, & Liao, 2011; Duong, Balaban, & Perera, 2015; Liao, Hu, Liao, Chen, & Wu, 2007).

In this study, the optimum DPCD treatment condition of pickled carrot was investigated. The sterilization effect, color, β-carotene content, hardness and pectin characteristics of pickled carrot treated by DPCD and traditional sterilization method, thermal technology was compared. The results from this study will provide a comprehensive technical support for the application of DPCD in pickled vegetable processing.

2. Materials and methods

2.1. Chemicals and reagents

Nutrient agar and MRS culture media were obtained from Beijing Aoboxing Biological Technology Company, Ltd. (Beijing, China). D-galacturonic acid standard (97.0% or higher) was purchased from Sigma Chemicals Company (California, USA). Lactic acid bacteria powder was obtained from Beijing Chuanxin Technology Co., Ltd. (Beijing, China). All the chemicals (anhydrous ethanol, sulfuric acid, acetone, petroleum ether, 1 g/L carbazole ethanol solution) were of analytical grade and were obtained from Beijing Chemical Reagent Company (Beijing, China). The chromatographic grade solvents (methanol, acetic acid, (TCA) were homogenized using a Philips handheld mixer (Philips Electronics Hong Kong Limited, Hong Kong, China) and filtered by four layers of cotton gauze. The pickled carrots (10 g) plus 90 mL, 100 g/L trichloroacetic acid (TCA) were homogenized using the following equation.

\[ P = \frac{(P_1 - P_0)}{(P_2 - P_0)} \times 100\% \]  

2.2. Preparation of pickled carrot

Fresh carrots were purchased from a local market, Guoguo Shidai, in Zhanghua Road, Haidian District, Beijing, China. The edible parts were cleaned with sterile water and cut into 1 × 1 × 3 cm strip using a sterile knife. Lactic acid bacteria powder (2 g) dissolved in sterile water (1.8 L). The addition amount was 1 kg of carrots: 2 g lactic acid bacteria powder. Carrot strips were soaked in 8% NaCl solution and lactobacillus solution in a fermentation jar (5 L). The jar was capped and put some water on the edge of the cap. The ratio was 1 kg of carrots:1.8 L solution containing 8% NaCl and 2 g of lactobacillus. The carrot strips were pickled for 4–6 days sat 20 °C when the total acid content reached 0.8%.

2.3. DPCD treatment and thermal treatment

Pickled carrots were subjected to different treatments in a DPCD equipment (Haian oil scientific instrument co., Ltd. Nantong, China). Using commercial grade liquid CO₂ of 99.5% purity (Beijing beiven gas factory, Beijing, China). The pickled carrots were placed in the vessel and subjected to pressures of 10, 15, 20 and 25 MPa at 20 °C with each DPCD treatment being repeated for 10, 20 and 30 min. Thermal treatment was carried out at 95 °C for 5 min. DPCD (20 Mpa/30 min) and thermal treated samples were packaged in sealed bags and stored at 37 °C until further analysis of the storage quality of pickled carrot.

2.4. Microbiological analysis

The pickled carrots (25 g) were immersed in 225 mL sterile 0.85% NaCl solution. Serial dilutions were made and plated onto appropriate culture media to determine microbiology. The total number of bacteria and lactic acid bacteria were detected by incubating in nutrient agar and MRS culture media at 37 °C for 48 h, respectively. Microbiological numbers were expressed as log₁₀ CFU/mL.

2.5. Determination of pickled carrots’ relative conductivity

The pickled carrots were cut into chips which diameter was about 1 cm with a sterile knife. Twenty of the chips were put in a triangular flask then 60 mL of distilled water added to it. The conductivity was measured as P₁ after stirring for 20 min on the magnetic stirrer. The triangular flask was boiled for 10 min, and then the conductivity was measured as P₂ when the temperature cooled with after cooling it. The conductivity of distilled water was as P₀. The relative conductivity was calculated by using the following equation.

\[ P = \frac{(P_1 - P_0)}{(P_2 - P_0)} \times 100\% \]  

2.6. Malondialdehyde content (MDA) detection

The pickled carrots (10 g) plus 90 mL, 100 g/L trichloroacetic acid (TCA) were homogenized using a Philips handheld mixer (Philips Electronics Hong Kong Limited, Hong Kong, China) and filtered by four layers of cotton gauze 80 cm×50 m (Caoxian Hualu Health Materials Co., Ltd.). The homogenate was centrifuged at 4°C, 12000 × g for 20 min using a centrifuge (Sigma 3-18K, Germany). Then, the supernatant collected and 3 mL of it was added to 3 mL, 0.67% sulfur barbituric acid (SBA) solution while the distilled water was added 0.67% TBA as blank as control. The mixed solution was boiled for 20 min, then cooled to room temperature. The blank values of supernatant were detected at 450, 532, and 600 nm, respectively, by using a UV-1800 spectrophotometer (Shimadzu Co. Japan). The MDA content was calculated using equation 2.
\[ C(\mu\text{mol}/100\text{g}) = \frac{[6.45 \times (\text{OD532} - \text{OD600}) - 0.56 \times \text{OD450}] \times V}{m \times 1000} \]  

(2)

where \( C \) was the MDA content; OD\text{532}, OD\text{532} and OD\text{600} were the absorbance values at 450, 532, and 600 nm; \( V \) was the total volume of the extracting solution in mL; \( V_s \) was the samples’ volume of extracting solution used in mL and \( m \) the sample mass in g.

### 2.7. Hardness analysis

Hardness measurement was performed using a TA-XT plus texture analyzer (Stable Micro Systems, Surrey, England). Operation mode was as follows: piercing mode, Probe: P/2; Parameter Settings: 10 mm/min rate before measuring, testing rate of 20 mm/min, post-test rate of 20 mm/min, piercing distance of 8 mm. The hardness was defined as the peak force at 50% strain.

### 2.8. Microstructure analysis

Pickled carrots were sliced to 1.0 mm thickness and fixed in the supplier of the 2% glutaraldehyde in 0.1 M phosphate buffer solution (PBS) at a pH of 7.4. The samples were stored for a minimum of 72 h at 3°C, washed with PBS and then fixed in 1% osmium tetroxide (OsO\text{4}) for 90 min. Pickled carrots were washed and dehydrated by using a graded ethanol series (50%, 70%, 80%, 95% and 100%) for 20 min, respectively. The dehydrated pickled carrots were subjected to critical point drying (Leica CPD030, Germany) and then sputter-coated with gold/palladium. A scanning electron microscope (SEM) (NovaNanoSEM 400, FEI Company, Hillsboro, OR) was used to scan at 5 kV and a magnification of 200.

### 2.9. Color measurement

The white and black calibration plates were used for standardization prior to measurement. Color quality was evaluated by using a color parameter (\( L^*, a^*, b^* \)) obtained from CM-3700d colorimeter (Konica Minolta, Japan). A small aperture was selected to measure CIE-\( L^*, a^*, b^* \) in the reflection mode. Fifteen points for each sample were measured. The mean and standard deviation were calculated.

### 2.10. \( \beta \)-carotene content analysis

Pickled carrots were homogenized for 20 s with a mixer (Philips Electronics Hong Kong Limited, Hong Kong, China). A 10 g sample was extracted using 25 mL of extraction solution (petroleum ether:acetone = 80:20, v/v) and the upper yellow liquid was collected. The extraction process was repeated until the aqueous phase was colorless. \( \beta \)-carotene was obtained after removing the solution by rotary evaporation (water bath temperature was set at 30 °C). The sample was dissolved in petroleum ether, and then adsorbed on an aluminum oxide column. \( \beta \)-carotene was eluted with the eluting solution (petroleum ether:acetone = 95:5, v/v). The filtrate was diluted with the eluting solution to 10 mL. Finally, the resulting solution was filtered through a 0.22 µm syringe filter for high-performance liquid chromatography (HPLC) analysis. The HPLC analysis was performed by an Agilent 1200 series liquid chromatograph system (Agilent Technologies, USA) with a \( \beta \)-carotene standard and an XBridge C\text{18} column (4.6 × 250 mm, 5 µm, Agilent Technologies, USA). The wavelength of the UV detector was set at 448 nm. The elution mode was isocratic with a mixture of 90% methanol and 10% acetonitrile as the mobile phase at a flow rate of 1.2 mL/min with an injection volume of 20 µL aliquot of sample for HPLC analysis. \( \beta \)-carotene standard curve was as follows:

\[ Y = 144.04X - 19.175, R^2 = 0.99953. \]

Where \( Y \) represented peak area, and \( X \) represented \( \beta \)-carotene concentration (µg/mL). \( \beta \)-carotene content was calculated using the following equation.

\[ X = \frac{V \times c \times \frac{1}{m}}{1000} \]  

(3)

where \( X \) is \( \beta \)-carotene content of sample (g/kg), \( V \) is volume of 10 mL, \( c \) is \( \beta \)-carotene concentration of standard (µg/mL), \( m \) is sample mass (g).

### 2.11. Pectin content analysis

Pickled carrots (1.0–5.0 g) were placed in a 50 mL centrifuge tube, and 35 mL of anhydrous ethanol was added at 75 °C in a water bath (Digital Utility Baths, StableTemp, USA). The centrifuge tube was heated in a water bath at 85 °C for 10 min, shocked fully, cooled in a water bath at 10 °C until it reached 25 °C and then topped with anhydrous ethanol to a total volume of 50 mL. The solution was centrifuged at 4000 r/min for 15 min. The supernatant was discarded. The process of heating and centrifugation using a centrifuge (Sigma 3-18K, Germany) was repeated until the supernatant contained none sugar. The precipitate was collected and dissolved in a sulfuric acid solution (pH 0.5), and then heated in a water bath (Digital Utility Baths, StableTemp, USA) at 85 °C for 60 min. The solution was cooled in a water bath at 10 °C until it reached 25 °C and fixed the volume to 100 mL with a sulfuric acid solution (pH 0.5), then the solution was filtered. The filtrate was stored at 4 °C for further use. One mL of filtrate was put in a 25 mL test tube, then added 0.25 mL of carbazole ethanol solution and 5 mL of sulfuric acid, and then heated in a water bath (Digital Utility Baths, StableTemp, USA) at 85 °C for 20 min. The absorbance of the solution was measured at 525 nm after cooling in a water bath at 10 °C until it reached 25 °C. The absorbance of the solution was measured at 525 nm after cooling in a water bath at 10 °C until it reached 25 °C (using a UV spectrophotometer, UV-1800 Shimadzu UV Spectrophotometer, Japan). Galactose acid content (pectin content) standard curve was as follows:

\[ Y = 0.00915X - 0.01105, R^2 = 0.9994. \]

Where \( Y \) represented absorbance value, and \( X \) represented pectin concentration (mg/L). Pectin content was calculated as the following equation.

\[ w = \frac{\rho \times V}{m \times 1000} \]  

(4)

where \( w \) pectin content of sample (g/kg); \( \rho \) is pectin concentration of standard (mg/L), \( V \) is volume of 100 mL, \( m \) is sample mass (g).

### 2.12. Pectin type analysis

The cell-wall materials and extraction of pectin were performed following the method by Christiaens et al. (2011) with some modifications. Pickled carrots (10 g) were ground rapidly in an ice-cold mortar, then 80% (v/v) ethanol...
(100 mL) added and heated in a water bath (Digital Utility Baths, StableTemp, USA) at 85 °C for 1 h, then, filtrated. This procedure was repeated until the filtrate did not contain sugar. After that, the residue obtained after filtration was dried in the oven (DHG-9240A, Beijing Yashilin Testing Equipment Co., Ltd.) at 40 °C for pectin crude extract.

The pectin crude extract was extracted sequentially to obtain water-soluble pectin (WSP), chelated pectin (CSP) and alkali-soluble pectin (NSP). A quarter gram of pectin crude extract was stirred at 28 °C for 4 h in 45 mL distilled water. The mixture was then filtered using 300 molybdenum gauze, and distilled water was added to the filtrate to a volume of 50 mL as WSP. The residue obtained from filtration was further extracted at 28 °C for 6 h with 45 mL 0.05 M EDTA which contained 0.1 M KAC (pH 6.5). The mixture was then filtered using molybdenum gauze as described above. The filtrate was collected and diluted with the extract liquor [0.05 M EDTA which contained 0.1 M KAC (pH 6.5)] to 50 mL as CSP. The residue obtained from filtration was further extracted at 4°C for 16 h in 45 mL 0.02 M NaBH₄. The residue obtained from filtration was subjected to additional extraction at 28 °C for 6 h. The mixture was filtrated using molybdenum gauze. The filtrate was collected and diluted with the extract liquor [0.05 M EDTA which contained 0.1 M KAC (pH 6.5)] to 50 mL as NSP.

2.13. FT-IR spectrum analysis

Pectin crude extract was ground into a fine powder using a pestle and mortar. Infrared spectra were recorded on a Nicolet 6700 FT-IR spectrometer (Thermo Nicolet Corporation, MA, USA) with an ATR detector. The conditions were as follows: scan range 400–4000 cm⁻¹, resolving was 4 cm⁻¹, the number of scans was 100 times.

2.14. Statistical analysis

All values were expressed as mean ± SD. All the data were repeated for three times. Data were analyzed by using one-way analysis of variance (ANOVA) followed by post-hoc Dunnett’s t-test using SPSS statistics 20.0 software (SPSS Inc., Chicago, USA). Differences with p < 0.05 were considered significant.

3. Results and discussion

3.1. DPCD treatment condition selection

The pickled carrots were treated with DPCD at 10, 15, 20 and 25 MPa at 20 °C with each DPCD treatment being repeated for 10, 20 and 30 min. The results indicated that the initial total bacterial number and lactic acid bacteria number of treatments under the pressure of 15 MPa for 10 min did not reduce significantly (p > 0.05), while other treatments were effective in inactivating microorganisms (Table 1). Sterilization is the first role of DPCD processing and therefore, treatment pressures of 20 and 25 MPa at 20 and 30 min were found to be the best treatments that could decrease the total bacterial number below 3 log₁₀ CFU/mL.

The relative electrolytic leakage is an important indicator of membrane integrity and permeability, while malondialdehyde is one of the products of cytomembrane peroxidization. Both relative electrolytic leakage and malondialdehyde can reflect the damage to the cell (Hilz, Lille, Poutanen, Schols, & Voragen, 2006). The relative electrolytic leakage and malondialdehyde content of pickled carrot was 67.6% and 0.43 μmol/L, respectively (Table 2). DPCD treatments increased the electrolytic leakage and malondialdehyde content. The relative electrolytic leakage of 25 MPa treatment was higher than that of 20 MPa, while there was no significant (p > 0.05) difference of the malondialdehyde content.

### Table 1. Effect of DPCD treatments on bacteria of pickled carrot.

| Microorganism | Time   | CK            | 10 MPa       | 15 MPa       | 20 MPa       | 25 MPa       |
|---------------|--------|---------------|--------------|--------------|--------------|--------------|
| Total bacteria| 10 min | 3.94 ± 0.54ᵃ   | 3.82 ± 0.43ᵃ  | 3.79 ± 0.23ᵃ  | 3.75 ± 0.33ᵇ  | 3.31 ± 0.38ᶜ  |
| Lactic acid bacteria | 10 min | 8.14 ± 0.74ᵃ   | 8.01 ± 0.63ᵇ  | 7.94 ± 0.53ᵇ  | 7.27 ± 0.43ᶜ  | 6.09 ± 0.55ᶜ  |

Note: The unit is log₁₀ CFU/mL. CK was the sample without DPCD treatment. Data are expressed as mean ± standard deviation of triplicate samples. Values in the same column sharing different letters are significantly different (p < 0.05).

Note: La unidad es log₁₀ CFU/mL. CK fue la muestra sin tratamiento con DPCD. Los datos se expresan como media ± desviación estándar de muestras en triplicado. Los valores en la misma fila que comparten letras distintas son significativamente diferentes (p < 0.05).

### Table 2. Effect of DPCD treatments on quality of pickled carrot.

| Treatments | The relative electrolytic leakage (%) | Malondialdehyde content (μmol/g) | Hardness (N) | Brittleness (N) | pectin content (g/kg) |
|------------|--------------------------------------|----------------------------------|-------------|---------------|---------------------|
| 0MPa 0min  | 67.6 ± 4.34ᵃ                         | 0.43 ± 0.07ᵇ                     | 9.4 ± 0.44ᵇ | 14.9 ± 0.76ᵇ  | 44.26 ± 2.45ᵇ       |
| 20MPa 0min | 86.3 ± 3.45ᵇ                         | 1.31 ± 0.13ᵇ                     | 9.1 ± 0.61ᵇ | 13.5 ± 0.92ᵇ  | 22.02 ± 1.17ᶜ       |
| 25MPa 0min | 82.4 ± 5.34ᵇ                         | 1.34 ± 0.19ᵇ                     | 8.5 ± 0.70ᵇ | 13.2 ± 0.67ᵇ  | 28.12 ± 2.49ᵇ       |
| 25MPa 30min| 92.3 ± 4.86ᵇ                         | 1.24 ± 0.13ᵇ                     | 8.2 ± 0.64ᵇ | 13.0 ± 0.87ᵇ  | 19.19 ± 1.38ᶜ       |
| 25MPa 30min| 93.5 ± 3.34ᵃ                         | 1.44 ± 0.22ᵇ                     | 7.2 ± 0.54ᵇ | 12.1 ± 0.74ᶜ  | 17.71 ± 0.73ᶜ       |

Note: DPCD treatment was at 20 Mpa for 30 min. Data are expressed as mean ± standard deviation of triplicate samples. Values in the same column sharing different letters are significantly different (p < 0.05).

Nota: El tratamiento con DPCD fue a 20 Mpa durante 30 min. Los datos se expresan como media ± desviación estándar de muestras en triplicado. Los valores en la misma columna que comparten letras distintas son significativamente diferentes (p < 0.05).
between different pressure treatments. Furthermore, the effect of DPCD treatment on hardness, brittleness of pickled carrot was studied, and the results are shown in Table 2. The hardness and brittleness of samples treated with DPCD decreased significantly (p ≤ 0.05) compared to the control group, while the texture property had no difference among the samples treated with DPCD at 20 Mpa for 20, 30 min and 30 Mpa for 20 min, which were stronger than samples treated at 25 Mpa for 30 min. Pectin content that affected texture property was estimated (Table 2). Pectin content is also an important nutrient that is known to promote digestion (Cabrera, Cambier, & Cutsem, 2011). DPCD treatments decreased the pectin content, with DPCD at 20 Mpa for 30 min exhibited the highest value than other groups treated with DPCD (Table 2). Furthermore, the pickled carrots SEM microstructures are displayed in Figure 1(a–e). The cell of the control sample was intact, which has a regular shape, whereas the flat cell was caused by brine immersion. The cell of the sample treated with DPCD at 20 MPa had no obvious damage. The boundary between cells that treated for 20 min was blunted, while the most cell stereoscopic perception increased to 30 min. The cell of the sample treated at 25 MPa was damaged, and loosely arranged, especially treated for 30 min, cells appear in fragments. Therefore, the optimum condition of DPCD treatment was 20 MPa for 30 min.

3.2. Influence of DPCD and thermal treatments on pickled carrot quality during storage

The inactivation of microorganisms plays a key role in achieving extended shelf life and maintaining the quality of pickled carrot. The DPCD (20 Mpa/30 min) and thermal treatment exhibited a significant inactivation on the total bacteria from 4.0 to 2.1, 2.3 log₁₀ CFU/mL and the lactic acid bacteria from 7.4 to 4.0, 3.3 log₁₀ CFU/mL, respectively (Figure 2). The numbers of lactic acid bacteria were higher than the total bacteria because of different culture media. The numbers of the total bacteria and lactic acid bacteria increased during storage except that there was a drop between day 12–20. DPCD treatment exhibited a higher degree of inactivation than thermal treatment during storage, with the total bacteria number being less than 5 log₁₀ CFU/mL and lactic acid bacteria ranging between 3.3 and 4.7 log₁₀ CFU/mL during storage for 40 days at 37 °C (Figure 2). The high-pressure treatments are known to disrupt the cell membranes and proteins of microorganism but the pressures used are typically over 200 MPa. In our case, the pressures were lower than high-pressure treatment but the implementation of the CO₂ treatment may have increased the antimicrobial effect.

Figure 1. Effect of DPCD treatments on the cell structure of pickled carrot. (A) Effect of DPCD treatments on hardness of pickled carrot; (B) The cell structure of pickled carrot without treatment; (C) The cell structure of pickled carrot with DPCD treatments for 20 min under 20 Mpa; (D) The cell structure of pickled carrot with DPCD treatments for 20 min under 25 Mpa; (E) The cell structure of pickled carrot with DPCD treatments for 30 min under 25 Mpa.

Figure 2. Effect of different sterilization treatments on the total bacterial number and lactic acid number of pickled carrots during storage. (A) Effect of different sterilization treatments on the total bacterial number; (B) Effect of different sterilization treatments on the total lactic acid number.

Figura 2. Efecto de diferentes tratamientos de esterilización en el número total de bacterias y el número de ácido láctico de las zanahorias en escabeche. (A) Efecto de diferentes tratamientos de esterilización sobre el número bacteriano total; (B) Efecto de diferentes tratamientos de esterilización sobre el número total de ácido láctico.
The results indicated that DPCD treatment could maintain good taste under a condition of microorganism’s safety because there is a certain fermenting effect, but not over fermentation at this number of colonies.

It is observed that pickled carrots subjected to different treatments exhibited different visual colors. On the initial day of storage, thermal treatment exhibited the lowest L* value, while DPCD treated samples had an L* value between that of samples treated with thermal treatment and the control. The L* value decreased with the storage period. DPCD treatment had the highest L* value at the end of storage (Table 3).

The parameters a* and b* were measured to assess the change in red and yellow color. a* increase means sample becoming redder while b* decrease means sample becoming yellower. a* value was affected by β-carotene content and browning degree. The a* and b* values of DPCD treated samples remained almost unchanged during storage. In comparison with DPCD treatment, a* value increased while b* values decreased progressively in control and thermal treated samples, indicating DPCD treatment maintained the visual color of pickled carrots (Table 3).

In comparison with the control group, DPCD treated samples had no significant (p > 0.05) difference in hardness, while thermal-treated samples exhibited a decline of the hardness (Table 3). The hardness of pickled carrot of DPCD treatment maintained the best texture which was in the range of 8.0–6.3 N during 48 days storage. The hardness of the control and thermal treated group were 5.3 N and 3.7 N after storage for 24 days, respectively, which were significantly (p ≤ 0.05) lower than the DPCD treatment group. After storage for 32 days, the control and thermal treated samples were soft, and their hardness values were undetectable when samples were stored for a longer time. DPCD treatment was one of the non-thermal sterilization technologies that could keep the texture of the sample (cocoyam, Peruvian carrot and sweet potatoes cylinders, etc.) as high-pressure technique (Oliveira, Tribst, Júnior, Oliveira, & Cristianini, 2015).

β-carotene is an important nutrient and color substance by a chromophore in carrots (Sarunggallo, Hariyadi, Andarwulan, Purnomo, & Wada, 2015). The content of β-carotene in pickled carrot was 8.7 mg/100g, which was increased to 9.1 after treatment by DPCD sterilization (Table 3), that is because high pressure and CO₂ medium could promote not only the extraction of β-carotene but also the transformation of some substances into β-carotene. The content of β-carotene decreased as the storage time prolonged because of degradation. The value of the sample treated by DPCD technology was in the range of 7.0–9.1 mg/100 g during storage which was significantly higher than other two groups, while the sample of thermal treatment exhibited the lowest of β-carotene. The possible reason for the lower degradation of β-carotene during storage was that DPCD technology inactivated the activities of β-carotene degradation-related enzymes, such as lipoxigenase, phenylalanine ammonia lyase, polyphenol oxidase and peroxidase (Balaban, 2012). Therefore, utilizing the combination of high pressure and CO₂, DPCD achieved not only a reduction in bacteria but also reduced the damage to sample cells and slowed down the loss of nutrients in pickled carrot.

### 3.3. Influence of DPCD treatment on pectin characteristics of pickled carrot

Texture is an important quality of vegetable products. The softening of texture and the decrease of hardness are mainly caused by changes in the structure and composition of cell wall, especially pectin polysaccharides during vegetable processing (Silva, Doungla, Smout, Ann, & Marc, 2006; Zheng et al., 2013). The pectin content of the pickled carrot is shown in Figure 3(a). The untreated group contained the highest (44.9 g/kg) pectin content and which decreased to 30.2 g/kg after DPCD treatment, while thermal treatment resulted in the lowest amount of pectin (22.5 g/kg).

According to the solubility of pectin, the pectin can be classified into WSP, CSP and NSP, which may be transformed into each other under certain conditions (Tangwongchail, Ledward, & Ames, 2000). Low methoxyl pectin transformation is formed from the catalytic conversion of pectin under the action of pectin methylesterase. The production of

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**Table 3.** Effect of different sterilized treatments on the physicochemical properties of pickled carrot during storage.

| Treatments          | Detection index | Storage time (d) |
|---------------------|-----------------|------------------|
|                     |                 | 1    | 8    | 16   | 24   | 32   | 40   | 48   |
| Untreated           |                 | L*   | 54.1 | 2.4  | 52.3 | 2.9  | 49   | 3.0  | 47.5 | 3.1  | 43.2 | 2.9  | 39.8 | 3.2  | 35.8 | 2.1  |
|                     |                 | a*   | 42.5 | 1.2  | 44.6 | 1.4  | 45.4 | 2.1  | 48.4 | 2.6  | 46.6 | 2.1  | 49.2 | 1.2  | 54.5 | 4.5  |
|                     |                 | b*   | 40.9 | 2.5  | 43.7 | 2.9  | 40.5 | 1.9  | 38.4 | 1.7  | 36.4 | 1.2  | 33.8 | 2.6  | 30.7 | 2.8  |
| Hardness            |                 | 7.9  | 0.9  | 7.0  | 0.6  | 6.0  | 0.9  | 5.1  | 0.3  | -     | -     | -     | -     | -     | -     |
| β-carotene          |                 | 5.7  | 0.2  | 5.2  | 0.3  | 4.6  | 0.2  | 4.4  | 0.3  | 4.1  | 0.2  | 3.7  | 0.3  | 3.3  | 0.3  |
|                     |                 | L*   | 52.1 | 2.8  | 50.4 | 2.9  | 49.6 | 2.4  | 47.8 | 2.1  | 47.1 | 1.7  | 46.2 | 2.9  | 44.5 | 1.2  |
|                     |                 | a*   | 36.9 | 1.2  | 39.3 | 2.1  | 37.2 | 3.1  | 34.2 | 2.4  | 36.9 | 2.9  | 37.2 | 1.7  | 35.5 | 2.8  |
|                     |                 | b*   | 38.9 | 3.0  | 38.3 | 1.9  | 40.8 | 1.4  | 39.4 | 2.6  | 37.5 | 4.2  | 36.4 | 2.6  | 35.2 | 3.0  |
| Hardness            |                 | 8.1  | 0.7  | 7.9  | 0.8  | 7.2  | 0.5  | 7.0  | 0.5  | 6.7  | 0.4  | 6.3  | 0.7  | 5.3  | 0.8  |
| β-carotene          |                 | 9.1  | 0.6  | 8.9  | 0.5  | 8.7  | 0.3  | 8.6  | 0.2  | 8.1  | 0.3  | 7.4  | 0.2  | 7.0  | 0.4  |
|                     |                 | L*   | 51.1 | 2.6  | 50.3 | 2.7  | 45.5 | 3.5  | 41.3 | 3.1  | 36.9 | 2.9  | 34.4 | 2.1  | 29.3 | 2.4  |
|                     |                 | a*   | 44.6 | 1.7  | 48.4 | 2.4  | 50.4 | 2.9  | 54.2 | 2.5  | 59.8 | 3.5  | 58.7 | 3.2  | 55.3 | 4.2  |
|                     |                 | b*   | 43.2 | 2.9  | 42.6 | 2.6  | 39.4 | 3.2  | 42.5 | 3.0  | 43.6 | 2.9  | 41.7 | 3.0  | 39.6 | 1.9  |
| Hardness            |                 | 6.7  | 0.6  | 6.0  | 0.9  | 4.9  | 0.2  | 3.5  | 0.4  | -     | -     | -     | -     | -     | -     |
| β-carotene          |                 | 3.3  | 0.3  | 2.6  | 0.3  | 2.0  | 0.2  | 1.5  | 0.2  | -     | -     | -     | -     | -     | -     |

Note: DPCD treatment was at 20 Mpa for 30 min. The unit of Hardness is N. The unit of β-carotene is mg/100g. Data are expressed as mean ± standard deviation of triplicate samples. Values in the same row sharing different letters expressed as significantly different (p < 0.05). – means no value detected.

Nota: El tratamiento con DPCD fue a 20 Mpa durante 30 min. La unidad de dureza es N. La unidad de β-caroteno es mg/100 g. Los datos se expresan como media ± desviación estándar de muestras en triplicado. Los valores en la misma fila que comparten letras distintas son significativamente diferentes (p < 0.05). – significa que no se detectó ningún valor.
Galacturonic acid is catalyzed by polygalacturonase and synthesized from low methoxyl pectin (Duvetter et al., 2009), which causes the samples to soften. The low methoxyl pectin can be cross-linked by calcium to form chelating pectin indicating the presence of calcium ions that inhibit pectin degradation to galacturonic acid (Buggenhout, Sila, Duvetter, Loey, & Hendrickx, 2009). The more water-soluble pectin content, the more serious the softening, while the more chelated pectin (CSP), the easier for treated samples to maintain their texture. Diverse types of pectin content are displayed in Figure 3. The content of WSP in thermal treated samples was 17.6 g/kg, which was significantly (p ≤ 0.05) higher than other treatments. Compared with the WSP and NSP content, CSP content was low, so pectin has been degraded and dissolved after thermal sterilization. The content of CSP increased, the content of WSP decreased and NSP content did not change significantly after treatment with DPCD, so the quality of pectin was remained (Yen & Lin, 1999), which was consistent with the results of hardness.

Infrared spectroscopy is widely used to study the molecular structure and chemical composition of substances. It has been used to measure the presence of a functional group of cellulose or pectin in plant cell walls (Chen, Wilson, & McCann, 1997; McCann, Wells, & Roberts, 1992). Infrared spectrum of pectin crude extract in pickled carrot is shown in Figure 3(e). All samples exhibited absorption peaks in the range 3500–3000 cm\(^{-1}\) which represented stretching vibration of O-H and C-H bonds; absorption peaks in 3000–2800 cm\(^{-1}\) represented stretching vibration of C-H in methoxyl group; absorption peaks in 1750 and 1650 cm\(^{-1}\) represented stretching vibration of C = O bonds.

Figure 3. Effect of different sterilization treatments on pectin characteristics of pickled carrot after treatment. (A) Pectin content; (B) Water-soluble pectin content; (C) Chelated pectin content; (D) Alkali soluble pectin content; (E) Effect of different sterilization treatments in IR spectrum of pectin.

Figure 3. Efecto de diferentes tratamientos de esterilización en las características de pectina de la zanahoria en escabeche después del tratamiento. (A) Contenido de pectina; (B) Contenido de pectina soluble en agua; (C) Contenido de pectina quelada; (D) Contenido de pectina soluble en álcali; (E) Efecto de diferentes tratamientos de esterilización en el espectro IR de la pectina.
in pectin ester group; absorption peaks in 1200–900 cm⁻¹ represented stretching vibration of C-C, C-O and C-O-H bonds in pectin polysaccharides group. There was no significant difference of pectin crude extract spectrum in absorption peak shape after different treatments. Although there were differences in the content of different kinds of pectin, pectin crude extract contained cellulose, hemicellulose, lignin and so on, the comprehensive results of changes in various bands showed that no significant (p < 0.05) difference among the treatments. The group composition of cell-wall material did not change during DPCD and thermal treatment.

4. Conclusions

In the present study, the results showed that DPCD technology exhibited a remarkable effect in sterilization and improvement of the quality of pickled carrot than thermal treatment. The optimum treating condition of DPCD technology was 20 Mpa for 30 min. The reason of high hardness values was shown to be caused by the fact that the total pectin and CSP content of DPCD treatment was higher and the WSP content was lower than thermal treatment. The results obtained in this study could potentially be useful in the application of DPCD technology in the processing of pickled vegetables.

Disclosure statement

No potential conflict of interest was reported by the author.

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