Effects of high pressure CO₂ treatments on microflora, enzymes and some quality attributes of apple juice

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A B S T R A C T

The inactivation of microorganisms and enzymes of cloudy apple juice, and some physico-chemical properties of the juice were investigated after continuous high pressure carbon dioxide (HPCD) at 25 MPa and 43 °C, for 2 min and at 22 MPa and 60 °C for 3, 5 and 10 min, respectively. The coliform bacteria were completely inactivated in all the cases. Total aerobic bacteria was reduced by 3.72 log cycles, pectin methylesterase was reduced by 54.3% and polyphenol oxidase was completely inactivated after 10 min treatment at 22 MPa and 60 °C. The yeasts and molds were completely inactivated and the turbidity increased at 22 MPa and 60 °C regardless of time, while the L and a value reduced, but browning degree did not change. The particle size distributions of the juice changed after HPCD but were regained as treatment time was prolonged. The pH was reduced at 22 MPa and 60 °C for 3 or 5 min.

1. Introduction

The cloudy apple juice has a growing share in the current market due to its sensory and nutritional qualities, which meets the consumers’ demand for fresh-like products with little or no degradation of nutritional and organoleptic properties (Yıldızgan and Bayndırli, 2004; Özğülü and Bayndırli, 2002). The main problem with cloudy apple juice during production and storage is the colour and cloud stability. The discoloration of cloudy apple juice is a result of enzymatic browning, which involves the action of polyphenol oxidase (PPO, EC 1.14.18.1) catalyzing oxidation of phenolic compounds (Joslyn and Ponting, 1951). The cloud stability of cloudy juices is closely related to the pectin methylesterase (PME, EC 3.1.1.11) activity. Undesired clarification is strongly influenced by demethylation of pectin by PME yielding acidic pectin with a lower degree of esterification (Assis et al., 2001; Cameron et al., 1994). Traditionally, thermal processes are applied in fruit juice processing to ensure its safety and to inactivate indigenous enzymes that can cause undesirable quality change. However, heat-sensitive nutrients and volatile compounds may be negatively affected by thermal processing.

High pressure carbon dioxide (HPCD) processing has been rapidly developing over the past decades as a novel, non-thermal pasteurization method for liquid food pasteurization. It has the ability to inactivate different microorganisms without exposing foods to adverse effects of heat and can retain their fresh-like physical, nutritional, and sensory qualities (Damar and Balaban, 2006). Its efficacy on at least 12 gram-positive bacteria, 10 gram-negative bacteria, 8 bacterial spores, and 8 fungi as filament or spores, has been studied over the last few years (Zhang et al., 2006). HPCD has also been proven effective to inactivate many indigenous fruit enzymes, including PPO (Pozo-Insfran et al., 2007; Gui et al., 2007; Chen et al., 1992; Liu et al., 2008), Peroxidase (Liu et al., 2008), Lipoxygenase (Liao et al., 2009) and PME (Arreola et al., 1991; Park et al., 2002; Kincal et al., 2006; Zhi et al., 2008; Zhou et al., 2009a).

However, there is only a limited number of published studies on the effects of HPCD on the quality of foods (Damar and Balaban, 2006), or the change of particle size distribution (PSD) of cloudy juice. Zhou et al. (2009b) investigated the effect of HPCD (batch system) on the PSD of carrot juice, concluding that its change depends on the treatment time. It was reported that the particle size was affected by the soluble solids concentration, which was attributed to conformational changes in juice particles aggregates, simultaneously with a reduction in particle solvation (Benitez et al., 2007). According to Genovese and Lozano (2000), cloudy juices are closely related to the pectin methylesterase (PME, EC 3.1.1.11) activity. Undesired clarification is strongly influenced by demethylation of pectin by PME yielding acidic pectin with a lower degree of esterification (Assis et al., 2001; Cameron et al., 1994). Traditionally, thermal processes are applied in fruit juice processing to ensure its safety and to inactivate indigenous enzymes that can cause undesirable quality change. However, heat-sensitive nutrients and volatile compounds may be negatively affected by thermal processing.

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apple juice is a colloidal dispersion where the dispersed matter is mainly formed by cellular tissue comminuted during fruit processing.

Most studies dealing with HPCD have applied the technology batch-wise. There are only a few studies that used continuous HPCD. Since a continuous system allows the flow of both CO₂ and liquid food to go through the system, it was effective in killing pathogens and spoilage bacteria in a short time (Damar and Balaban, 2006; Kincal et al., 2005; Lecky and Balaban, 2005; Yagiz et al., 2005). Parton et al. (2007) proved continuous HPCD as an effective way for the pasteurization of natural grape must and tomato paste. Kincal et al. (2005) showed that the natural microflora in orange juice significantly decreased from 10^{2} to 10^{3} CFU/mL to <10 CFU/mL following continuous HPCD at room temperature. Studies also showed that the quality of fruit juice was well maintained after continuous HPCD treatment (Kincal et al., 2006; Lecky and Balaban, 2005; Yagiz et al., 2005). Yagiz et al. (2005) found that continuous HPCD enhanced cloudiness of mandarin juice up to 38.4%, increased its L and b value, and decreased its a value without changing its pH value and the content of total soluble solids (TSS).

The purpose of this work was to investigate the effectiveness of continuous HPCD processing of cloudy apple juice on its natural microorganisms flora, as well as PPO and PME activity. At the same time the change of colour, particle size distribution, turbidity, viscosity, and other physico-chemical properties of the juice due to continuous HPCD was studied.

2. Materials and methods

2.1. Preparation of cloudy apple juice

Apples (Fuji, Yantai, Shandong, China) were purchased from the local market in Beijing (China) and stored in a cold warehouse at an average temperature of 6 °C. The apples were washed, diced and squeezed with a screw juice extractor (T6G7, Zhejiang Light Industries Machinery Plant, Zhejiang, China) and were then filtered with 4 layers of cheesecloth. To prevent the browning of the juice, 0.7‰ L-ascorbic acid (Aoboxing Biotechnical Co., Beijing, China) was added to the freshly squeezed juice. Because the valves and pipelines in the HPCD system might clog if the juice was very rich in pulp, all the juice was continuously centrifuged in 9720 rpm (Westfalia centrifuge, Germany) after being filtered and was then mixed to ensure its uniformity. The juice was later evenly divided into different sterilized containers, each with volume of 10 L, and stored at the cold warehouse until the experiment started.

2.2. Continuous HPCD system

A pilot-scale continuous HPCD system constructed by China Agriculture University is presented in Fig. 1. Firstly, the CO₂ (purity 99.5%, Beijing Jing Cheng Co.) is cooled below 4 °C. Then liquid CO₂ and juice sample are simultaneously pumped to the preheating vessels and then to the reactor by plunger-type variable speed pumps (Pumps 1 and 2, Fig. 1) in different flow rates. Blended in the entrance of the reactor, the CO₂/juice mixture is to flow through the holding tube (i.d. 9 mm × 10 m) for HPCD treatment. Installed within the holding tube are 2 thermocouples that measure the temperature of the mixture at the middle and end of the holding tube. The reactor is maintained at constant temperature and pressure which is manually controlled by the back pressure regulator. The final section of the system consists of 2 separators for depressurization and separation of carbon dioxide and juice. After processing, the treated juice is collected with aseptic manipulation in a clean bench. The separated CO₂ passes through a tube and is either vented to the air or recycled to the chiller for cycling treatment. The frequency of the pumps, temperatures of the reactor and two preheating vessels can also be set up and recorded by the computer system. Since there is no monitor in this system for recording the holding time in the reactor, the holding time is recorded manually as below:

\[ \text{Holding time} = T_0 - T_t \]

where \( T_0 \) means the time from the fluids being pumped to the fluids flowing out of the separator 1. \( T_t \) means the time from the fluids being pumped to the CO₂/juice mixture reaching the entrance of the reactor. The frequencies of the two pumps are adjusted, respectively, to reach the desirable holding time. The frequencies of Pumps 1 and 2 are 9 Hz and 15 Hz for 2 min (at 5 MPa, 43 °C), respectively, and are both 14 Hz, 8 Hz and 6 Hz for 3, 5 and 10 min (at 22 MPa, 60 °C), respectively.

2.3. HPCD processing

2.3.1. Untreated juice as a control

Freshly squeezed juice which was pumped through the HPCD system without CO₂ or heating under atmospheric pressure was used as the control to test its natural microorganisms, enzymes and other quality parameters.

2.3.2. Processing of cloudy apple juice using HPCD

Effects of HPCD on natural microorganisms, PPO, PME and related qualities of cloudy apple juice were discussed firstly at 43 °C for 2 min, under 10 MPa and 25 MPa, respectively. Then, effects of HPCD on apple juice at 60 °C and 22 MPa for longer holding time from 3 min to 10 min were studied. Finally, the treated juices were stored at 6 °C or frozen at −80 °C for further analysis. The microbial inactivation and quality changes of apple juice were studied immediately after HPCD. The PPO and PME activity was measured after being thawed from the frozen sample in the following days after treatment.

Before and after each experiment, the HPCD system was rinsed by hot water and pasteurized with 75% ethanol in continuous flow for more than 10 min. All the experiments were repeated in triplicate.

2.4. Microbiological enumeration

To detect the viable cells of total aerobic bacteria (TAB) and yeasts and molds (Y&M) in apple juice, the pour-plating method was used. Treated and untreated samples were serially diluted with sterile 0.85% NaCl solution and 1.0 mL of diluted (or non-diluted) samples was plated into duplicated plates of appropriate agar. The Nutrient Agar (NA, Beijing Aoboxing Biological Technology Co. Ltd., Beijing, China) was used for detecting the viable cells of the TAB and the plates were incubated at 37 °C for 48 ± 2 h. The Rose Bengal Agar (RBA, Beijing Aoboxing Biological Technology Co. Ltd., Beijing, China) was used for detecting the viable cells of the Y&M, the plates were incubated at 28 °C for 3–5 days. After incubation, plates of 30–300 CFU for TAB, 10–150 CFU for Y&M were chosen for enumeration. Log \( N \) was calculated to determine the inactivation effect, where \( N \) represented the number of microorganisms colonies of the untreated or treated samples.

Most probable number (MPN) method was used to detect the coliforms in apple juice (Suwansonthichai and Rengpipat, 2003). In the pre-fermentation test, samples were diluted into three appropriate series, and 1.0 mL of each diluted (or non-dilute) samples was inoculated in triplicate tubes of 10 mL lauryl sulfate tryptone (LST) broth (Beijing Luqiao Biological Technology Co. Ltd., Beijing, China). Inoculated tubes were incubated at 37 °C for 48 ± 2 h. In the later re-fermentation test, loopfuls of samples from inoculated LST broth, which were positive for turbidity and gas for-
mation, were inoculated in tubes of 10 mL brilliant green lactose bile (BGLB) broth (Beijing Luqiao Biological Technology Co. Ltd., Beijing, China). After incubated at 37 °C for 48 ± 2 h, the positive tubes with colour and turbidity changes and gas formation were counted. Results of the test were reported as the MPN per 100 mL of sample.

2.5. Enzyme activity determination

2.5.1. PPO assay

The activity of PPO was assayed by a spectrophotometric method (Weemaes et al., 1997) with some modifications. Catechol was chosen as the substrate, and 0.05 M catechol substrate solution was prepared with 0.05 M phosphate buffer (pH 6.0). The juice was centrifuged in 9000 g for 15 min (GL-166-A, Shanghai Anting Scientific Equipment Factory, Shanghai, China) and the supernatant was taken as tested sample. All samples were analyzed by adding 500 L centrifuged juice into 2.5 mL substrate solution. The increase in absorbance at 420 nm was monitored at intervals of 0.1 s immediately after incubation with a Cary 50 spectrophotometer (Varian Co. Ltd., California, USA), which was equipped with a peltier thermo-statted cell holder, a water pump (Varian Co. Ltd., California, USA) to keep temperature at 30 ± 0.1 °C and an in-built electromagnetic stirring to mix up the substrate and juice. Prior to measurement, a pre-equilibrium of the substrate solution and the juice at 30 °C by the peltier thermo-statted cell holder was obtained. The slope of the very first linear part of the reaction curve was taken as the PPO specific activity (Abs/min). The RA of PPO was estimated with the following equation:

\[ \text{Residual activity} = \frac{\text{PPO specific activity after HPCD treatment}}{\text{PPO specific activity in the control}} \times 100\% \]  

(2)

2.5.2. PME assay

The activity of PME was measured at pH 7.5 and 30 °C according to the method proposed by Kimball (1991), which was based on carboxyl group titration. 5 mL cloudy apple juice was mixed with 20 mL of 1% apple pectin (DE 70–75%, Andre Co., Shandong, China) containing 0.1 M NaCl at 30 °C and was incubated at 30 °C. The mixture solution was adjusted to pH 7.0 with 2.0 N NaOH, and then the pH of the solution was readjusted to pH 7.5 with 0.05 N NaOH. After the pH reached 7.5, 0.05 mL of 0.05 N NaOH was added. The time was measured for the solution's pH to return to 7.5. PME activity (A) expressed in pectin methylesterase units (PMEU) was calculated by the following:

\[ A = \frac{[\text{NaOH}]V_{\text{NaOH}}}{V_{\text{sample}}t_0} \]

where [NaOH] was NaOH concentration (0.05 N), V_{NaOH} was the volume of NaOH used (0.05 mL), V_{sample} was the volume of sample used (=5 mL cloudy apple juice), and t' was the time (in minutes) needed for pH to return to 7.5 after the addition of NaOH

\[ \text{Residual activity (RA)} = \frac{A}{A_0} \times 100\% \]

where A_0 represented the mean activity of PME in the control, A represented the mean activity of PME after HPCD treatment.

2.6. Determination of colour changes

2.6.1. Colour assessment

Colour assessment was conducted at room temperature with a colour difference meter (SC-80, Kangguang Co., Beijing, China) in the reflectance mode. The L, a, and b values of cloudy apple juice were measured. \( \Delta E \) was calculated as below:

\[ \Delta E = [(L_0 - L_t)^2 + (a_0 - a_t)^2 + (b_0 - b_t)^2]^{1/2} \]

where the L_0, a_0 and b_0 meant the L, a and b value of cloudy apple juice after HPCD treatment, respectively. And the L_t, a_t and b_t meant the L, a and b value of the control, respectively.

2.6.2. Determination of browning degree (BD)

The BD of cloudy apple juice was analyzed with a spectrophotometric method described by Roig et al. (1999). Cloudy apple juice
was centrifuged with a refrigerated Centrifuge (GL-166-A, Shanghai Anting Scientific Equipment Factory, Shanghai, China) at 9000g at 4°C for 30 min, and then passed through a 0.45 μm cellulose nitrate membrane (Beijing Bomex Co., Beijing, China). The BD was determined by measuring the A (absorbance at 420 nm) value by a UV−762 spectrophotometer (T6, PG General, Beijing, China) with a cell of 1 cm path length at ambient temperature (20 ± 1°C).

2.7. Turbidity determination

The turbidity of cloudy apple juice was measured at room temperature with the method proposed by Reiter et al. (2003). A digital photoelectrical turbidimeter (WGZ-200, Shanke Instrument Factory, Shanghai, China) with 5 mm cuvette at colour correction mode was applied. Cloudy apple juice was diluted in 1/20 with distilled water. Turbidity was expressed in nephelometric turbidity units (NTU).

2.8. Determination of PSD

PSD of juice was determined by a LS 230 particle size analyzer (Beckman Coulter, Inc., Florida, USA). A laser light with a wavelength of 750 nm was applied in the system, to measure particles from 0.4 to 2000 μm by light diffraction. Fourier optics collected the diffracted light and the PSD was calculated by Fraunhofer model.

Firstly, distilled water from a tank was pumped into a sample cell at the speed of approximately 8 L/min until the cell was full. The juice was added into the cell using a pipette and mixed with distilled water. Particles in juice were dispersed and suspended in distilled water. The measurement started when the obscuration percentage increased from 0 to 10%. Data obtained were analyzed by software LS v3.29. Volume mean diameter D[4,3] and surface mean diameter D[3,2] were determined for all samples.

2.9. Some physico-chemical properties

2.9.1. Viscosity determination

The viscosity of cloudy apple juice was determined by a rotational viscometer (Visco Basic Puls L, Fungilab S.A., Barcelona, Spain) in LCP mode at 100 rpm. The temperature was kept at 30°C by constant-temperature water bath circulation device (HX-1050, Beijing De-Tianyou Technology Development Co., Ltd, Beijing, China).

2.9.2. Determination of total soluble solids (TSS)

The TSS of cloudy apple juice was determined as °Brix at ambient temperature (20 ± 1°C) by WAY-25 digital Abbe Refractometer (Shanghai Precision and Scientific Instrument Co., Shanghai, China).

2.9.3. pH Determination

The pH was measured at 25°C using digital Thermo Orion 555A pH meter (Thermo Fisher Scientific Inc., MA, USA). 10-ml cloudy apple juice was inserted with a pH electrode (Thermo Orion Ross 9103BN, MA, USA) and pH was recorded after stabilization.

3. Results and discussions

3.1. Inactivation of natural microorganisms

High temperatures stimulate the diffusivity of CO2, and increase the fluidity of the bacterial cell membrane, which enhances penetration easier (Lin et al., 1993; Hong et al., 1997, 1999). Several studies have proved that CO2 at supercritical state (>31°C and >7.34 MPa) attained higher inactivation efficacy (Lin et al., 1992; Shimoda et al., 1998; Iensenwid et al., 1995; Ishikawa et al., 1997). With low viscosity and better diffusivity and dissolving power, it is more effective for supercritical CO2 to penetrate into cells, extract essential substances from cells or membranes, and to disorder the conformation of cytoplasmic membranes and organelles, resulting in the disruption of biological system in the cell (Shimoda et al., 1998; Tomasula et al., 2003). Therefore, HPCD experiments were carried out at temperatures higher than ambient temperature in this study.

The inactivation of the TAB, Y&M and coliforms in cloudy apple juice by HPCD is illustrated in Table 1. The coliform bacteria in cloudy apple juice were very sensitive to HPCD and were completely inactivated in all the cases. 1.30 and 2.32 log cycles reduction of the TAB and Y&M was attained at 10 MPa and 43°C for 2 min, whereas it was about 3.03 and 2.61 log cycles reduction at 25 MPa and 43°C for 2 min, indicating the important role that the pressure plays for inactivation of the TAB and Y&M. Similar results were reported in previous studies (Hong et al., 1999; Ballestra et al., 1996; Dillow et al., 1999; Erkmen and Karaman, 2001; Lin et al., 1992; Spiilimbergo et al., 2003; Liao et al., 2007). Higher pressure enhances CO2 solubility to facilitate its contact with and penetration into cells (Hong et al., 1997). The inactivation of the TAB and Y&M induced by HPCD at 22 MPa and 60°C was, therefore, studied as a function of time from 3 min to 10 min in this study. The TAB significantly decreased with the increasing of the treatment time. Chen et al. (2010) reported the effect of time on microorganisms under HPCD treatment at 35 MPa and 55°C, concluding that long treatment times resulted in significant TAB reduction. The Y&M were completely inactivated by HPCD at 22 MPa and 60°C, regardless of treatment time, in this study, suggesting that HPCD brought about better inactivation on Y&M than on the TAB at 60°C. This observation was in accordance with findings from some previous studies. Pozo-Insfran et al. (2006) showed that compared with TAB, Y&M were inactivated at a significantly higher rate under identical processing conditions of HPCD. Liao et al. (2010) also observed that the Y&M in cloudy apple juice were more susceptible to a batch HPCD treatment than TAB. Microbial inactivation was highly dependent on the type of microorganisms presented in the food matrix due to distinct microbial cell microstructure. The inactivation of microorganisms in cloudy apple juice without CO2 at 22 MPa and 60°C for 10 min was investigated as the heat control (data not shown in the Table 1). 3.07 and 4.3 log cycles reduction were attained for TAB and Y&M, respectively, which were less than the HPCD group. Liao et al. (2010) study showed HPCD increased the susceptibility of natural microorganisms to temperature and enhanced their microbial inactivation.

3.2. Inactivation of PPO and PME in cloudy apple juice

The residual activity (RA) of PPO and PME in cloudy apple juice after HPCD treatment is shown in Fig. 2. The RA of PPO in cloudy apple juice was 47.29% when the juice was subjected to HPCD at 25 MPa and 43°C for 2 min, which was very similar to the case at 22 MPa and 60°C for 3 min. Moreover, the activity of PPO in cloudy apple juice reduced significantly with an increasing treatment time at 22 MPa and 60°C. Complete inactivation of the enzyme was
found when the treatment time was 10 min. The effects of HPCD treatment temperature and time on PPO activity in juice or matrices have been previously studied. For example, Gui et al. (2007) showed that higher temperature and longer treatment time resulted in higher inactivation of PPO. The highest enzyme reduction in cloudy apple juice was greater than 60% when applying batch HPCD at 30 MPa and 55 °C for 60 min. Liu et al. (2008) reported that the activity of PPO in red beet extract also decreased as HPCD temperature and time increased. For example, the PPO original activity was reduced by 95% with a batch HPCD at 22.5 MPa and 55 °C for 60 min. Chen et al. (1992) reported in their study, that purified Florida lobster, brown shrimp and potato PPOs activities declined as treatment time proceeded with a batch HPCD at 5.8 MPa and 43 °C. Park et al. (2002) found that a batch HPCD treatment at 4.9 MPa and 5 °C for 10 min was responsible for 61% reduction of PPO activity in carrot juice. HPCD treatment at 30 °C and 27.6 MPa for 6.25 min resulted in a 75% decrease in PPO activity in muscadine juice with a batch system (Pozo-Insfran et al., 2007). None of these studies reported complete inactivation of PPO by HPCD. Different inactivation levels of PPO could be attributed to different HPCD systems, treatment conditions, PPO sources and cultivars. For example, PPO from the potato was more resistant to HPCD than that from the spiny lobster and shrimp (Chen et al., 1992). In this study, PPO was completely inactivated by continuous HPCD at 22 MPa and 60 °C for 10 min, whereas in Gui’s study (Gui et al., 2007), the maximal reduction of PPO from the same apple cultivar was more than 60% with a batch HPCD at 30 MPa and 55 °C for 60 min. Although the HPCD conditions in this study were less intense, the difference in inactivating PPO was possibly attributed to the higher efficiency of continuous HPCD system than that of a batch HPCD system.

As shown in Fig. 2, PME activity was increased after HPCD treatment at 25 MPa and 43 °C for 2 min. To our best knowledge, no study has reported on HPCD-induced activation of PME in cloudy apple juice. However, the activation of PME in cloudy apple juice by thermal processing (70 °C, 50–100 s) has been previously reported (Krapfenbauer et al., 2006; Denes et al., 2000). The assumed reason lies in the fragmentation of cell wall, which might possibly occur during HPCD processing. The fragmentation could favor enzyme accessibility and thus increase the apparent PME activity since PME is ionically-bounded to the cell wall. Thus, more studies are needed to investigate the effect of mild HPCD treatment on PME. When the temperature was increased to 60 °C, PME activity was slightly reduced. After 5 min HPCD treatment, the PME activity in cloudy apple juice was reduced significantly, and after 10 min it was reduced by 58.3%. Similar to PPO inactivation, the results also showed that temperature and time played an important role in HPCD inactivation of PME. Zhi et al. (2008) reported that better inactivation of apple PME with an increase of temperature from 35 to 55 °C at 30 MPa and the maximal reduction of apple PME activity (in Tris-chloride buffer, pH 7.5) was 94.57% at 30 MPa and 55 °C for 60 min. Zhou et al. (2009a) also showed that the activity of carrot and peach PME was reduced as the treatment time extended. Several other studies also studied the inactivation of PME in juices by HPCD treatment. Kincal et al. (2006) showed that the highest inactivation (46.3%) of PME in orange juice was achieved when the pressure was 107 MPa and no heat was applied with a continuous HPCD. Park et al. (2002) reported that the RA of PME in carrot juice was about 40% after a batch HPCD treatment at 4.90 MPa and 5 °C for 10 min. Arreola et al. (1991) observed that a batch HPCD at 29 MPa and 50 °C for 4 h resulted in complete inactivation of PME in orange juice.

A complete inactivation of PPO in this study was found while the maximum reduction of PME was only 58.30%, indicating that PPO is more susceptible to HPCD than PME. This might be attributed to their structural difference. PPO is assumed to have 3 or 4 subunits (Van Gelder et al., 1997; Heidmal et al., 1994) with a molecular weight of 46 kDa (Janovitz-Klapp et al., 1989) whereas PME has one subunit (D’Avino et al., 2003) with a molecular weight of 36 kDa (Macdonald and Evans, 1996). In general, the larger an enzyme and the more complex its structure is, the more susceptible it is to high temperature (Yang et al., 2004). Similarly, it could be reasoned that the greater sensitivity of PPO to HPCD treatment than PME is caused by the difference in its structure. Furthermore, the inactivation of PPO and PME induced by HPCD could also result from the alterations in secondary and tertiary structures (Park et al., 2002; Zhou et al., 2009a).

3.3. Change of colour in cloudy apple juice

Table 2 shows the colour change of cloudy apple juice after continuous HPCD treatment. The L value of cloudy apple juice exhibited no alteration after 2 min HPCD at 25 MPa and 43 °C, but it was significantly reduced after HPCD treatment at 60 °C regardless of the treatment time. The a value of cloudy apple juice significantly decreased after all treatments, indicating that the apple

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**Table 1**

<table>
<thead>
<tr>
<th>Treatment conditions</th>
<th>Aerobic bacteria (Log N)</th>
<th>Yeasts and molds (Log N)</th>
<th>Coliforms (MPN/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>HPCD</td>
<td>Control</td>
</tr>
<tr>
<td>10 MPa, 43 °C, 2 min</td>
<td>4.78 ± 0.10</td>
<td>3.48 ± 0.08</td>
<td>4.24 ± 0.01</td>
</tr>
<tr>
<td>25 MPa, 43 °C, 2 min</td>
<td>4.45 ± 0.01</td>
<td>1.42 ± 0.03</td>
<td>4.39 ± 0.16</td>
</tr>
<tr>
<td>22 MPa, 60 °C, 3 min</td>
<td>5.56 ± 0.04</td>
<td>2.32 ± 0.09</td>
<td>4.17 ± 0.08</td>
</tr>
<tr>
<td>22 MPa, 60 °C, 5 min</td>
<td>5.20 ± 0.01</td>
<td>1.77 ± 0.13</td>
<td>4.19 ± 0.03</td>
</tr>
<tr>
<td>22 MPa, 60 °C, 10 min</td>
<td>5.07 ± 0.01</td>
<td>1.35 ± 0.01</td>
<td>4.77 ± 0.04</td>
</tr>
</tbody>
</table>

All data were the means ± SD, n = 3. ND = not detectable.

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Fig. 2. Residual activity of PPO and PME in cloudy apple juice by HPCD (A: 25 MPa, 43 °C, 2 min; B: 22 MPa, 60 °C, 3 min; C: 22 MPa, 60 °C, 5 min; D: 22 MPa, 60 °C, 10 min). All data were the means ± SD, n = 3.
juice lost some redness after HPCD. The $b$ value did not change except at 25 MPa and 43 °C for 2 min. Although the colour difference $\Delta E$ of cloudy apple juice increased when the treatment temperature slightly increased from 43 to 60 °C, it showed no significant change for different treatment times at 22 MPa and 60 °C ($\Delta E < 2$). BD of cloudy apple juice increased significantly with HPCD at 25 MPa and 43 °C for 2 min, and changed insignificantly after HPCD at 22 MPa and 60 °C, which was in accordance with the inactivation of PPO.

Other studies measured colour change of fruit juice after HPCD treatment as well. Gui et al. (2006) found that the $L$, $a$ and $b$ value of cloudy apple juice remained the same after HPCD treatments (55 °C, 8–30 MPa, 60 min). However, in Kincal et al. (2006) study, the $L$ value of orange juice significantly increased, the $a$ value significantly decreased, and the $b$ value exhibited no change after continuous HPCD treatment (room temperature, 38, 72, and 107 MPa for 10 min). Arreola et al. (1991) also found that the HPCD-treated orange juice was brighter, less red and more yellow than the untreated sample. Zhou et al. (2009b) found that the $L$ value of HPCD (25 °C, 10–30 MPa, 15–60 min) treated carrot juices increased significantly, the $a$ value increased with the increasing of the treatment time, while the $b$ value of HPCD-treated juice did not change. The difference on the change of $L$, $a$ and $b$ value between these previous studies and ours was probably attributed to the different investigated subjects and/or treatment conditions (such as temperature).

3.4. Change of PSD of cloudy apple juice

Fig. 3 illustrates the PSDs of cloudy apple juice as a function of the treatment time with HPCD at 22 MPa and 60 °C. The PSD of HPCD-treated juice at 25 MPa and 43 °C for 2 min was not discussed due to the difficult comparison upon the trilaminated effects of time, temperature and pressure. The PSDs of untreated cloudy apple juice showed two peaks, which were characterized by a large peak and small peak at approximately 1 and 7 µm, respectively. After HPCD treatment, the PSDs changed as the volume number of the larger peak decreased and that of the small peak increased. However, the PSDs were gradually regained as the treatment time was prolonged. In Table 3, the change of $D_4(3)$ (volume mean diameter) and $D_2(3,2)$ (surface mean diameter) of cloudy apple juice after HPCD is demonstrated. It indicated an increase after 3 min and 5 min and then a decrease after 10 min.

This observation was in agreement with Zhou et al. (2009b) study. Precipitation caused by pH shift is characteristic of proteins (Roig et al., 1999). As shown in Table 4, the pH of HPCD-treated juices declined after HPCD treatment at 22 MPa and 60 °C due to the dissociation of carbonic acid, which is formed by CO2 dissolution into the juices. The HPCD-induced increase of the average particle size of the juice was possibly a result of the acid-induced protein coagulation, while the decrease in particle size of HPCD-treated juices after longer treatment time was probably attributed to the HPCD-induced homogenization effect (Zhou et al., 2009b). Longer treatment resulted in a stronger homogenization effect of the HPCD treatment. Therefore, it was assumed that the alteration of the PSD for HPCD-treated juices was an interaction between the acid-induced protein coagulation and the HPCD-induced homogenization, which was affected by the pH change of the juice. After a short HPCD treatment (3 and 5 min), the pH was reduced and the acid-induced protein coagulation seemed to dominate, while the HPCD-induced homogenization effect alternated for a longer treatment (10 min) as the pH remained unchanged. On the other hand, the increase of the average particle size of HPCD-treated cloudy apple juice at 25 MPa, 43 °C, and 2 min (shown in Table 3) was higher than that treated at 22 MPa, 60 °C, and 3 min, although the treatment time of the former was shorter and pH stayed unchanged. This might be attributed to the finding that a higher temperature (60 °C compared to 43 °C) helped to accelerate the HPCD homogenization effect. In addition, the different ratio of CO2 to the juice, caused by the different the flows/frequencies of the pumps, was probably another influential factor which needed further investigation.

![Fig. 3. Volume change of PSDs in cloudy apple juice as a function of treatment time induced by HPCD (A: 22 MPa, 60 °C, 3 min; B: 22 MPa, 60 °C, 5 min; C: 22 MPa, 60 °C, 10 min). The PSD of HPCD-treated cloudy apple juice at 25 MPa, 43 °C, and 2 min was not included. All data were the means, n = 3.](image)
3.5. Change of turbidity, viscosity, TSS and pH of cloudy apple juice

The change of turbidity of cloudy apple juice after HPCD treatment is shown in Table 4. The turbidity of cloudy apple juice after HPCD treatment at 25 MPa and 43 °C for 2 min did not change, whereas it increased with HPCD at 22 MPa and 60 °C regardless of treatment time. However, the particle size increased under these conditions, apart from the one unchanged at 22 MPa and 60 °C for 10 min. It seems that the turbidity is not closely related to the particle size. Mc Clements (1999) found that the turbidity of dispersions depended on the concentration, size, and relative refractive index of their particles. It is therefore reasoned that the treatment conditions in this study probably favors the effect of any of these factors on the turbidity. Kincal et al. (2006) suggested that the increase of turbidity could be attributed to the decrease of particles of the juice colloid due to homogenization, which thereby increased cloud stability to PME attack. However, the PSD of HPCD-treated juice was not reported in this study.

It was found that the cloud destabilization process and the cloud loss were caused by PME (Castaldo et al., 1997). However, active PME in cloudy apple juice after HPCD treatment was also observed in this study (Fig. 2) and the RA of PME in cloudy juice was at least 41.70% after HPCD. Kincal et al. (2006) also found that the cloudiness of orange juice was increased by up to 846% of its original value with continuous HPCD at 38 MPa, despite active PME still present. Therefore, the turbidity of cloudy apple juice is not necessarily associated with the RA of PME.

Table 4 also shows the change of some physico-chemical properties of cloudy apple juice induced by continuous HPCD. No significant differences in viscosity and TSS were observed. This was in accordance with previous studies (Arreola et al., 1991; Kincal et al., 2006; Yagiz et al., 2005). However, Zhou et al. (2009b) showed that the viscosity of juices increased significantly. In this study, the pH of cloudy apple juice was reduced significantly after HPCD treatment at 22 MPa and 60 °C for both 3 and 5 min. The decline of pH in HPCD-treated juices is caused by the dissociation of carbonic acid formed by the CO2 dissolution into the juices. A higher temperature and/or higher pressure may accelerate this process by enhancing the diffusivity of CO2 into juice (Lin et al., 1993; Hong et al., 1997), but a longer treatment time possibly retards this process. As discussed in the PSD section above, this observation was in accordance with what was found for the PSD change. However, most studies indicated that HPCD treatment did not cause a detectable pH change in orange juices (Arreola et al., 1991; Kincal et al., 2006), except for Park et al. (2002) and Zhou et al. (2009b), which reported a final pH drop in the carrot juice.

4. Conclusions

Microorganisms and native enzymes in cloudy apple juice showed enhanced inactivation with increasing treatment temperature, pressure and time when subjected to continuous HPCD processing. The extend of colour (such as BD) was related to the level of PPO inactivation. However, no correlation of the juice’s turbidity was found with the change of RA of PME, or particle size distribution of the juice. The pH of the juice was reduced significantly after HPCD treatments for 3 and 5 min at 60 °C and 22 MPa, which seemed to influence the PSD of cloudy apple juice. This study also showed that the decrease of the juice’s pH is higher at elevated temperature (e.g. 60 °C), which possibly led to higher protein coagulation, while a longer treatment time may retard these effects by enhancing pressure-induced homogenization. The quality characteristic of continuous HPCD-treated cloudy apple juice was good but further studies are needed on storage stability and sensory attributes are needed to prove its acceptance by consumers.

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References


