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Influence of Thermal and Dense-Phase Carbon Dioxide Pasteurization on Physicochemical Properties and Flavor Compounds in Hami Melon Juice

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The influence of thermal and dense-phase carbon dioxide (DP-CO_2) pasteurization on physicochemical properties and flavor compounds in Hami melon juice was investigated. Melon juice was pasteurized using DP-CO_2 treatment and compared to a conventional high-temperature−short-time (HTST) method. The DP-CO_2 treatment was carried out using a DP-CO_2 unit (55 °C, 60 min, and 35 MPa). The thermal pasteurization was performed at 90 °C for 60 s with an adapted laboratory setup. Effects of variations to both treatments on pH and concentrations of microbes, β-carotene, ascorbic acid, sugars, organic acids, and volatile compounds were investigated. The changes of pH and organic acid and sugar concentrations were not significant. There were significant differences between treatments in microbial count, vitamin C, β-carotene, and volatile compound concentrations. In general, DP-CO_2 treatment had less of an effect on the measured variables than the thermal treatment.

KEYWORDS: Thermal and dense-phase carbon dioxide; pasteurization; physicochemical properties; flavor compounds; Hami melon juice

INTRODUCTION

Thermal pasteurization is efficient in preventing microbial spoilage of fruit juice, but the applied heat may also cause undesirable changes to biochemical and nutritional properties that may affect the overall quality of the final product. In contrast, dense-phase carbon dioxide (DP-CO_2) treatment is a promising nonthermal processing method that may radically change liquid food preservation technology. In recent years, many studies have reported innovations in the use of DP-CO_2 to preserve liquid food. Several of these papers focused on microbiology, enzyme inactivation, and effects on food quality (*1*). These confirmed the feasibility and effectiveness of the technique (*2*). Fraser (*3*) proposed a technique for collecting *Escherichia coli* content by bursting cells in liquid culture with a sudden release of pressurized Ar, N_2, N_2O, or CO_2 and suggested that pressurized CO_2 could be used for the inactivation of *E. coli*. Spilimbergo et al. (*4*) reported that microbial inactivation was a crucial parameter in product safety and shelf life. Several papers report on the effects of DP-CO_2 treatment on the food system. The majority of testing has been on orange juice (*5*−*8*). Other juices that have been studied include carrot (*9*), grape (*1*), and apple products (*10*). Physical and chemical properties, e.g., pH, Brix value, and titratable acidity, of orange juice do not appear to be influenced by CO_2 treatment. Yellowness and lightness seem to decrease (*5*, *7*, *9*).

Flavor components in fruit juice are numerous, and flavor identification is considered complex because of the aromatic nature of fruits. Hami melon or cantaloupe (*Cucumis melo* var. *reticulatus*) is a classical fruit produced in the Xinjiang Uigur Autonomous Region, People's Republic of China, which is highly appreciated for its nutritional quality and special flavor. The flavor quality is of utmost importance because it is the key factor for consumer acceptance. The sterilization of Hami melon juice is difficult because it is heat-sensitive (*11*, *12*). It is therefore important to select a suitable method for melon juice pasteurization. Ma, Wang, and others (*11*, *12*) reported changes in the aroma components of melon juice after freezing and irradiation.

Thus far, there is little information available regarding the effects of DP-CO_2 on the physicochemical properties and composition of volatile chemical compounds responsible for the aroma and flavor of melon juice. There are few direct comparisons of DP-CO_2 and high-temperature−short-time (HTST) treatments, in terms of microbial inactivation and general quality attributes.

This paper compares DP-CO_2 and HTST pasteurization of melon juice, focusing on their effects on microbial inactivation, physicochemical properties, and retention of flavor components.

MATERIALS AND METHODS

Chemicals. All chemicals used in the experiment were analytical- or high-performance liquid chromatography (HPLC)-grade, purchased from

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Sigma. They were fructose, sucrose, glucose, malic acid, citric acid, and oxalic acid. The authentic standards of volatiles [ethyl acetate, ethyl propanoate, ethyl butyrate, butyl acetate, ethyl-2-methyl butyrate, hexanal, (*Z*)-6-nonenal, nonanal, (*Z*)-nonel-3-ol, (*E*,*Z*)-3,6-nonadien, 2-nonenal, (*Z*)-nonel-6-ol, nonanol, dimethyl disulfide, dimethyltrisulfide, 2-methylpiperazine, and *N*-ethyl-methylthiamine] were used as authentic compounds to identify the compounds.

Materials. The melon samples studied were Xiangfei Mi cantaloupe (*Cucumis melo* var. *reticulatus*, Hami melon), a variety planted in the Xinjiang Uigur Autonomous Region, People's Republic of China. All melon samples were fully ripe without any quality deterioration or decay.

Preparation of Freshly Squeezed Melon 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, 10 melons were cut into pieces and squeezed into juice. The melon juice was filtered through a 4-layer cheese cloth and stored at −20 °C in darkness until use. The initial pH of the cloudy cantaloupe juice was 6.36. Samples were thawed at ambient temperature before use.

DP-CO_2 Pasteurization and HTST Treatment. All experimental runs were carried out at a constant temperature and pressure (55 °C and 35 MPa). Previous studies (*10*) demonstrated that these conditions were optimal for increasing the efficiency of the process while minimizing effects on the product quality. Each time, melon juice (50 mL) was introduced to vessels (V_{max} = 500 mL) and exposed to supercritical gas (35 MPa, 55 °C) for 60 min. A total of 10 consecutive experimental runs were performed on the condition above to produce the total volume of 500 mL needed for aroma and chemical analyses.

HTST treatment: Melon juice (50 mL) was introduced to sanitary containers, which were held at 90 °C for 1 min and then cooled to room temperature with cold water. A total of 10 consecutive experimental runs were performed to produce the total volume of 500 mL needed for aroma and chemical analyses.

Chemical and Physical Analyses. Total bacterial count: The dominant micro-organisms in the melon juice were isolated and cultivated on media (10 g of glucose, 3 g of beef extract, 5 g of sodium chloride, 5 g of peptone, 15 g of agar, and 1000 mL of water), adjusted to pH 7.2, and sterilized (121.8 °C for 30 min) according to standard methods (*13*). The plate count method stipulated by GB4789.2-94 was used to calculate the amount of bacteria. This plate count method is standard in microbiological analysis (*14*).

The pH was measured with a digital pH meter (Thermo Orion 555A, Waltham, MA) equipped with a microelectrode (Thermo Orion ROSS 9103BN, Waltham, MA) at 25 °C.

Ascorbic acid content was analyzed using a titrimetric method based on 2,6-dichloroindophenol (*15*).

Sugars and organic acids were determined by HPLC (*16*).

β-Carotene content was analyzed according to the method of Speek et al. (*18*).

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 of NaCl, to minimize the loss of volatile components and to avoid browning. Volatiles were sampled by manual headspace SPME at 40 °C while stirring. The fiber [100 mL of polydimethylsiloxane (PDMS), Supelco] was inserted into the injection port of gas chromatography/mass spectrometry (GC/MS) after 30 min of sampling and then desorbed at 250 °C for 10 min. Each analytical sample was measured in triplicate.

GC/MS Setup. 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 inner diameter × 30 m × 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 performed in triplicate. Identification of compounds was based on a comparison of their spectrum and relative abundance with NIST 98/Wiley Registry of Mass Spectral Data (Hewlett-Packard Co., Palo Alto, CA).

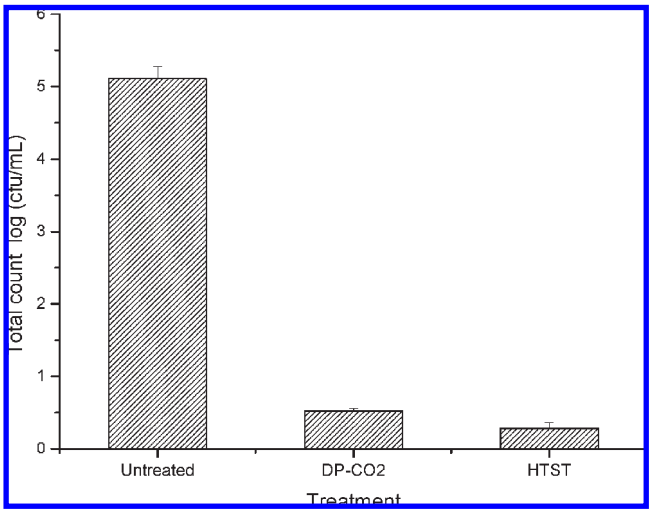

Figure 1. Effect of DP-CO_2 and HTST treatment on bacterial survival in Hami melon juice. All data are the means ± standard deviation (SD) (n = 3).

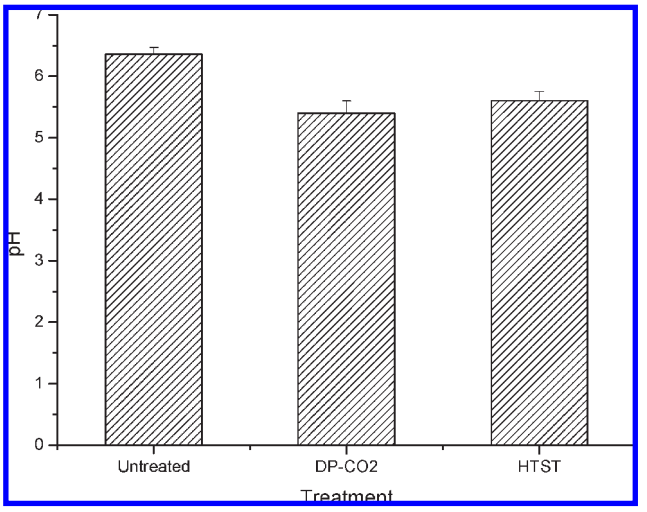

Figure 2. Effect of DP-CO_2 and HTST treatment on the pH of Hami melon juice. All data are the means ± SD (n = 3).

For identification of the ethyl acetate, ethyl propanoate, ethyl butyrate, butyl acetate, ethyl-2-methyl butyrate, hexanal, (*Z*)-6-nonenal, nonanal, (*Z*)-nonel-3-ol, (*E*,*Z*)-3,6-nonadien, 2-nonenal, (*Z*)-nonel-6-ol, nonanol, dimethyl disulfide, dimethyltrisulfide, 2-methylpiperazine, and *N*-ethyl-methylthiamine, the GC/MS identification were confirmed by comparing GC retention times with authentic compounds.

Statistical Analysis. The results were statistically evaluated by one-way analysis of variance (AVOVA) using the software Microcal Origin 7.5 (Microcal Software, Inc., Northampton, MA). Statistical differences with p values under 0.05 were considered significant. All of the physical and chemical indicators in the experiments were replicated 3 times.

RESULTS AND DISCUSSION

The composition of microorganisms in the melon juice was relatively complex, and different species of microorganisms had different levels of resistance. Pasteurization significantly reduced the microbial counts in melon juice. The total microbial count in the untreated melon juice was 5.11-log cycle (**Figure 1**). After DP-CO_2 and HTST pasteurization, the total microbial count was 0.5- and 0.28-log cycle, respectively. However, effects on other physicochemical quality indices must be considered when determining the optimal treatment.

There was no significant difference in the pH value between the untreated melon juice and either DP-CO_2-treated or HTST-pasteurized samples (**Figure 2**). There was a slight reduction in the pH of DP-CO_2-treated samples. It has been reported for several other products including orange, carrot, apple juices, and red beet extract (*6*, *18*, *9*, *19*) and was most likely due to the CO_2 dissolved in the product under high pressures. The pH of HTST-treated samples declined to 5.6 (**Figure 2**).

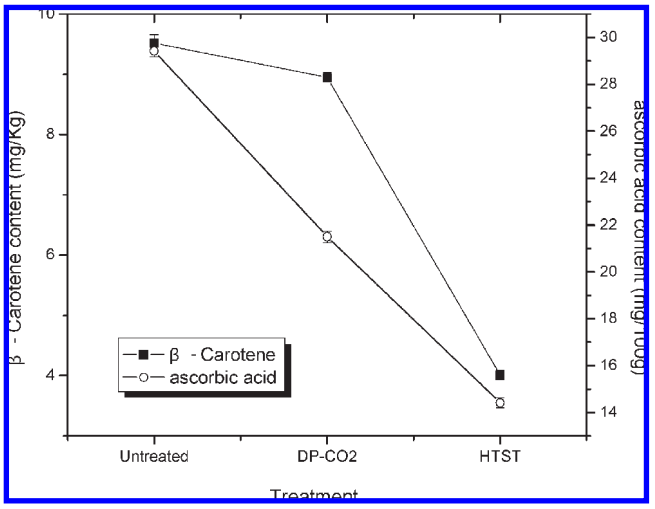

Figure 3. Effect of DP-CO_2 and HTST treatment on β-carotene content in Hami melon juice. All data are the means ± SD (n = 3).

Table 1. Changes of the Main Sugar and Organic Acid in Melon Juice after Treatment[a]

composition	untreated sample (mg/mL)	DP-CO_2 (mg/mL)	HTST (mg/mL)
sucrose	122.25 ± 3.25	120.42 ± 3.07	118.03 ± 3.01
fructose	75.00 ± 2.43	75.28 ± 2.51	64.21 ± 2.25
glucose	66.47 ± 2.10	61.58 ± 2.23	56.45 ± 2.19
malic acid	3.47 ± 0.27	4.41 ± 0.29	4.62 ± 0.32
citric acid	2.42 ± 0.16	2.79 ± 0.22	3.01 ± 0.25
oxalic acid	12.7 ± 1.01	15.51 ± 1.34	16.96 ± 1.37

[a] All data are the means ± SD (n = 3).

The β-carotene content of different foods is of nutritional importance and additional interest because β-carotene is believed to have a protective role against cancer (*20*). The main carotenoid compound found in fresh melon juice was β-carotene (9.52 mg/kg). Its content varied in the different processing samples with different treatment. **Figure 3** shows a total retention of β-carotene in the DP-CO_2-treated melon juice (8.95 mg/kg), and there was no significant loss of β-carotene compared to the initial fresh melon juice. There was a reduction of 57.87% in β-carotene with HTST treatment (4.01 mg/kg).

Ascorbic acid content following the two pasteurization methods varied. The HTST treatment caused a considerable loss of ascorbic acid (51%) compared to the DP-CO_2 treatment, which only caused a 13.3% reduction (**Figure 3**). CO_2 can lower pH when dissolved in the aqueous part of food. Ascorbic acid has higher stability at low pH and oxidizes easily when oxygen is present in the environment.

Sugars and organic acids are important to melon juice flavor. In the melon juice samples, glucose, fructose, and sucrose were main sugars. The levels were not remarkable (**Table 1**). A total of 10 organic acids were measured in the melon juice samples. Oxalic, malic, and citric acids were the dominant organic acids. Concentrations of these three organic acids increased in HTST-treated samples but were in fresh melon juice and DP-CO_2-treated melon juice (**Figure 2**).

The volatile components of muskmelon have been analyzed by a number of authors (*21*), 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

Table 2. Changes of Volatile Compounds in Melon Juice Treated by Different Methods[a]

retention time (min)	compounds	untreated sample (%)	DP-CO_2 (%)	HTST (%)
		Esters		
2.26	ethyl acetate	10.72 ± 0.32	9.47 ± 0.27	2.48 ± 0.25
3.38	ethyl propanoate	2.11 ± 0.20	3.05 ± 0.05	1.8 ± 0.05
5.32	ethyl butyrate	2.77 ± 0.20	2.78 ± 0.21	3.09 ± 0.23
5.69	butyl acetate	1.31 ± 0.11	1.33 ± 0.12	2.86 ± 0.21
6.74	ethyl-2-methyl butyrate	1.02 ± 0.05	1.05 ± 0.05	2.32 ± 0.07
		Aldehydes		
6.93	hexanal	6.96 ± 0.25	5.93 ± 0.27	2.07 ± 0.25
17.27	(*Z*)-6-nonenal	2.79 ± 0.06	2.71 ± 0.06	0.27 ± 0.05
17.38	nonanal	1.83 ± 0.02	1.51 ± 0.02	0.91 ± 0.01
17.27	(*Z*)-6-nonenal	2.79 ± 0.06	2.71 ± 0.06	0.27 ± 0.05
17.38	nonanal	1.83 ± 0.02	1.51 ± 0.02	0.91 ± 0.01
19.18	2-nonenal	1.77 ± 0.05	1.16 ± 0.05	0.87 ± 0.03
		Alcohols		
18.98	(*Z*)-nonel-3-ol	27.51 ± 3.31	24.6 ± 3.25	7.44 ± 0.51
19.46	(*Z*)-nonel-6-ol	0.81 ± 0.01	0.73 ± 0.01	2.06 ± 0.12
19.50	nonanol	5.56 ± 0.35	4.54 ± 0.30	3.13 ± 0.25
		Others		
19.04	(*E*,*Z*)-3,6-nonadien	19.24 ± 2.25	12.07 ± 2.23	5.76 ± 0.47
3.06	dimethyl disulfide	nd[b]	nd	1.18 ± 0.23
6.83	dimethyltrisulfide	nd	nd	1.10 ± 0.19
8.73	2-methylpiperazine	nd	nd	1.06 ± 0.12
9.83	*N*-ethyl-methylthiamine	nd	nd	0.32 ± 0.01

[a] All data are the means ± SD (n = 3). [b] nd = not detected.

aldehydes and alcohols (*22*). Esters, alcohols, and aldehydes containing a nine-carbon straight chain have been shown to be important in muskmelon aroma (*23*−*27*). There are few reports in the literature regarding the effects of DP-CO_2 treatment on Hami melon aroma.

A total of 45 volatile compounds were analyzed by SPME/GC/MS (**Table 2**). There was no change in ester composition (ethyl acetate, ethyl propanoate, ethyl butyrate, butyl acetate, and ethyl-2-methyl butyrate) after DP-CO_2 treatment. There were slight changes in alcohols and aldehydes, such as (*Z*)-nonel-3-ol, (*E*,*Z*)-2,6-nonadien, (*Z*)-nonel-6-ol, nonanol, hexanal, (*Z*)-6-nonenal, nonanal, and 2-nonenal. The flavor of DP-CO_2-treated melon juice was similar to fresh melon juice. After HTST treatment, the ethyl acetate and ethyl propanoate contents decreased, but the ethyl butyrate, butyl acetate, and ethyl-2-methyl butyrate contents increased. The six- and nine-carbon alcohol and aldehyde contents decreased significantly. The flavor of HTST-treated melon juice had a cooked-off odor, and no green flavor from the sensory. New aroma compounds, such as dimethyl disulfide, dimethyltrisulfide, 2-methylpiperazine, and *N*-ethyl-methylthiamine, were produced. These compounds were not found in fresh or DP-CO_2-pasteurized melon juices. These compounds may be responsible for the cook-off odor.

In conclusion, DP-CO_2, a nonthermal preservation technique to pasteurize melon juice, proved to efficiently induce microbial inactivation as well as preserved some quality attributes, including most of the aroma compounds in the melon juice. Although conventional HTST pasteurization inactivated microorganisms in the melon juice, it caused significant losses in ascorbic acid, β-carotene, six- and nine-carbon alcohol, and aldehyde contents. Some new compounds were produced in HTST-pasteurized melon juice, which were associated with a cooked-off odor. DP-CO_2-treated melon juice retained more volatile compounds, ascorbic acid, and β-carotene. DP-CO_2 can be considered as a promising alternative to thermal pasteurization of melon juice. Further studies are needed to optimize the process, study the chemistry of melon juice flavor compounds, reduce the loss of volatile compounds, and enhance process stability.

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