Journal of Bioscience and Bioengineering
VOL. 111 No. 6, 675–681, 2011

www.elsevier.com/locate/jbiosc

Effects of aqueous chlorine dioxide treatment on nutritional components and shelf-life of mulberry fruit (*Morus alba* L.)

Zhao Chen,[1,†] Chuanhe Zhu,[2,*,†] and Ziqiang Han[3]

College of Life Sciences, Shandong Agricultural University, Daizong Street 61, Taian, Shandong 271018, PR China,[1] College of Food Science and Engineering, Shandong Agricultural University, Daizong Street 61, Taian, Shandong 271018, PR China,[2] and Pingyin Vocational School of Jinan, Yinzhuang, Jinan, Shandong 250400, PR China[3]

Received 4 November 2010; accepted 12 January 2011
Available online 8 February 2011

Effects of aqueous chlorine dioxide (ClO_2) treatment on nutritional components and shelf-life of mulberry fruit (*Morus alba* L.) were investigated. Mulberry fruit were immersed into 20, 60, and 80 mg/l ClO_2 solutions for 5, 10, and 15 min, respectively. Mulberries were then rinsed with potable tap water for 1 min and stored at −1°C for 14 d. ClO_2 treatment was effective in retention of flavonoid, ascorbic acid, reducing sugar, and titratable acid. ClO_2 concentration and treatment time were significant factors affecting ClO_2 treatment. The shelf-life of the samples treated by 60 mg/l ClO_2 for 15 min was extended to 14 d compared to 8 d for the control. No ClO_2, ClO_2^-, or ClO_3^- residues were detected in samples treated by 60 mg/l ClO_2 for 15 min. These results indicated that ClO_2 treatment was a promising approach to preserve mulberry fruit with no significant risks of chemical residues.

[**Key words:** Chlorine dioxide; Mulberry; *Morus alba* L.; Nutritional components; Shelf-life; Chemical residue; Chlorite; Chlorate; Fruit; Preservation]

Mulberry fruit are a traditional Chinese medicine, which can be used for the treatment of sore throat, fever, hypertension, and anemia (1). They must be harvested at full maturity to achieve the maximum quality according to flavor, texture, and nutritional value. Mulberry fruit are perishable after harvest due to the high water content of more than 70% (2). And because of their short harvesting season and sensitivity to storage, mulberry fruit are mainly processed and consumed locally, during which a large part is damaged, causing a great economic loss. Conventional thermal processing, which is an effective method for preserving fruit and vegetables, is not suitable for thermo-sensitive products such as mulberries (3). In addition to the desirable effect of microbial and enzyme inactivation, it results in the loss of organoleptic and nutrient level of the produce. An alternative technology for the preservation of mulberry fruit is sun drying, which can reduce the moisture content to inhibit the growth of microbes and hinder quality losses (4). However, the sensory quality of mulberries can be seriously damaged during exposure to sunlight. Therefore, application of new preservation methods to improve storage quality and shelf-life of mulberry fruit is necessary for enhancing levels of commercial sales.

Chlorine dioxide (ClO_2) is a powerful sanitizing agent that has broad and high germicidal activity. It is more stable and has a higher oxidizing capability than chlorine. Moreover, unlike chlorine, ClO_2 does not react with nitrogen-containing compounds or ammonia that results in potentially carcinogenic byproducts and it remains largely constant in a wide range of pH (5). ClO_2 is legally permitted in PR China and USA for sanitizing fruit and vegetables (6, 7), though there are no regulations of the European Union regarding the use of chlorine dioxide for fresh produce washing (8). There are numerous reports in the literature describing the beneficial effects of ClO_2 treatment on fruit and vegetables, such as apricot, pepper, lettuce, alfalfa, clover sprouts, carrot, strawberry, cantaloupe, potato, and fig (9-22). And ClO_2 treatment has been proven to be a novel and effective non-thermal technology for prolonging the shelf-life of fresh and fresh-cut produce. Mahmoud and Linton (15) extended the shelf-life of strawberries to 16 d compared to 8 d for the untreated control by ClO_2 treatment when stored at 4°C. And the shelf-life prolongation of cantaloupe from 3 to 9 d was achieved during storage at 22°C (17). Gómez-López et al. (21) reported that treatment with ClO_2 on minimally processed carrots resulted in one additional day of shelf-life at 7°C. Our pervious study showed that a shelf-life extension of 10 d at 4°C was obtained in fresh-cut asparagus lettuce treated by ClO_2 (9).

To our knowledge, there have been no studies on the effects of ClO_2 treatment on nutritional components and shelf-life of mulberry fruit. The objective of this work was to evaluate the effects of aqueous ClO_2 treatment on flavonoid, ascorbic acid, reducing sugar, titratable acid, and shelf-life of mulberry fruit. And chemical residues of ClO_2, ClO_2^-, and ClO_3^- were analyzed after treatment. This study would provide valuable information for preserving mulberry fruit.

* Corresponding author. Tel.: +86 538 8249157; fax: +86 538 8242850.
E-mail address: chhzhu@sdau.edu.cn (C. Zhu).
† Equal contributors.

1389-1723/$ - see front matter © 2011, The Society for Biotechnology, Japan. All rights reserved.
doi:10.1016/j.jbiosc.2011.01.010

MATERIALS AND METHODS

Fruit material and preparation Mulberry fruit (*Morus alba* L.) were harvested at full maturity at a local orchard (Taian, PR China). The berries were selected for uniformity of weight (approx. 3 g) and color (purple red), and fruit with apparent injuries were removed. Fruit were then randomly divided into twelve groups, each group containing 3 kg of fresh fruit.

ClO_2 preparation A commercially available brand of ClO_2 powder (Charmstar, Tianjin Charmstar Technology Development Co., Ltd., Tianjin, PR China) was dissolved in deionized water to prepare a stock solution (approx. 200 mg/l) according to manufacturer's procedures. The concentration of ClO_2 was measured by a standard method using iodimetry immediately before use (23). The ClO_2 stock solution was then diluted with deionized water to desired concentrations (20, 60, and 80 mg/l).

ClO_2 treatment on mulberry fruit Mulberries were immersed into ClO_2 solutions at different concentrations (20, 60, and 80 mg/l) for different time (5, 10, and 15 min) with a ratio of 1 kg:5 l (Mulberry:ClO_2 solution) at 22 ± 2°C. Samples washed with potable tap water were used as the control. Following treatments, berries were rinsed in tap water for 1 min, solution residues on the fruit surface drained off. Each group was packaged into an aseptic polyethylene bag (350 mm × 250 mm) and stored at −1°C for 14 d for subsequent assay. The storage temperature was chosen according to Li and Ma, who indicated that −1°C was the optimal condition (24). The bag was 0.02 mm thick with properties specified by the manufacturer (Zhongda, Nanjing Zhongda Package Material Co., Nanjing, PR China) as follows: O_2 permeability of 210–1615 ml m^{-2} h^{-1} MPa^{-1} (23°C, 0% RH) and CO_2 permeability of 1060–8075 ml m^{-2} h^{-1} MPa^{-1} (23°C, 0% RH).

Determination of flavonoid, ascorbic acid, reducing sugar, and titratable acid contents The flavonoid content of mulberry fruit was determined using the aluminum chloride colorimetric method described by Ercisli and Orhan (2). Briefly, 0.1 g of fruit samples were dissolved into 1 ml of deionized water. Then, 0.5 ml of this solution was mixed with 1.5 ml of 95% alcohol, 0.1 ml of 10% aluminum chloride hexahydrate, 0.1 ml of 1 mol/l potassium acetate, and 2.8 ml of deionized water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415 nm with deionized water as the blank sample. Quercetin was chosen as a standard. Flavonoid content in mulberry fruit was determined using a seven point standard curve (0–50 mg/l). The results were expressed as mg of quercetin equivalents per 100 g of mulberry fruit.

The ascorbic acid, reducing sugar, and titratable acid contents of mulberry fruit were determined according to Li et al. (25). The ascorbic acid was titrated by using 2,6-dichloroindophenol titration method. Briefly, 50 g of fruit samples were homogenized in 50 ml of a 0.02 g/ml oxalic acid solution and centrifuged at 15,000g and 4°C for 15 min. Afterwards, 10 ml of supernatant were titrated to a permanent pink color by 0.1% 2,6-dichlorophenolindophenol titration. Ascorbic acid content was expressed as mg/100 g of mulberry fruit. The content of reducing sugar was determined by the Fehling's method. Fruit samples (50 g) were homogenized for the determination of reducing sugar. Then, 25 g of the homogenates were transferred to a beaker containing 150 ml of distilled water. The mixture was heated in a water bath at 80°C for 30 min. Aliquots of 10 ml were titrated and reducing sugar content was expressed as g of glucose per 100 g of mulberry fruit. The content of titratable acid was obtained by titration with 0.1 mol/l sodium hydroxide to pH 8.2 and expressed as g of citric acid per 100 g of mulberry fruit.

Shelf-life study After the analyses of effects of ClO_2 treatment on flavonoid, ascorbic acid, reducing sugar, and titratable acid contents, the ideal ClO_2 treatment condition for maintaining nutritional components of mulberry fruit was obtained to conduct the shelf-life study. Control and ClO_2 treated samples were packaged and stored at −1°C for 14 d. Mulberries were taken for microbial growth assay and sensory quality evaluation during storage. Samples without washing by tap water or ClO_2 (raw fruit) were used to determine the inherent background microflora. The end of the shelf-life was defined as when the population of a microbial group reached an unacceptable level or the sensory quality evaluation panelists rejected the sample.

To perform microbial enumeration, 25 g of mulberry fruit sample were homogenized for 2 min in 225 ml of sterile buffered peptone water using a Stomacher 400 Circulator (Steward Ltd., London, UK). Ten-fold dilution series were made in peptone saline solution for plating. The following media and conditions were used for microorganism incubation: Plate Count Agar (Hope, Qingdao Hope Bio-Technology Co., Ltd., Qingdao, PR China) for total aerobic mesophilic bacteria incubated at 30°C for 3 d and also for total aerobic psychrotrophic bacteria incubated at 22°C for 5 d; de Man-Rogosa-Sharpe medium containing 0.14% sorbic acid (Qingdao Hope Bio-Technology) for lactic acid bacteria incubated at 30°C for 3 d; Rose Bengal Agar (Qingdao Hope Bio-Technology) for yeasts and moulds incubated at 30°C for 3 d. Colonies were counted and results expressed as log cfu/g. The following microbiological specifications were used to determine the end of the shelf-life (12): 8 log cfu/g for aerobic mesophilic bacteria and aerobic psychrotrophic bacteria, 7 log cfu/g (plus sensory analysis) for lactic acid bacteria, and 5 log cfu/g for yeasts and moulds.

Sensory quality was evaluated by a semi-trained panel of six graduate students. Overall visual quality (OVQ) was scored based on a modification of the 9-point hedonic scale reported by Wright and Kader (26): 9 = excellent, extremely fresh; 7 = very good, marketable; 5 = good, limit of marketability; 3 = fair, limit of usability; 1 = poor, unusable. The following sensory quality attributes were also evaluated according to Gómez-López et al. (12): off-odor (1 = none, 3 = acceptable, 5 = severe); flavor (1 = fresh, 3 = acceptable, 5 = spoiled); texture (1 = fresh, 3 = acceptable, 5 = spoiled); white blushing (1 = none, 3 = acceptable, 5 = severe). Off-odor, flavor, and texture were scored under red light; under white light OVQ and white blushing were evaluated. The end of the shelf-life from the sensory quality point of view was reached when at least one of the mean scores was above the acceptability limit.

Analysis of chlorine dioxide, chlorite, and chlorate residues The most effective ClO_2 treatment condition for extending shelf-life of mulberry fruit was used to determine ClO_2, ClO_2^-, and ClO_3^- residues.

Immediately after ClO_2 treatment and rinse step, fruit sample was homogenized with deionized water 5 times their weight for 30 s. The water extract was filtered through a 0.22 μm filter. The filtrate was collected to detect whether ClO_2, ClO_2^-, and ClO_3^- residues existed. The residual concentration of ClO_2 was determined by iodimetry standard method with the detection limit of 0.1 mg/l of filtrate (0.6 mg/kg of mulberry fruit). The byproducts of ClO_2, including ClO_2^- and ClO_3^-, were analyzed using ion chromatography method (19, 27). The IonPac AS9-SC analytical column (Dionex, Dionex China Ltd., Beijing, PR China) was eluted with a 1.4 mmol/l Na_2CO_3/0.2 mmol/l $NaHCO_3$ mobile phase at a flow rate of 1.5 ml/min. The detection limit was 0.05 mg/l of filtrate (0.30 mg/kg of mulberry fruit). The samples washed with tap water were used as the control. The results were expressed as mg/l of filtrate and then converted to mg/kg of mulberry fruit.

Statistical analysis All assays were performed in triplicate. Differences among samples were determined by least significant differences (LSD) test using SigmaPlot 11.0 (SigmaPlot, Systat Software Inc., San Jose, CA, USA), and were considered to be significant when $P<0.05$.

RESULTS

Flavonoid, ascorbic acid, reducing sugar, and titratable acid contents The effects of ClO_2 treatment on flavonoid content of mulberry fruit are shown in Fig. 1. Flavonoid contents of the control and ClO_2 treated samples decreased over time. In the first 2 d, flavonoid contents of the control were higher than those of the ClO_2 treated samples. The treatment with 20 mg/l ClO_2 for 5 min was similar with the control during storage ($P>0.05$), which was not effective in maintaining flavonoid. After 6 d, the treatments with 60 and 80 mg/l ClO_2 were more effective and significantly different from the control and 20 mg/l ClO_2 treatments ($P<0.05$). The 60 and 80 mg/l ClO_2 treatments were very similar ($P>0.05$). For the 60 and 80 mg/l ClO_2 treatments, contents of the 15 min ClO_2 treated samples were higher than those of 5 and 10 min treated samples ($P<0.05$). ClO_2 concentration and treatment time had significant effects on flavonoid contents for all the samples ($P<0.05$).

In view of mulberry as a source of ascorbic acid, the ascorbic acid content of mulberry fruit during storage was determined. As shown in Fig. 2, a remarkable decrease in ascorbic acid content was detected in all samples. Contents of the ClO_2 treated samples were lower than those of the control in the first 6 d. From day 10, contents of ClO_2 treated samples became higher comparing with the control ($P<0.05$). ClO_2 concentration and treatment time had significant effects on ascorbic acid content ($P<0.05$). The 60 and 80 mg/l ClO_2 treatments were more effective than the 20 mg/l ClO_2 treatments in retaining ascorbic acid ($P<0.05$). For the 60 and 80 mg/l ClO_2 treatments, ascorbic acid contents of the 15 min ClO_2 treated samples were higher than those of the 5 and 10 min ClO_2 treated samples ($P<0.05$). The 60 mg/l ClO_2 treatment was similar with the 80 mg/l ClO_2 treatment ($P>0.05$).

The effects of ClO_2 treatment on reducing sugar content in mulberry fruit are shown in Fig. 3. There were no significant differences in initial reducing sugar contents among all samples ($P>0.05$). Reducing sugar contents of the control and ClO_2 treated samples decreased as storage time was prolonged. Though the changing patterns in all treatments were similar, contents of the ClO_2 treated samples were higher than those of the control ($P<0.05$). The reducing sugar content became higher with increases in ClO_2 concentration and treatment time ($P<0.05$). Compared to the 20 mg/l ClO_2 treatments, reducing sugar contents of the 60 and 80 mg/l ClO_2 treated samples were higher during storage ($P<0.05$). The 60 mg/l ClO_2 treatments were similar with the 80 mg/l ClO_2 treatments