



Decontamination of chokeberries (*Aronia melanocarpa* L.) by cold plasma treatment and its effects on biochemical composition and storage quality of their corresponding juices

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Abstract To improve the microbiological quality of chokeberries, corona discharge plasma jet (CDPJ) was employed as a decontaminating agent. The mean levels of aerobic bacteria and yeast and molds contaminants in chokeberries were 4.78 and 4.87 log CFU/g, respectively; the viable counts were decreased by 2.09 and 1.83 log units following CDPJ treatment for 3 min, respectively. A first-order kinetic model was well-suited for explaining the inactivations. The concentration of total polyphenols of chokeberries was unaffected (up to 3 min treatment), whereas DPPH radical scavenging activity was significantly decreased by CDPJ treatment (2 min and more). CDPJ treatment (all tested times) of chokeberries led to a significant decrease of monomeric anthocyanin content of their corresponding juices, whereas soluble solids content (Brix) and pH were unaffected. After storage at 4 °C for 72 h, juices obtained from CDPJ-treated berries possessed relatively low levels of contaminants with no significant alterations in pH and soluble solids content when compared with controls. In conclusion, CDPJ treatment for 2 min was optimal to improve the microbiological quality of the berries without negatively affecting biochemical qualities of their corresponding juices.

Keywords Chokeberry · Corona discharge plasma · Inactivation · Kinetics · Microbial contaminants · Polyphenols

Introduction

As derivatives of oxygen, reactive oxygen species (ROS) such as hydroxyl radical (HO•), superoxide anion (O₂^{•−}), hydrogen peroxide (H₂O₂) and others are more reactive than molecular oxygen. The ROS can exert oxidative stress when their production levels overwhelm the cellular antioxidant defense system, and this, in turn, may lead to different pathological conditions such as neurodegeneration, carcinogenesis, diabetes, aging and atherosclerosis (Ray et al., 2012).

Detrimental effects of ROS can be prevented by consuming antioxidant-rich foods. One such plant food is chokeberry fruit, and it is known to contain a high content of polyphenols, especially anthocyanins, which act as antioxidants (Denev et al., 2012). Chokeberries belong to the plant family *Rosaceae*, native to North America, including Eastern Canada and Eastern and Central America. They are largely cultivated in Poland, Denmark, and the Netherlands. Chokeberries were introduced and have been cultivated in Korea since 2007 (Shin and Choe, 2015).

Chokeberry fruits contain relatively more antioxidants than other berries due to their richness in anthocyanins. The anthocyanins are composed of cyanidin-3-glucoside, cyanidin-3-galactoside, cyanidin-3-xyloside and cyanidin-3-arabinoside, which are glycosides of cyanidin and have various physiological functions (Lee et al., 2014). These berries have a limited shelf-life and therefore they are generally subjected to freezing after harvesting and distributed in frozen state. However, the freezing of

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chokeberries may result in excessive tissue softening (Delgado and Rubiolo, 2005). In addition, the freezing can induce alterations in biochemical and physical characteristics of the berries. Furthermore, freeze–thaw cycle may adversely affect the microbiological safety (Opoku-Nkoom, 2015). Alternatively, the application of advanced methods such as low-temperature drying (e.g. spray drying, freeze drying and vacuum drying) to increase shelf-life and/or preserve nutritive quality (especially bio-actives) of chokeberries has been recommended (Horszwald et al., 2013).

Non-thermal methods are increasingly used for food preservation, and such techniques could be adopted to sanitize or decontaminate heat-sensitive foods like chokeberries. Low-temperature plasma or cold plasma, which is one of the novel non-thermal decontamination agents, is rich in reactive charged particles, such as radicals and ions, besides other species including ultraviolet (UV) rays. Plasma chemical species (especially oxygen containing radicals) have been shown to inactivate microorganisms possibly through oxidation of DNA, protein, and cytoplasmic membrane (Gallagher et al., 2007). A positive influence of plasma treatment on polyphenols (e.g. anthocyanins) stability in fruit juices has been shown (Bursac Kovacevic et al., 2016).

Among various cold plasmas, corona discharges generated in atmospheric air are well suited for food decontamination purposes. This is particularly due to simplicity in their production. Corona discharge plasma jet (CDPJ) has been applied to the preservation or decontamination of different food products, such as dried laver (Kim et al., 2015), fresh and frozen pork (Choi et al., 2016), semi-dried squid (Choi et al., 2017a), and dried squid shreds (Choi et al., 2017b). However, corona plasmas have rarely been used for fruit decontamination purposes. Therefore, in this study, CDPJ was tested for microbial (natural contaminants) decontamination of chokeberries. Also, the effects of cold plasma treatment on biochemical quality of chokeberries and storage quality of their corresponding juices were investigated.

Materials and methods

Chokeberries

Chokeberry (*Aronia melanocarpa* L.) fruits used for this experimentation were purchased in August 2018 from Danyang Aronia Agricultural Cooperative Federation (Danyang, Korea). They were stored at 4 °C and samples were analyzed within 48 h of purchase.

Determination of microbial contaminants

For chokeberries taken in filter stomacher bags (approx. 5 g each) (3 M, Seoul, Korea), sterile 0.9% NaCl (w/v) solution (45 mL) was added to each bag. Later, the samples were homogenized using a paddle blender (Masticator, IUL Instruments, Barcelona, Spain) for 2 min at 8.0 strokes/s. Thereafter, 1 mL aliquots (each) were aseptically taken from the bag filtrate(s), serially diluted in 0.9% sterile saline, and 1 mL aliquots of each dilution were pipetted into 90 mm diameter Petri plates containing either general purpose or selective agar media. The plates were then incubated at 37 °C for 24–48 h. The pour plate technique was used to determine viable counts. General-purpose as well as selective growth media were used to detect microbial contaminants of chokeberries. All microbial culture media used in this study were procured from Becton Dickinson and Co. (Sparks, MD, USA). The contaminants were quantified according to the standard plate count method (KFDA, 2011). Plate count agar and potato dextrose agar (both are general-purpose media) were used for determining the counts of aerobic bacteria and yeast and molds, respectively. Selective enrichment media used include Baird-Parker agar for *Staphylococcus aureus*, eosin-methylene blue agar for *Escherichia coli*, xylose–lysine–deoxycholate agar for *Salmonella* spp., and mannitol–egg yolk–polymyxin agar for *Bacillus cereus*.

CDPJ generation and decontamination

Schematic diagram of cold plasma system used in this study is shown in Fig. 1. Electrical current applied to the electrodes was fixed at 1.5 A, and the plasma generated between the discharge electrodes was driven downwards by an air-blowing device, thereby the jet of the plasma was formed. Chokeberry samples were treated by fixing the gap between the bottom of the electrode and the sample treatment table at 25 mm. The chokeberries were subjected to

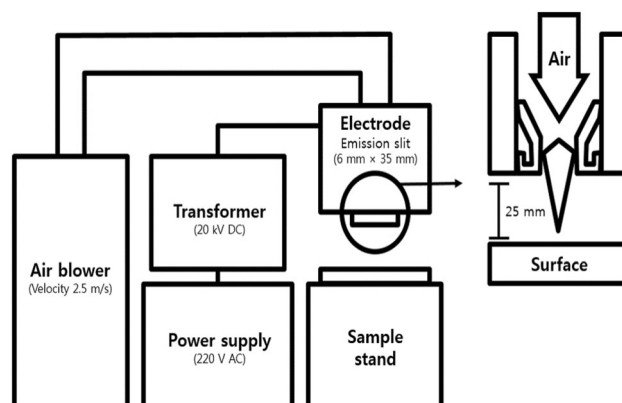


Fig. 1 Schematic diagram of CDPJ treatment system

the CDPJ treatment at different intervals (0, 1, 2, and 3 min). Each treatment was conducted in triplicate.

Modeling inactivation kinetics

First-order inactivation model is generally employed to explain survival curves of microorganisms exposed to lethal agents, assuming a linear logarithmic decrease in survivor count over treatment period, as given in Eq. (1).

$$\log \frac{N_0}{N} = \frac{k}{2.303} \cdot t \quad (1)$$

where,

N_0 is initial microbial counts.

N is microbial counts at time t .

k is inactivation rate constant.

t is exposure time (min)

$$D = \frac{2.303}{k} \quad (2)$$

where,

D is decimal reduction time.

Chokeberry extracts and their biochemical analysis

Chokeberries were ground using a grinder (HR2860, Philips, Seoul, Korea) for 3 min and subsequently freeze-dried using a lyophilizer (FD5505, Ilshin Lab Corporation, Yangju, Korea) for 24 h. The freeze-dried samples were extracted with a mixture of distilled water, methanol, and 5 N HCl (26:50:24) at 30 °C for 2 h. Thereafter, the extracts were centrifuged at $2500 \times g$ at 4 °C for 10 min to remove undissolved material. The extraction was repeated for two more times, and the resulting supernatants were combined and stored at 4 °C until used. The extracts were used for determining total phenolic content and DPPH radical scavenging activity.

Phenolic content

Total phenolic content of chokeberries was measured by using Folin-Ciocalteu reagent, according to the procedure described earlier (Singleton and Rossi, 1965). Briefly, to 0.5 mL of the extract, 2 mL of 7.5% Na_2CO_3 (w/v) and 2.5 mL of 10% Folin-Ciocalteu (v/v) reagent were added, mixed well. Then, the mixture was heated at 45 °C for 15 min in a constant-temperature water bath, cooled to room temperature and finally, using a spectrophotometer, the absorbance of the reaction mixture was recorded at 765 nm. The concentration range of calibration curve was 0–100 $\mu\text{g/mL}$. Total polyphenol content of chokeberry

juice was expressed in terms of gallic acid equivalent (mg GAE/100 g).

DPPH radical scavenging activity

DPPH radical scavenging activity of extracts from chokeberries was determined according to the method described by Blois (1958). Briefly, 0.2 mM DPPH solution was prepared using absolute ethanol. Then, this solution was mixed with chokeberry extract (1:1 ratio) and the resultant mixture was incubated in the dark for 30 min. Thereafter, centrifuged at 4500 rpm for 3 min, and the resultant supernatant was taken for spectrophotometric analysis at 517 nm.

DPPH radical scavenging activity (%)

$$= \frac{(Abs_{blank} - Abs_{sample})}{Abs_{blank}} \times 100 \quad (3)$$

Chokeberry juices and their analysis

Juices were prepared by crushing of untreated and CDPJ-treated (0, 1, 2, and 3 min) chokeberries for 1 min using a blender (HR 2860, Philips, Seoul, Korea). In the process of juicing, following the blending, the resultant mixtures were squeeze-filtered through 4 layers of cheese-cloth to separate the liquid (juice) and fiber fractions. Then the juices were sealed in airtight polypropylene bags (200 mL capacity) before storing at 4 °C. The bags were kept unopened at this temperature until analysis within 24 h after juicing.

Soluble solids content and pH levels

Soluble solids content and pH levels of chokeberry juices were determined using a digital refractometer (SCM-1000, HM Digital, Seoul, Korea) and a pH meter (PHS-3BW, Bante Instruments Limited, Shanghai, China), respectively.

Total monomeric anthocyanin content

Total monomeric anthocyanin concentration in chokeberry juices was determined according to a pH differential method, which has been recommended by Lee, Durst, and Wrolstad (2005). Briefly, two dilutions of chokeberry juice were prepared, one with KCl buffer 0.025 M (pH 1.0) and the other with CH_3COONa buffer 0.4 M (pH 4.5), by diluting the juice in 1:10 (v/v) ratio. The samples were incubated at 4 °C for 24 h. Absorbance was measured simultaneously at 520 and 700 nm by UV–Vis spectrophotometer. The diluted test portions were read versus a

blank cell filled with distilled water. The pigment content was expressed in mg cyanidin-3-glucoside equivalents per liter of juice, and calculated as follows:

$$\text{Monomeric anthocyanin pigment (mg/L)} = \frac{A \times MW \times DF \times 1000}{\epsilon \times l} \quad (4)$$

$A = (A_{520} - A_{700})$ at pH 1.0— $(A_{520} - A_{700})$ at pH 4.5
 $MW = 449.2$ g/mol (molecular weight of cyanidin-3-glucoside).

DF = dilution factor.

1000 = factor for conversion g to mg.

$\epsilon = 26\,900$ molar extinction coefficient, in $L \times \text{mol}^{-1} \times \text{cm}^{-1}$, for cyanidin-3-glucoside.

l = path length of cuvette in cm.

Statistical analysis

All experiments were performed in triplicate. All statistical analyses were conducted using the SAS statistical software package (version 9.3, SAS Institute Inc., Cary, NC, USA). Significant difference ($p < 0.05$) between values obtained was tested using a one-way ANOVA, followed by Duncan's multiple range test.

Results and discussion

Microbial contaminants

Chokeberry fruits were found to be contaminated with bacteria, and yeast and molds; the average levels of these were 4.78 and 4.87 log CFU/g, respectively (Table 1). However, bacterial pathogens including *E. coli*, *B. cereus*, *S. aureus* and *Salmonella* spp. were not detected in the berries.

In a study, it has been shown that chokeberries collected from different locations in Bulgaria were contaminated with molds, predominantly *Penicillium* sp., followed by

Botrytis and *Alternaria* species. Four out of eighteen randomly chosen *Penicillium* isolates have shown the capability to produce mycotoxin, namely ochratoxin A (Genchev et al., 2015). In another study, fruit rot of black chokeberry after harvest by *Fusarium tricinctum* has been shown (Lee et al., 2016). These studies confirmed that microbiological contamination of harvested chokeberry fruit is very common; however, the presence of some toxin-producing strains needs the application of effective decontamination methods.

Inactivation of contaminants and kinetics

Berry samples were treated using the CDPJ at different intervals (0, 30, 60, 90, 120, 150, 180 s). The inactivation efficiency of the plasma was increased with increasing treatment time as shown in Fig. 2. Log inactivation of aerobic bacteria as well as yeast and molds increased linearly with plasma exposure time. As high as > 2 log CFU/g reduction of aerobic bacteria was observed following the plasma treatment for 180 s, whereas yeast and molds were reduced by 1.8 log CFU/g during the same treatment time. Relatively low plasma susceptibility of yeast and molds could be due to their eukaryotic nature, multicellular nature (molds), and variation in cell wall composition compared with bacteria.

In general, the pattern of microbial inactivation by different lethal agents follows first-order kinetics, assuming that a linear logarithmic decrease in survivor count over treatment period. Hence, such an inactivation can be represented by a single rate constant 'k' or its reciprocal, the linear 'D-value', which is regarded as a measure of resistance to an applied inactivating agent. For modeling the inactivation of the aerobic bacteria and yeast and molds, the first-order kinetics were well suited. Decimal reduction times (D-value) for the aerobic bacteria and yeast and molds by CDPJ treatment were 87.82 and 85.47 s, respectively.

Effect of plasma treatment on biochemical quality

The impact of CDPJ treatment of chokeberries on their biochemical quality was studied. CDPJ treatment of chokeberries exerted negative effect on total monomeric anthocyanin content of their corresponding juices. Compared with untreated controls (461.89 ± 1.20 mg/L), there was a significant ($p < 0.05$) reduction in the levels of monomeric anthocyanins in juices obtained from CDPJ-treated (440.18 ± 2.34 , 421.15 ± 3.76 , and 433.50 ± 2.61 mg/L for 1, 2, and 3 min treatment time) chokeberries (Table 2).

Compared to untreated controls, no significant ($p > 0.05$) reduction in the levels of total polyphenol

Table 1 Microbial contaminants of fresh chokeberry fruits

Microorganisms	Mean $\pm$ SD (n = 10) (Unit: log CFU/g)
Aerobic bacteria	4.78 ± 0.11
Yeast and molds	4.87 ± 0.21
<i>Bacillus cereus</i>	ND
<i>Escherichia coli</i>	ND
<i>Salmonella</i> spp.	ND
<i>Staphylococcus aureus</i>	ND
ND not detected	

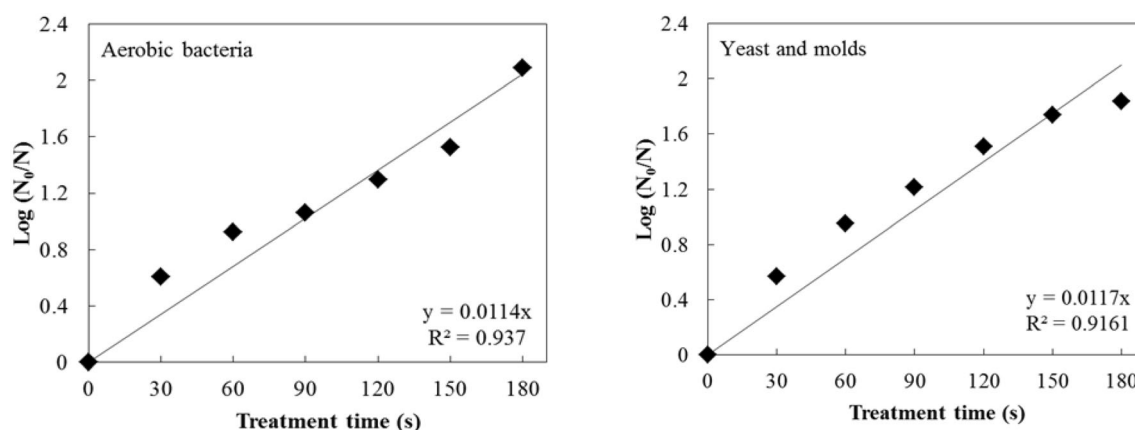


Fig. 2 Levels and pattern of inactivation of aerobic bacteria and yeast and molds in chokeberry fruits by CDPJ treatment

Table 2 Levels of monomeric anthocyanin content, total phenolics, and DPPH radical scavenging activity in untreated and CDPJ-treated chokeberries

Parameters	CDPJ treatment time (min)			
	0	1	2	3
Total monomeric anthocyanin content (mg/L juice)	461.89 ± 1.20 ^a	440.18 ± 2.34 ^b	421.15 ± 3.76 ^d	433.50 ± 2.61 ^c
Total polyphenol content (mg GAE/100 g)	32.78 ± 0.35 ^a	32.97 ± 1.68 ^a	33.75 ± 1.79 ^a	33.64 ± 3.62 ^a
DPPH radical scavenging activity (%)	84.44 ± 1.91 ^a	84.39 ± 1.60 ^a	80.88 ± 2.01 ^{ab}	78.86 ± 2.33 ^b

Values followed by the same letter within the same row are not significantly different ($p > 0.05$)

content of extracts prepared from CDPJ-treated berries (1, 2, and 3 min) was observed. The average levels of total polyphenol content ranged between 32–34 mg GAE/100 g.

The DPPH radical scavenging assay measures the antioxidant capability of a test substance based on its reducing power against the DPPH radical. The DPPH radical scavenging activity of chokeberry extracts from CDPJ-treated berries (up to 2 min) was not significantly ($p > 0.05$) different compared to untreated controls; however, the radical scavenging capacity of extracts from the berries treated for 3 min was significantly decreased (Table 2). Therefore, it can be concluded that CDPJ exposure of chokeberries for 3 min negatively affected their antioxidant capacity.

Changes in soluble solids content and pH of chokeberry juice

Compared to untreated controls, CDPJ treatment (1, 2, and 3 min) of chokeberries resulted in non-significant ($p > 0.05$) reductions of soluble solids content and pH level in their corresponding juices (Fig. 3). During the storage of juices obtained from both untreated and CDPJ-treated berries at 4 °C for 72 h, no significant variations in the levels of soluble solids and pH were noted. Soluble

solids content (Brix score) ranged between 12.17–13.23% and pH levels were in the range of 3.57–3.66 (Fig. 3).

Changes in microbial quality of chokeberry juice

The initial counts of aerobic bacteria as well as yeast and molds in juices obtained from untreated chokeberries were 4.8 and 4.7 log CFU/mL, respectively. During the storage of the juices at 4 °C for 72 h, the levels of aerobic bacteria as well as yeast and molds were increased to 5.5 and 7.2 log CFU/mL, respectively (Fig. 3). On the contrary, initial (0 h storage) aerobic bacterial count of juices obtained from CDPJ-treated (1, 2, and 3 min) chokeberries were 2.8, 2.5, and 2.4 log CFU/mL, respectively; and after storage for 72 h at 4 °C, the counts of aerobic bacteria were raised (at all treatment times) to a maximum of 4 log CFU/mL. In the case of yeast and molds, initial counts were 2.7 log CFU/mL (1 min-treated), 2.5 log CFU/mL (2 min-treated), and 2.3 log CFU/mL (3 min-treated). At the end of storage period (72 h), the counts of yeast and molds in chokeberry juices (obtained from CDPJ-treated berries) were in the range of 3.5–6.0 log CFU/mL. These results indicate that CDPJ treatment of chokeberries can extend the shelf-life of their corresponding juices via microbial load reduction.

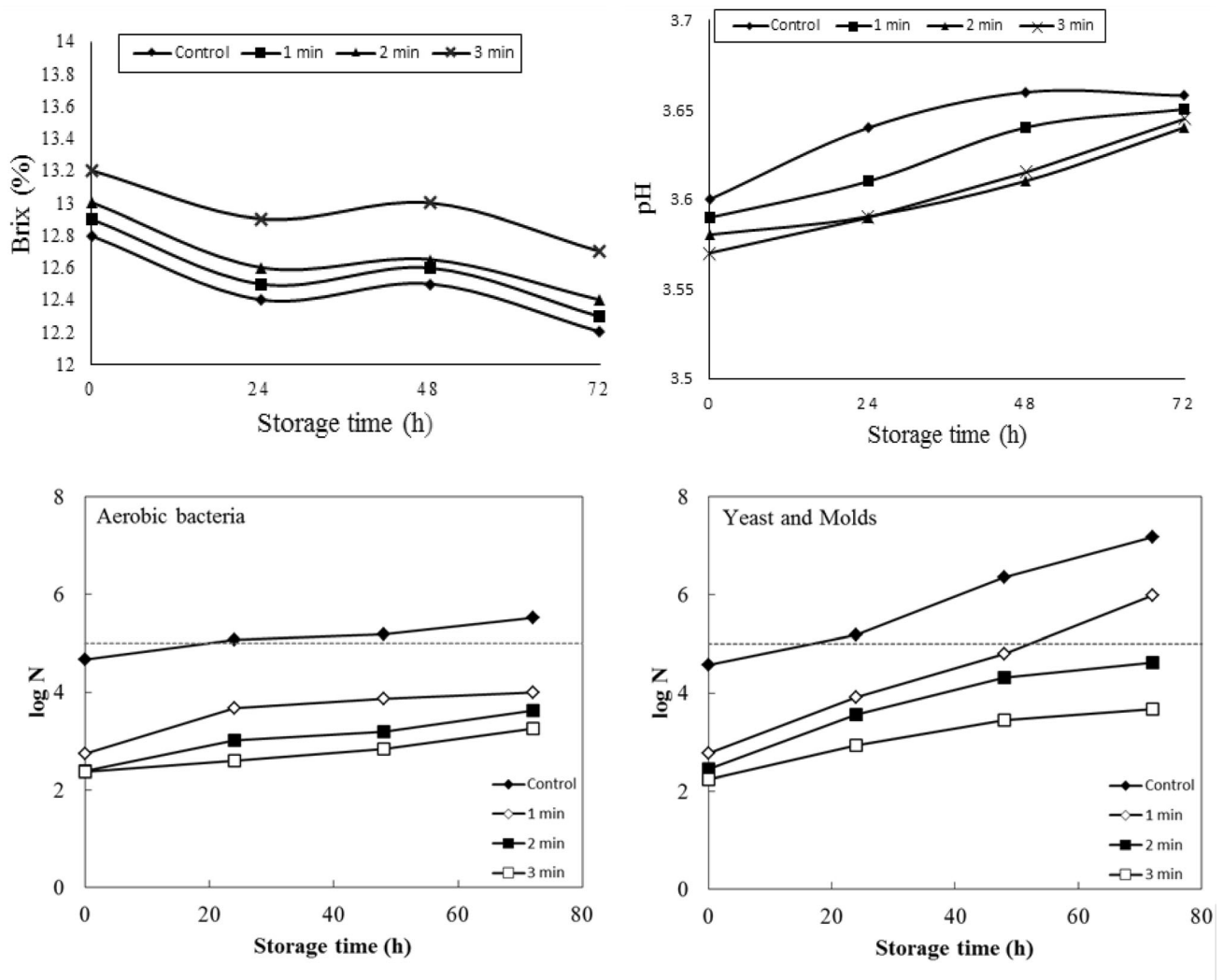


Fig. 3 Levels of soluble solids (Brix, %), pH, and microbial contaminants load in juices obtained from untreated and CDPJ-treated chokeberries during storage

In conclusion, moderate levels of contamination by aerobic bacteria as well as yeast and molds were observed in chokeberries. CDPJ treatment of chokeberries caused appreciable levels of contaminant inactivation ($> 2 \log$ CFU/g) in a short time (3 min). This indicates that nearly 99% of the initial contamination level can be decreased by CDPJ treatment. A linear increase in contaminant inactivation was noted with the increase of treatment time. The first-order kinetic model was better suited to describe the inactivation curves of the contaminants. CDPJ treatment for 2 min was found to be optimal since treatment periods beyond 2 min negatively affected the biochemical quality of the berries. The obtained results indicated that CDPJ treatment of chokeberries can effectively improve the microbiological quality of corresponding juices.

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Compliance with ethical standards

Conflicts of interest The authors declare no conflicts of interest.

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