

Quality and antioxidant properties of mixed fruit juice as affected by cold plasma treatment

Soğuk plazma uygulamasının karışık meyve suyunun kalite ve antioksidan özelliklerine etkileri

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ABSTRACT

This study aimed to assess the effects of cold plasma treatment on some quality and antioxidant properties of mixed fruit juices. Fresh mixed juice of apple, black carrot, and strawberry was subjected to dielectric barrier discharge cold plasma (DBDCP) treatment (40 kV) for 10 (CP10), or 20 min (CP20) or heat treated (HT) at 95 °C for 2 min. The samples which were not DBDCP- or heat-treated were used as the control. The changes in the titratable acidity, pH, total soluble solids, color, natural microbial load, total phenolic content (TPC), and antioxidant activity upon the treatments were evaluated. The DBDCP treatment did not cause any significant differences in the titratable acidity, pH, total soluble solids content and TPC, while the heat treatment led to a decrease in the titratable acidity and TPC. Also, the total color difference was higher upon the heat treatment than the DBDCP treatment. The HT samples demonstrated lower L* value and higher a*, b*, and C* values than the other samples. Furthermore, there was no change in the cupric ion-reducing antioxidant capacity (CUPRAC) of DBDCP-treated samples, but the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was lower in CP20 than that was in the control and CP10. On the other hand, HT samples demonstrated lower antioxidant activity than the control and DBDCP-treated samples. On the other hand, the yeast-mold count was not changed by the DBDCP treatment but reduced to below the detection limit upon the heat treatment. Thus, it can be said that DBDCP treatment of mixed fruit juice can be used to enhance the antioxidant activity, but more studies are required to guarantee microbial safety.

Key Words: Cold plasma, fruit juice, antioxidant activity, quality

ÖZ

Bu çalışmada, soğuk plazma uygulamasının karışık meyve suyunun bazı kalite antioksidan özelliklerine etkilerinin araştırılması amaçlanmıştır. Taze karışık meyve suyu elma, siyah havuç ve çilek suyu kullanılarak hazırlanmış ve 10 (CP10) veya 20 dk (CP20) boyunca dielektrik bariyer boşaltım soğuk plazma (DBDCP) (40 kV) veya 95 °C'de 2 dk ısıl işlem (HT) uygulanmıştır. DBDCP veya ısıl işleme tabi tutulmayan örnekler kontrol olarak kullanılmıştır. Uygulanan işlemlerin ardından örneklerin titre edilebilir asitlik, pH, toplam çözünür katı madde, renk, mikrobiyal yük, toplam fenolik içerik (TPC) ve antioksidan aktivitesinde meydana gelen değişiklikler incelenmiştir. DBDCP işlemi titrasyon asitliği, pH, toplam çözünebilir katı madde içeriği ve TPC'de önemli bir farka neden olmazken, ısıl işlem sonucunda titrasyon asitliği ve TPC'de azalma görülmüştür. Ayrıca, toplam renk değişimi ısıl işlem uygulanmış örneklerde daha yüksek bulunmuştur. HT örneklerinin diğer örneklere göre daha düşük L* ve daha yüksek a*, b* ve C* değerlerine sahip olduğu görülmüştür. Ek olarak, DBDCP uygulanan örneklerin bakır iyonu indirgeyici antioksidan kapasitesinde (CUPRAC) bir değişiklikle olmazken, 2,2difenil-1-pikrilhidrazil (DPPH) radikal süpürücü aktivitesi CP20'de kontrol ve CP10'a göre daha düşük bulunmuştur. Dahası, HT örneklerinin kontrol ve DBDCP uygulanmış örneklerden daha düşük antioksidan aktivitesine sahip olduğu görülmüştür. Öte yandan, DBDCP maya-küf sayımında bir değişime neden olmazken ısıl işlem uygulanan örneklerde tespit limitinin altına düştüğü görülmüştür. Bu nedenle DBDCP uygulamasının karışık meyve sularında antioksidan aktiviteyi artırmada kullanılabileceği söylenebilir ancak mikrobiyal güvenliğin garanti altına alınması için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Soğuk plazma, meyve suyu, antioksidan aktivite, kalite

Introduction

Fruit juices are frequently consumed food products with high consumer demand. They are rich in micro- and macronutrients and contain high concentrations of bioactive components. In addition, the consumer demand for minimally processed foods with high nutritional content and good sensory properties has been increasing (Ozen & Singh, 2020).

Although fruit juices contain high levels of bioactive compounds, they need to be decontaminated to prevent microbial spoilage and pathogenic growth. However, thermal treatment can cause losses in the bioactive composition, sensory properties, and some quality parameters. Nonthermal food processing technologies can decrease heat-induced quality loss while providing microbial and enzymatic inactivation. Thus, investigating the utilization of nonthermal food processing in fruit juice products is important (Noguera, Lima, Filho, Fonteles, & Rodrigues, 2021).

Cold plasma technology can be regarded as one of the promising methods as an alternative to thermal processing. Cold plasma is generated by the ionization of the gaseous phase (Niemira, 2012). The utilization of the cold plasma process of various fruit juice products in different conditions has been studied by several researchers. For instance, plasma jet cold plasma treatment of aronia juice limited the decrease in the hydroxycinnamic acid content, but it also reduced the anthocynanin content (Bursać Kovačević et al., 2016). In addition, it was observed in another study that dielectric barrier discharge cold plasma (DBDCP) inactivated Escherichia coli O157:H7, and it did not cause any significant changes in quality properties (Liao et al., 2018). The cold plasma processing of food products can exhibit varying

effects on the contents of total phenolics, anthocyanins, and vitamins and the quality parameters, such as pH, color, sensory properties, and titratable acidity depending on several factors, such as the cold plasma system, gas, voltage, frequency, treatment time, sample amount, and matrix of the fruit juice (Ozen & Singh, 2020). Thus, the number of research studies that investigate the effects of different cold plasma systems, setups, and treatment conditions, such as the power, voltage, frequency, and treatment time, on different fruit juices should increase for the optimization and industrial adaptation of the technology (Ozen & Singh, 2020).

In addition, although there are several studies evaluating the effect cold plasma on the quality of the juices from different fruits, there have been no studies with black carrot juice or mixed fruit juices to the best of our knowledge. Besides, it has been reported that mixed juices are advantageous for the aroma, flavor, nutritional, and antioxidant properties (Bhardwaj & Pandey, 2011; Schiassi et al., 2018).

The objective of this study was to assess the effects of cold plasma treatment on the quality and antioxidant properties of mixed fruit juice of apple, black carrot, and strawberry.

Material and Method

Fruit juice preparation

The apples (Starking), black carrots, and strawberries were obtained from a local market between August 2022 and December 2022 and washed under water in the laboratory. The black carrot samples were peeled and diced before use. Then all samples were separately juiced in a juicer (Sinbo, Türkiye) and filtered using a filter paper (Whatman 1). Then equal amounts (approximately 50 mL) of the apple, black carrot, and strawberry juices were mixed in a beaker. The same procedure was repeated three times. Then, some of the samples (7 mL) were separated for heat treatment at 95 °C for 2 min (HT) using three replications, while the remaining was treated with cold plasma. All samples were prepared freshly before the analysis (titratable acidity, pH, total soluble solids, color, microbial analysis) and extraction.

Cold plasma treatment

A 7 mL of the mixed juice was poured into a glass petri plate and placed between two parallel electrodes (316 stainless steel, 0,4 cm thickness, 95 mm diameter). The top electrode was coupled to a glass barrier (2 mm thickness, 140 mm x 140 mm). The space between the glass barrier (top) and the surface of the fruit juice was 12 mm. The dielectric barrier cold plasma (DBDCP) treatment was performed using a pulsed direct current (DC) power source (Asentek, Türkiye) at 40 kV (56 Hz, 10 mA) for 0 min (control), 10 min (CP10), or 20 min (CP20). All treatments were repeated three times. The parameters used in the cold plasma treatment were selected according to the findings of the preliminary runs.

Titratable acidity, pH, and total soluble solids

The titratable acidity (TA) of the fruit juice samples was determined by the protocol described by AOAC International (2005) with some modifications. The fruit juice samples were titrated with 0.1 N NaOH. Then, the TA of mixed juice samples was calculated using *Equation 1*.

$$TA(\%) = \frac{V_b \times C_b \times f \times 100}{m} \tag{1}$$

where V_b is the volume of the NaOH solution consumed, C_b is the density of the NaOH solution, f is the acidity factor (0.06 for malic acid), and m is the juice volume.

The pH of the samples was measured using a pH meter (Testo 206, Germany) at approximately 13-14 °C.

The total soluble solids content of the mixed juice samples was determined by using a hand refractometer (Loyka ATC 0-32, Türkiye).

Color

The color values (L*, a*, b*) of the mixed fruit juice samples were measured by using a color measurement device (CR-400, Konica Minolta, Japan). Then the chroma (C*), the hue angle (h°), and the total color difference (ΔE) were calculated using the following equations:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2}$$
(2)

$$h^{\circ} = \arctan\left(\frac{b^{*}}{a^{*}}\right) \tag{3}$$

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 (\Delta b^*)^2}$$
(4)

Microbial analysis

One mL of the mixed fruit juice samples was diluted in 9 mL peptone water and further serial dilutions were prepared (Aneja, 2001) and spread onto the plate count agar (PCA) and dichloran rose bengal chloramphenicol (DRBC) agar for the total viable and yeast-mold count. The inoculated PCA and DRBC agar in petri plates were incubated at 37 °C for 24-48 h and at 25 °C for 3-5 days, respectively.

Total phenolic content

Methanolic extracts of the juice samples were prepared as described before with some modifications (Devi Ramaiya et al., 2013). A 1mL of fresh fruit juice was added with 25 mL of methanol (75% in water) and homogenized at 25 °C for 30 min in an ultrasonic bath (Isolab, Germany). Then the samples were centrifuged at 4000 rpm for 20 min (Universal 320R, Hettich, Germany). After centrifuging, the aliquots were separated and stored at -18 °C.

The TPC of the samples was assessed by the Folin-Ciocalteu assay as described by Spanos and Wrolstad (1990). The TPC of the fruit juice samples was expressed as mg gallic acid equivalents (GAE)L⁻¹.

Antioxidant activity

The same extracts prepared for the TPC were used for the antioxidant activity analysis. The

antioxidant properties of fruit juice were determined by the cupric ion reducing antioxidant capacity (CUPRAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assays using the methods described previously (Apak et al., 2007; Kumaran et al., 2006). The antioxidant activity was expressed as mg 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid equivalents (TE)L⁻¹.

Statistical analysis

The data were evaluated using analysis of variance (ANOVA) and Duncan's multiple range test using statistical software (IBM SPSS Statistics 27, USA). The correlations between different parameters were determined using Pearson's correlation coefficients.

control (P > 0.05).

An increase in the TA of apple juice by increasing DBDCP power between 30 and 50 W was reported by Liao et al. (2018). On the other hand, Pankaj et al. (2017) reported that the TA of grape juice after cold plasma (at 80 kV for 1-4 min) and thermal treatment were decreased, and those of the cold plasma- and heat-treated samples were not different. The decrease in the TA was explained by the production of the hydroxyl radicals by the cold plasma treatment (Pankaj et al., 2017), while the production of nitrogenous acids and the oxidation of the aldehydes in the juice by the plasma generated species could increase the acidity (Liao et al., 2018).

Results and Discussion

Titratable acidity, pH, and TSS

The heat-treated samples exhibited lower titratable acidity (TA) values than that of the others (P < 0.05) as shown in Table 1. The TA of DBDCP-treated samples was not different from that of the

Sample	Titratable acidity (%)	рН
Control	$0.34 \pm 0.02^{\circ}$	4.51 ± 0.01°
CP10	0.31 ± 0.06^{a}	4.50 ± 0.03°
CP20	$0.35 \pm 0.01^{\circ}$	$4.44 \pm 0.08^{\circ}$
HT	0.18 ± 0.02^{b}	4.48 ± 0.01 ^a

Control: not DBDCP- or heat-treated, CP10: DBDCP-treated for 10 min, CP20: DBDCP-treated for 20 min, HT: heat-treated. Values labeled with different letters in a column are significantly different (P < 0.05).

None of the treatments affected the pH of the samples (P > 0.05, Table 1). Similarly, the pH of the grape juice processed by the DBDCP at 80 kV for 1-4 min was not different from those of the control and heat-treated (Pankaj et al., 2017). Also, it was reported in another study that cold plasma treatment at 45 V for up to 5 min did not affect the pH of tomato juice (Ali et al., 2021). On the contrary, a decline in the pH of apple juice with increasing DBDCP power and treatment time was observed (Liao et al., 2018). Almeida et al. (2015) also declared that the pH of prebiotic juice decreased upon direct and indirect cold plasma

treatment.

The total soluble solids content of all samples was similar (P > 0.05) and approximately 10.5 °Brix. Similarly, the total soluble solids content of apple juice (Liao et al., 2018), grape juice (Pankaj et al., 2017), and orange juice (Shi et al., 2011) did not change upon cold plasma processing. However, it was stated that the total soluble solids content of tomato juice was increased after processing with cold plasma (Ali et al., 2021).

Color

The L*, a*, b*, C*, and h° values of the DBDCP-

treated samples were not altered compared to that of the untreated (P > 0.05), but they had lower a*, b*, and C* values than the heat-treated samples (P < 0.05) as demonstrated in Table 2. Also, the L* value of the control and the 10 min DBDCP-treated samples was above that of the heat-treated (P < 0.05). In addition, the total color difference caused by the DBDCP treatment was lower than that caused by the heat treatment (P < 0.05). However, the ΔE value significantly increased by approximately 12 folds as the treatment time increased (P < 0.05). The changes in the color values of juices could be associated with the enzymatic browning and degradations in the pigments and bioactive components (Ozen & Singh, 2020; Waghmare, 2021). In addition, a decrease in the L* value and an increase in the a* value were reported to be linked with the browning in juice (Illera et al., 2019). Thus, it could be said that the color change in the mixed juice samples caused by DBDCP at 40 kV for up to 20 min were only minor, and the treatment did not cause a significant loss in this quality parameter.

Table 2. Color values of the	mixed fruit juice as affected	by the DBDCP treatment.
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Sample	L*	a*	b*	C*	h°	ΔE
Control	26.65 ± 0.90 ^a	3.80 ± 0.82^{b}	0.99 ± 0.65 ^b	4.47 ± 0.12^{b}	17.09 ± 7.03 ^a	
CP10	26.96 ± 1.03 ^a	4.99 ± 0.88^{b}	1.65 ± 0.43 ^b	5.27 ± 0.89 ^b	16.04 ± 2.98 ^a	0.34 ± 0.02 ^c
CP20	25.20 ± 4.03 ^{ab}	4.61 ± 1.02^{b}	1.29 ± 0.51^{b}	4.79 ± 1.12^{b}	15.28 ± 2.49 ^a	4.21 ± 1.31 ^b
HT	21.32 ± 1.85 ^b	22.41 ± 1.34^{a}	8.47 ± 0.67 ^a	23.96 ± 1.49 ^a	20.69 ± 0.37 ^a	21.48 ± 0.42^{a}

Control: not DBDCP- or heat-treated, CP10: DBDCP-treated for 10 min, CP20: DBDCP-treated for 20 min, HT: heat-treated. Values labeled with different letters in a column are significantly different (P < 0.05).

It was stated by Kovačević et al. (2016) that cold plasma application led to decreases in color values $(L^*, a^*, b^*, C^*, h^\circ, and \Delta E)$ of pomegranate juice in comparison to the untreated samples, but the difference did not depend on the treatment time. In addition, Paixão et al. (2019) declared that glow discharge cold plasma application caused a slight increase in the L*, a*, and b* values of siriguela juice. In another study, variations in the color values of blueberry juice with increasing O2 concentration and treatment time were observed, but the change in the color values was greater when heat treatment was used (Hou et al., 2019). In contrast to the findings of the current study, the cold plasma processing of grape juice at 80 kV for up to 4 min caused color changes compared to the heat-treated and control samples (Pankaj et al., 2017). Also, Almeida et al. (2015) stated that the L* and chroma values of orange juice increased after cold plasma treatment, while the h° was reduced, and the values also differed depending on the treatment time.

Microbial analysis

The total viable count of all samples was below the detection limit (< 2 log cfu mL⁻¹). The yeastmold count of the control and DBDCP-treated samples ranged between 3.76 - 3.85 log cfu mL⁻¹ as given in Table 3 and were not different from each other (P > 0.05), whereas that of the heattreated samples were below 2 log cfu mL⁻¹.

Table 3. Yeast and mold count of the mixed fruit juice as affected by the DBDCP treatment.

Sample	Yeast-mold count (log cfu mL ⁻¹)	
Control	3.76 ± 0.08 ^a	
CP10	3.79 ± 0.18^{a}	
CP20	$3.85 \pm 0.02^{\circ}$	
HT	< 2	

Control: not DBDCP- or heat-treated, CP10: DBDCP-treated for 10 min, CP20: DBDCP-treated for 20 min, HT: heat-treated. Values labeled with different letters in a column are significantly different (P < 0.05).

Cold plasma can inactivate microorganisms mainly by cell leakage, intracellular damage, and

DNA damage (Han et al., 2016; Liao et al., 2018; Pankaj et al., 2017). However, the inactivation can

vary due to various factors, such as the cold plasma system, treatment time, gas, gas flow rate, frequency, voltage, and microbial population (Mir et al., 2020). It was also reported that the cold plasma-induced inactivation of spores and yeasts could be more difficult due to the polysaccharide layer in their cell wall (Mravlje et al., 2021). For instance, the yeast-mold count of fresh tomato juice did not change after gliding arc cold plasma processing at 3.8 kV for up to 600 s (Starek-Wójcicka et al., 2022). Also, Mehta et al. (2019) stated that the cold plasma processing at 60 kV for 10-15 min resulted in an approximately 1 log reduction in the yeast-mold count of a tomatobased beverage. It can be said that more studies investigating the effects of different cold plasma parameters to improve microbial inhibition in mixed fruit juice are required.

Total phenolic content

The changes in the TPC of the samples are shown in Table 4. There were no significant differences caused by the cold plasma treatment (P > 0.05), while the TPC values of the heat-treated samples were below the others (P < 0.05). Similarly, the TPC of blueberry juice increased after cold plasma with increasing treatment time and O₂ concentration compared to that of the heattreatment samples (Hou et al., 2019). Moreover, it was reported that DBDCP treatment at 20 kV and frequencies between 50 - 900 Hz increased the phenolic content of apple juice (Farias et al., 2022). Also, the TPC of cloudy apple juice increased by cold plasma treatment as stated by Illera et al. (2019). In addition, the TPC of apple juice was increased by cold plasma processing with increasing treatment time and power (Liao et al., 2018). The phenolic components (gallic acid, chlorogenic acid, catechin, and quercetin) of kiwifruit juice was higher after cold plasma treatment than heat treatment (Kumar et al., 2024). However, cold plasma treatment at 80 kV for up to 4 min and heat treatment decreased the TPC of grape juice (Pankaj et al., 2017). The effect of cold plasma processing on the TPC of juices depends on several factors, including the plasma system, gas, gas flow rate, power, voltage, frequency, and treatment time (Almeida et al., 2015; Farias et al., 2020; Farias et al., 2022; Illera et al., 2019; Kumar et al., 2023; Liao et al., 2018).

Table 4. TPC of the mixed fruit juice as affected by the DBDCP treatment.

Sample	TPC (mg GAE L ⁻¹)
Control	823.44 ± 11.17 ^a
CP10	801.06 ± 13.03 ^a
CP20	783.95 ± 25.93ª
HT	703.22 ± 31.84 ^b

Control: not DBDCP- or heat-treated, CP10: DBDCP-treated for 10 min, CP20: DBDCP-treated for 20 min, HT: heat-treated. Values labeled with different letters in a column are significantly different (P < 0.05).

The cold plasma-induced changes in the TPC of juices could be related to the changes in color, as cold plasma can cause changes in pigments and phenolic components (Ozen & Singh, 2020). As a matter of fact, the total phenolic content positively correlated with the titratable acidity and L* value (P < 0.05). Moreover, negative correlations between the TPC and the a*, b*, and C* values were noted (P < 0.05).

Antioxidant activity

The antioxidant activity of the mixed fruit juice as affected by the DBDCP and heat treatment is exhibited in Table 5. The CUPRAC and DPPH scavenging activity-based antioxidant activity was lower in heat-treated samples than in the control and DBDCP treated samples (P < 0.05). In addition, the DPPH scavenging activity of the 20 min DBDCPtreated samples was lower than that of the untreated and 10 min DBDCP-treated samples (P < 0.05). Also, the DPPH-based antioxidant activity positively correlated with the TPC (P < 0.05).

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Table 5. Antioxidant activity of the mixed fruit juice as affected by the DBDCP treatment.

Sample	CUPRAC (mg TE L ⁻¹)	DPPH (mg TE L ⁻¹)	
Control	3346.67 ± 615.20 ^a	1872.66 ± 36.49 ^a	
CP10	3370.84 ± 180.14 ^a	1848.08 ± 53.87ª	
CP20	3573.85 ± 406.35°	1656.39 ± 64.30 ^b	
НТ	2544.32 ± 517.00 ^b	1424.13 ± 22.65 ^c	

Control: not DBDCP- or heat-treated, CP10: DBDCP-treated for 10 min, CP20: DBDCP-treated for 20 min, HT: heat-treated. Values labeled with different letters in a column are significantly different (P < 0.05).

The decrease in the DPPH scavenging activity after the 20 min treatment could be explained by the reaction of the antioxidant components with the plasma-generated species. It was reported that prolonged exposure to cold plasma treatment can decrease antioxidant activity because of the formation of plasma-induced substances (Ali et al., 2021; Fernandes & Rodrigues, 2021; Pankaj et al., 2018). It was observed by Farias et al. (2022) that the antioxidant activity of apple juice varied depending on the cold plasma system, frequency, treatment time, and gas flow rate. Almeida et al. (2015) also claimed that the DPPH scavenging activity of prebiotic orange juice was not affected by the cold plasma processing at 70 kV for up to 60 s. On the contrary, the antioxidant activity of grape juice decreased upon cold plasma treatment at 80 kV for 4 min in comparison to the untreated, but it was similar to that of the heat-treated samples (Pankaj et al., 2017).

The variation between the findings obtained by the CUPRAC and DPPH methods was also observed in previous studies (Sethi et al., 2020). Apak et al. (2007) claimed that CUPRAC method has advantages over other electron transfer based assays.

Conclusion

The treatment of mixed fruit juice with DBDCP at 40 kV for 10 and 20 min did not cause a notable influence on the TA, pH, or total soluble solids content. The treatment also did not change the L*, a^* , b^* , C^* , or h° values. Also, the ΔE value after the 20 min DBDCP process was below that after the heat treatment. The plasma-induced changes in the TPC, yeast-mold count, and antioxidant activity except for the 20 min treatment were not significant. Besides, the heat treatment negatively affected the TPC and antioxidant activity but reduced the yeast-mold count to below 2 log cfu mL⁻¹. In conclusion, the use of DBDCP in the treatment of mixed fruit juice can improve the antioxidant activity, but more studies are required to guarantee microbial safety.

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Conflict of interest

The authors declare that there are no known conflicts interest.

Author contributions

EŞ performed the analysis and investigation, data analysis and interpretation and drafting the article. CKG designed and supervised the study and participated in drafting the article. All authors approved the final version of the manuscript.

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