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The Effect of Cold Plasma on the Enzymatic Activity and Quality Characteristics of Mango Pulp

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Abstract
The ability to produce cold plasma in the atmosphere provides new opportunities for the decontamination of biological materials including fresh food. This technology is also used to inactivate endogenous enzymes, especially polyphenol oxidase and peroxidases, which are responsible for browning reactions. This study investigated the effect of Dielectric Barrier Discharge Plasma (DBDP) on the inactivation of enzymatic activity and some quality parameters in mango pulp. Results showed that DBDP treatment up to 10 min resulted in a reduction of polyphenol oxidase (10.85%), peroxidase (5.15%), and pectin methyl esterase (5.25 %) activities, aerobic plate count (16.6%), and yeast and mold count (18.8%) activities. An improvement was observed in physicochemical (especially viscosity and firmness values) and phytochemical (i.e. ascorbic acid, total phenol content) profiles as well as color values with increase DBDP treatment time until 6 min. This study provides the impact of DBDP time on the enzymatic activities and quality characteristics (especially phytochemical profiles) of mango pulp. Therefore, it is possible to use it as a new non-thermal alternative technology for pasteurizing mango pulp instead of thermal treatment.

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Introduction
Mango pulp is one of the valuable mango (*Mangifera indica* L.) products, which is a good source of bioactive compounds (i.e. mainly carotenoids as well as ascorbic acid and phenolic contents) (Ribeiro & Schieber, 2010). Mango pulp may be subject to many reactions that cause deterioration such as enzymatic activities, microbial spoilage, ascorbic acid degradation, and changes in flavor and color during the processing steps and storage time. All of these reactions led to the loss of product quality (Tharanathan, Yashoda, & Prabha, 2006).

One of the main problems faced by mango pulp processing is enzymatic activities that result in undesirable changes during pulps or juices processing (Abedelmaksoud, Mohsen, Duedahl-Olesen, Elnikeety, & Feyissa, 2018). Pectin methyl esterase (PME) is one of these enzymes that cause the separation of layers (in the juice after processing). Also,

browning occurs after pulp or juice extraction due to oxidation of polyphenols by polyphenol oxidase (PPO) and peroxidase (POD) (Abedelmaksoud, Mohsen, Duedahl-Olesen, Elnikeety, & Feyissa, 2018; Zhiqing, Li, Liu, Cheng, & Wang, 2015). As a result of using high temperatures in juices treatment, undesirable changes will occur in some of the quality parameters such as changes in color, texture, and loss of some nutrients (for example ascorbic acid degradation) (Abedelmaksoud *et al*., 2018; Ayaseh, Alizadeh, Esmaiili, Mehrdad, & Javadzadeh, 2014; Kaleem *et al*., 2016). For this reason, researchers tend to find new alternative technologies (thermal or non-thermal) for food processing and preservation. Cold plasma (CP) is one of the non-thermal technologies (green method), which identified as an ionized gas characterized by active particles i.e. ions, free radicals, atoms, and electrons that are produced by applying energy to gas or mixture of gas (Ramazzina *et al*., 2015).

Recently, there is a significant interest in using the CP process as a green method for food processing and preservation. There are many advantages of this technology such as being versatile, economical, environmentally friendly natural and non-thermal. In addition, CP has been effective in the inactivation of undesirable enzymes and main food-borne pathogenic microorganisms as well as food decontamination, toxin removal (Shashi K. Pankaj, Wan, & Keener, 2018).

CP has a high effect on the oxidative enzymes such as POD in tomatoes and PPO in fresh-cut apples (Tappi *et al*., 2014). From the previous studies, most of the researches focused on the effect of CP on microbial decontamination, with limited researches focused on quality attributes. On the other hand, the effect of CP on orange, pomegranate, chokeberry, apple, and white grape juices was investigated (Ramazzina *et al*., 2015).

However, there exist no study exists about the effect of Dielectric Barrier Discharge Plasma (DBDP), which is the electrical discharge between two electrodes separated by an insulating dielectric barrier (Dhali & Sardja, 1989), on the quality characteristics of mango pulp. Therefore, the main objective of this study was to study the impact of DBDP treatment on the quality properties of mango pulp.

Materials and methods

Chemicals

All chemicals used in this study were purchased from the chemical companies of Merck KGaA (Darmstadt, Germany) and Sigma (St. Louis, Mo., USA).

Mango pulp preparation

Fresh mango (*Mangifera indica* L., cv. Langra.) fruits were purchased from a local market in Semnan, Iran. These fresh fruits were washed with tap water, dried with paper towels; hand peeled, sliced, and pulped using a household blender. The extracted pulps were packed in plastic bags (3 kg per bag) and stored at -18 °C before all treatments and further research was performed (Ahmed, Ramaswamy, & Hiremath, 2005).

Plasma processed air treatment

The dielectric barrier discharge plasma (DBDP) was chosen as the plasma generation mode during this study. Two aluminum plate electrodes (45×10 cm) are covered with dielectric layers (made of mica) to assure the uniform micro discharges (Fig. 1). Approximately 5 g samples were placed in Petri dishes of 50 mm diameter and then subjected to plasma treatment (due to the capacity of this equipment, 4 Petri dishes (20 g) were kept for one treatment). All treatments were conducted using a discrete voltage of 25 kV and 2±0.2 A applied for 0, 2, 4, 6, 8, and 10 min, respectively using ambient air at atmospheric pressure conditions. The justification for using a discrete voltage of 25 kV is that the breakdown voltage is a function of the gas pressure and the gap between electrodes for plasma production.

For example, an air breakdown voltage of 1 cm between electrodes at atmospheric pressure requires a voltage of around 30 kV (Bárdos & Baránková, 2008). In turn, increasing the voltage increases the energy transmitted into the plasma, which contributes to higher plasma species production (Ziuzina, Patil, Cullen, Keener, & Bourke, 2013). Keener *et al.* (2012) reported that the ozone generation rate in the air by DBD plasma at 13.5 kV was 1200 ppm/min, which increased to 3750 ppm/min at 80 kV (Keener *et al.*, 2012; Pankaj *et al.*, 2018).

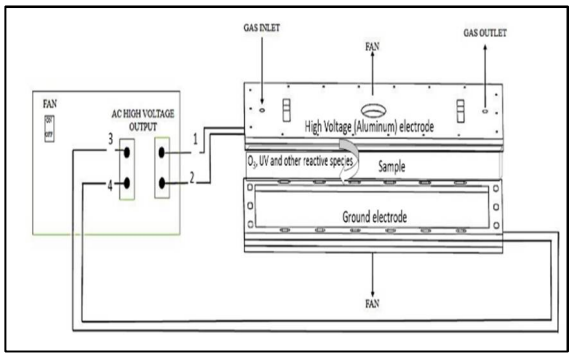

Fig. 1. Set up of dielectric barrier discharge plasma (DBDP) device

Many studies have shown that the overall efficacy of the desired results, such as microbial decontamination, surface alteration, and chemical degradation, is often improved by increasing the applied voltage. According to the characteristics of our device and the pretreatment, 25 kV was chosen for this study. In addition, a similar condition was applied to maximize the inactivation of enzymes (Tolouie, Mohammadifar, Ghomi, Yaghoubi, & Hashemi, 2018).

Physicochemical profiles

The content of dry matter of mango pulp samples has been gravimetrically determined according to AOAC (1990b). Total soluble solids (TSS) were measured by a digital refractometer at 20 °C (Model: PAL-1; Make: Atago, Japan). The pH value was measured by an Orion 868 pH meter at 25 °C (Thermo Fisher Scientific, Inc., Agawam, MA, USA). The standard buffer solutions at pH 4.0 and 7.0 were used for the calibration of the pH meter. Titratable acidity (TA) was determined using a method (942.15) described by AOAC (1990a).

The color values L* (lightness), a* (redness), and b* (yellowness), as well as total color difference (ΔE), were measured by a Hunter-Lab (color Flex EZ, 45°/0°, USA). The instrument calibration conducted by a white standard calibration plate (Kaushik, Kaur, Rao, & Mishra, 2014). The ΔE value was calculated by Eq. (1):

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5} \quad (1)$$

Where, $\Delta L^* = (L^*_1 - L^*_0)$; $\Delta a^* = (a^*_1 - a^*_0)$; and $\Delta b^* = (b^*_1 - b^*_0)$.

Subscript '0' refers to the color value of the untreated sample and subscript '1' refers to the color value of the sample being analyzed. In addition, other color values (yellowness index (YI) and browning index (BI)) were evaluated (Hirschler, 2012; Pathare, Opara, & Al-Said, 2013). YI was calculated using Eq. (2) and BI was calculated by Eq. (3):

$$YI = 142.86\, b*/L* \quad (2)$$

$$BI = [100(x \times 0.31)]/0.172 \quad (3)$$

Where, x= (a*+1.75L*)/(5.645L*+a*-3.012b*).

Viscosity value of mongo pulp was measured using a rotary viscometer (DV-I Brookfield, Stoughton, MA) with spindle No. 30 at 5 °C and 100 rpm.

Phytochemical profiles

The assessment of the content of ascorbic acid in mango pulp samples was done using the visual titration process 2,6-dichlorophenol-indophenol (DCPIP). 5 g sample mixed with 3% metaphosphoric acid to stop any degradation of ascorbic acid and then filtered (Whatman No. 1). The resulting filtrate was titrated with a standardized dye solution (DCPIP) to a pink color as the endpoint of this titration

(persist of pink color should be ≥15 s). The obtained results were expressed as mg of ascorbic acid / 100 g sample.

The determination of total phenol content was conducted by the Folin-Ciocalteu method, as described by Wang *et al*. (2012) with some modifications by Liu, Liao, & Wang (2016). The obtained results were expressed as mg of Gallic acid equivalent / 100 g sample.

Antioxidant activity by DPPH radical-scavenging was determined as a method described in Khademi Pour, Sharifan, & Bakhoda (2021).

Enzymatic activity

The protocols for the determination of PPO, POD, and PME activities were followed by the method described in Liu *et al*. (2016).

Microbial Count

Microbial Count (i.e. aerobic plate counts and yeast and mold count) was carried out according to Shahidi Noghabi, Niazmand, Sarraf, & Shahidi Noghabi (2019). The results were expressed as log (CFU/mL).

Statistical analysis

The statistical analysis of the collected data in this study was carried out using SPSS system v.22 (SPSS Inc., Chicago, IL, USA). Duncan's multiple regression tests performed a mean comparison; variations were found to be significant at $P<0.05$.

Results and discussion

Effect of DBDP treatment time on the enzymatic activities of mango pulp

The obtained results in Fig. (2) showed the effect of DBDP treatment time on residual activity (%) of PPO, POD, and PME activities in mango pulp. The residual activity (%) of control (fresh mango pulp extracted from mangoes on the day of analysis) for all enzymes was 100%. The results also showed that the residual activity (%) of these enzymes decreased with increasing the time of DBDP treatment (at a discrete voltage of 25 kV and 2±0.2 A applied) gradually from 2 to 10 min. This decrease in enzyme activity could be due to the interaction between plasma reactive species and amino acids (Li *et al*., 2014) and damage of α-helix and β-sheet in the secondary structure of the protein (Segat, Misra, Cullen, & Innocente, 2016). Furthermore, many factors affecting the protein denaturation and inactivation of enzymes by DBDP such as protein/enzyme type, plasma type, reactive gas, parameters of processing, enzyme media, and volume of sample (Pankaj *et al*., 2018).

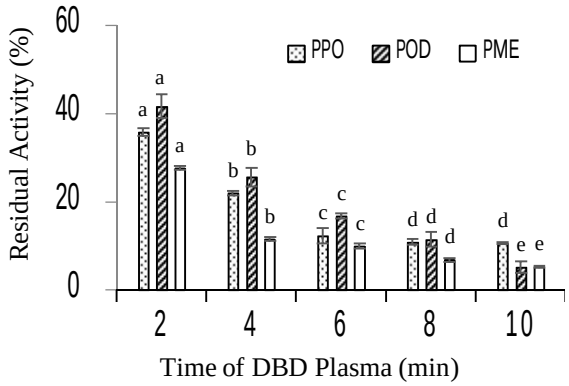

Fig. 2. Effect of DBDP on residual activity (%) of PPO, POD and PME activities in mango pulp; residual activity (%) of control for all enzymes was 100%

Physicochemical profiles

The obtained results in Table (1) showed the effect of DBDP treatment time on mango pulp samples, including dry matter, Brix, pH, TA, viscosity, and firmness. As seen from Table (1), a significant increase in the dry matter at the initial of DBDP treatment then no significant change after 6 min treatment. A significant increase for Brix value of mango pulp samples after being treated by DBDP at 2, 4, and 6 min, but there was no significant different among the samples that were treated at 2, 4, and 6 min (the highest value was observed at 6 min). In addition, there were no significant changes in samples treated at 8 and 10 min.

Table 1. Effects of DBDP treatment on physicochemical properties in mango pulp

Treatment time (min)	Dry matter	Brix	pH	TA (%)	Viscosity (Pa.s^n)
Control	16.21±0.00^d	15.2±0.1^b	4.11±0.01^a	0.475±0.005^d	874±4.24^d
2	16.24±0.01^c	15.7±0.1^a	4.01±0.01^c	0.520±0.000^b	927±9.90^c
4	16.32±0.03^b	15.9±0.1^a	3.98±0.00^d	0.535±0.005^a	1089±18.38^b
6	16.42±0.01^a	15.9±0.1^a	4.00±0.02^c	0.530±0.010^a	1162.5±14.85^a
8	16.41±0.02^a	15.3±0.2^b	4.13±0.00^a	0.470±0.000^d	912.5±3.54^c
10	16.43±0.00^a	15.4±0.1^b	4.09±0.00^b	0.485±0.005^c	811±18.38^e

Different letters (a, b, and c) mean statistical significant variation (P<0.05); the findings reflect the mean ± standard deviation; TA: Titratable acidity: grams of citric acid per 100 mL of mango pulp.

Titratable acidity % (TA %) of fresh mango pulp was 0.475%. An increase in TA% from 0.475 to 0.530 after treatment of the samples by DBDP for 6 min then decreased at 8 and 10 min as observed. This decrease in the acidity after 8 min may be due to the solubilization of the hydroxyl radical generated during the filamentary discharges. On the other hand, a decrease in pH from 4.11 to 4 after treatment of the samples by DBDP for 6 min then increased to 4.09 at 10 min. This decrease in pH value is related to the increase in acidity (Mohsen, Murkovic, El-Nikeety, & Abedelmaksoud, 2013). A previous study reported a significant decrease in the pH value after being treated by DBDP in both prebiotic orange and apple juices (Liao *et al.*, 2018). On the other hand, the changes in the acidity of mango pulp might be due to the production of acidogenic molecules (i.e. NOx) during DBDP treatment (Stoffels, Sakiyama, & Graves, 2008). Also, the changes in both pH and acidity after treatment by plasma were due to the interaction between plasma reactive gases and moisture content in the food (Pankaj *et al.*, 2018).

The viscosity value in fresh mango pulp was 874 (Pa.s^n). An increase was observed in viscosity values at 2, 4, 6, and 8 min, while after the DBDP treatment for 6 min (1162.5 Pa.s^n) the values decreased during treatment at 8 and 10 min. This increase in viscosity could be due to the inactivation of pectic-enzymes that leads to an increase in the viscosity values (Chou & Kokini, 1987). On the other hand, the decrease in viscosity value after 10 min was an agreement with Tappi *et al.* (2014) who reported that treatment by gas plasma leads to reduce viscosity value in fresh-cut apples with increase the time of treatment (10-30 min).

Phytochemical profiles

Table (2) shows the effects of DBDP treatment time (from 2 to 10 min) on phytochemical profiles (i.e. ascorbic acid, total phenolic content, and antioxidant activity) in mango pulp. Table (2) shows the ascorbic acid content of the fresh and treated mango pulp samples. The ascorbic acid value of mango pulp samples slightly decreased with increased plasma treatment time, which was 10.65 mg/100mL at 10 min compared to 12.05 mg/100mL for the control sample. The decrease in ascorbic acid with increasing DBDP time may be due to the interaction of ascorbic acid with some species of oxidizing plasma, where the critical factors for the degradation of ascorbic acid were plasma gas and processing time (Pankaj *et al.*, 2018). In this respect, the ascorbic acid content decreased in cut carrots, cucumber, and pears after being treated by DBDP (Wang *et al.*, 2012). In addition, Wang *et al.* (2012) reported that ascorbic acid content of cut fruits and vegetables decreased up to 4% after being treated with plasma. In addition, the ascorbic acid of orange juice reduced after CP treatment (Xu, Garner, Tao, & Keener, 2017).

Table 2. Effects of DBDP on phytochemical profiles in mango pulp

Treatment time (min)	Ascorbic acid (mg/100mL)	Total phenolic (mg GAE/100mL)	Total antioxidant (DPPH Capacity %)
Control	12.05±0.05^{a}	18.28±0.07^{c}	42.05±0.05^{c}
2	11.65±0.15^{b}	18.92±0.12^{b}	42.35±0.65^{c}
4	11.40±0.10^{c}	19.56±0.14^{a}	49.95±1.05^{a}
6	11.30±0.20^{c}	19.39±0.13^{a}	46.35±0.15^{b}
8	11.20±0.10^{c}	17.40±0.53^{d}	39.10±0.00^{d}
10	10.65±0.05^{d}	16.43±0.03^{e}	38.60±0.00^{d}

Different letters (a, b, and c) mean statistical significant difference (P<0.05); the results represent the mean ± standard deviation.

Table (2) also shows an increase in total phenolic content with increase DBDP treatment time until 6 min then decreased. This increase in phenolic content due to phenylalanine ammonialyase activity that acts on phenolic synthesis (this is correlated with increases in the phenolic content in mango fruits) (González-Aguilar, Zavaleta-Gatica, & Tiznado-Hernández, 2007). On the other hand, Brandenburg *et al*. (2007) reported that the increase in phenolic compounds (such as peroxyl radicals, hydroxyl radicals, singlet oxygen, and atomic oxygen) is related to the direct reaction of it with plasma species. The increase in phenolic content is also due to plasma depolymerization and dissolution of cell wall polysaccharides that lead to the extraction of the phenolic conjugated compounds (Sarangapani *et al*., 2016).

In general, antioxidant activity does not contribute directly to the quality parameter in the food industries, where it is related to phenolic contents, flavonoids, and flavanols that are present in the food (including fruit pulps or juices) (Shan, Cai, Sun, & Corke, 2005).

As shown in Table (2), the antioxidant activity (DPPH capacity %) of fresh mango pulp (38.60 %) increased when the DBDP treatment time increased until 4 min and then decreased. The decrease or increase in total antioxidant (DPPH capacity %) of mango pulp is mainly related to the total phenolic content in mango pulp (Shan *et al*., 2005). In addition, many other critical factors could affect the antioxidant activity such as the kind of food, source of plasma, and exposure mode (Pankaj, Misra, & Cullen, 2013).

Color values

Color is a very important factor that consumers can judge the quality of fruit juice or pulp (Brasil & Siddiqui, 2018). The changes in L*, a*, b* and ΔE* values in mango pulp treated by DBDP were tabulated in Table (3). As shown in Table (3), the L*, a*, and b* values of the fresh mango pulp were 50.22, 12.24, and 47.07, respectively. The L* and a* values increased with increasing the time of DBDP treatment until 8 min and then decreased. This decrease in color values at 10 min was probably due to the oxidation of colored pigments during DBDP treatment by reactive species, meaning a decrease in lightness and red intensities in mango pulp (Bursać Kovačević *et al*., 2016). The b* value decreased with increasing the time of DBDP until 4 min and then increased, meaning an improvement in yellowness intensity color in mango pulp. In general, the total color difference (ΔE) increased when the DBD treatment time increased from 2 to 8 min. This phenomenon caused the mango pulp color improvement. The ΔE values decreased at 10 min, meaning start the deterioration in the color quality. The results in Table (3) also showed a decrease in browning index (BI) values of mango pulp with increased DBDP treatment time compared to fresh mango pulp (117.22). This increase in the BI value of mango pulp is primarily related to a value (redness), which have a positive relationship between.

Table 3. Effect of DBDP treatment on the color values in mango pulp

Treatment time (min)	L*	a*	b*	ΔE*	Browning index
Control	50.22±0.11^{e}	12.24±0.01^{e}	47.07±0.02^{d}	-	117.22±0.28^{a}
2	50.03±0.05^{e}	12.10±0.02^{e}	47.065±0.02^{d}	0.17±0.05^{e}	117.58±0.15^{a}
4	51.53±0.09^{d}	13.89±0.10^{d}	46.25±0.07^{e}	2.33±0.21^{d}	113.36±0.29^{d}
6	52.63±0.20^{c}	14.49±0.17^{b}	47.63±0.09^{c}	3.42±0.39^{c}	114.28±0.15^{c}
8	54.17±0.04^{a}	14.86±0.13^{a}	48.975±0.05^{b}	5.19±0.11^{a}	114.15±0.14^{c}
10	53.59±0.38^{b}	14.22±0.01^{c}	49.75±0.15^{a}	4.81±0.51^{b}	116.64±0.46^{b}

Different letters (a, b, and c) mean statistical significant difference (P<0.05); the results represent the mean ± standard deviation.

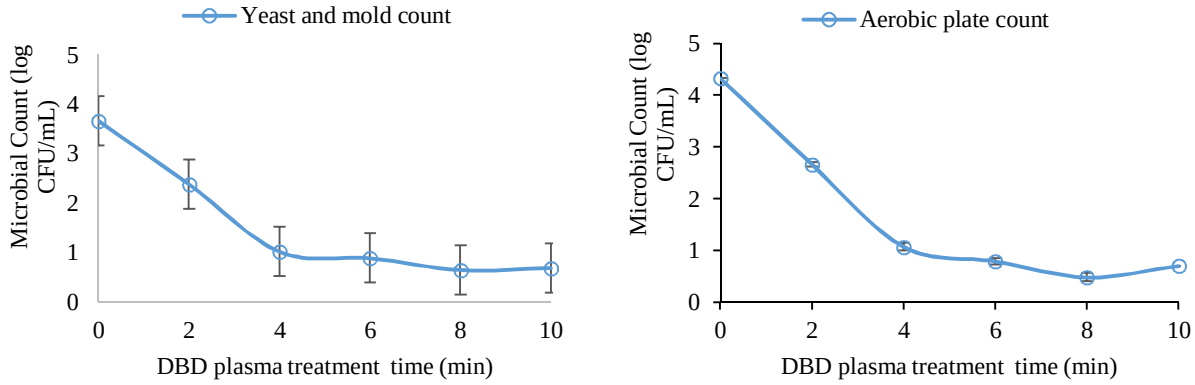

Fig 3. Microbial Count (log CFU/mL) of fresh and treated mango pulp

Microbial Count

The obtained results in Fig. (3) showed that the effects of DBDP treatment time (from 2 to 10 min) on microbial load i.e. aerobic plate count (APC) and mold and yeast (M/Y) of mango pulp. In fresh mango pulp, the APC and M/Y values were 4.33 and 3.66 log CFU/mL, respectively. A significant decrease in APC and M/Y values were observed in plasma treated samples with an increase in time of DBDP treatment. This reduction in microbial load either aerobic plate count (APC) or mold and yeast (M/Y) of mango pulp by DBDP treatment is due to the etching process by DBDP, where this process acting on exposure to an intense bombing by the radicals most likely provoking surface lesions that the living cell cannot repair sufficiently faster (Pignata, D'Angelo, Fea, & Gilli, 2017).

Conclusions

Based on the obtained results of this study, the results indicated that DBDP treatment has an effective impact on the inactivation of enzymes and microbial load in mango pulp with minimal quality degradation. The results showed that DBDP treatment up to 10 min resulted in about 10.85, 5.15 and 5.25 % reduction of PPO, POD and PME activities in mango pulp, respectively. The results also showed that DBDP treatment up to 10 min resulted in about 16.6 and 18.8% reduction in both aerobic plate count and yeast and mold count activities, respectively. The ascorbic acid value of mango pulp samples slightly decreased with increased plasma treatment time. An increase in total phenolic content with increase DBDP treatment time until 6 min then decreased. An increase in L* and a* values with increasing the time of DBDP treatment until 8 min and then decreased with 10 min. An improvement for yellowness intensity color in mango pulp. A decrease in browning index (BI) values of mango pulp with increased DBDP treatment time. Overall, an improvement of quality characteristics of mango pulp was achieved using DBDP. For the next researches, the optimization and scale-up for DBDP applications should be taken into consideration in the food industries to decrease the cost.

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تأثیر پلاسمای سرد بر فعالیت آنزیمی و ویژگی‌های کیفی پالپ انبه

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چکیده

توانایی تولید پلاسمای سرد در شرایط اتمسفری فرصت‌های جدیدی برای ضدعفونی مواد بیولوژیکی ازجمله غذای تازه فراهم می‌کند. این فناوری همچنین برای غیرفعال‌سازی آنزیم‌های درون‌زا، به‌ویژه پلی‌فنول اکسیداز و پراکسیدازها که مسئول واکنش‌های قهوه‌ای‌شدن هستند، استفاده می‌شود. این مطالعه به بررسی تأثیر پلاسمای سرد (DBDP) در غیرفعال‌سازی فعالیت آنزیمی و برخی ویژگی‌های کیفی در پالپ انبه پرداخته است. نتایج نشان داد که تیمار DBDP تا 10 دقیقه منجربه‌کاهش فعالیت‌های پلی‌فنول اکسیداز (10/85 درصد)، پراکسیداز (5/15 درصد) و پکتین متیل‌استراز (5/25 درصد)، شمارش میکروارگانیسم‌های هوازی (16/6 درصد) و تعداد کپک و مخمر (18/8 درصد) شد بهبود در ویژگی‌های فیزیکوشیمیایی (به‌ویژه ویسکوزیته و سفتی) و فیتوشیمیایی (مثل اسید آسکوربیک و فنول) و همچنین پارامتر رنگ با افزایش زمان تیمار DBDP تا 6 دقیقه مشاهده شد. بنابراین این مطالعه تأثیر زمان DBDP بر فعالیت‌های آنزیمی و خصوصیات کیفی پالپ انبه را فراهم کرده است. نتایج نشان می دهد که می‌توان از این فناوری به‌عنوان یک فناوری نوین جایگزین غیرحرارتی برای پاستوریزاسیون پالپ انبه به‌جای عملیات حرارتی استفاده کرد.

واژه‌های کلیدی: پروفایل کیفیت، پلاسمای تخلیهٔ سد دی‌الکتریک، فعالیت آنزیمی، ویژگی‌های فیتوشیمیایی، ویژگی‌های فیزیکوشیمیایی