International Food Research Journal 29(5): 1005 - 1019 (October 2022)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effects of aqueous ozone treatment on the nutritional attributes of mango (*Mangifera indica* L.) fruit juice

[1]Supian, N. A. M., [1,2]*Shah, N. N. A. K., [1]Shamsudin, R. and [1]Sulaiman, A.

[1]Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Halal Products Research Institute, Jalan Putra Infoport, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Article history

Received:
24 February 2021
Received in revised form:
28 September 2021
Accepted:
27 January 2022

Keywords

aqueous ozone, fruit juice, physicochemical, antioxidant properties

Abstract

The global fruit juice market is expanding alongside the exponentially growing demand for a healthy lifestyle. Fruit juice is a preferred drink among all age groups as it contains numerous essential nutrients that benefit human health. The safety aspects of fruit juice are equally important as its healthy features. The conventional method of thermal pasteurisation has been known to produce fruit juice of inferior quality. Hence, ozone is being considered as an alternative, non-thermal form of pasteurisation. With its strong oxidation potential, ozone exhibits antimicrobial characteristics and produces no toxic by-products. However, for ozone to be successfully adopted by juice producers, the synergistic effects of the composition of fruit juice and ozone treatment must be adequately evaluated. Therefore, the present work subjected various concentrations of Chokanan mango juice (MJ), diluted with distilled water (DW) at 100MJ:0DW, 75MJ:25DW, and 50MJ:50DW to aqueous ozone treatment at different ozone doses. The effects of these treatments on the physicochemical and antioxidant properties of the MJ were evaluated. Ozone was found to be effective in decreasing the pectin methylesterase (PME) activity arising from the de-esterification of the pectin molecules, and increasing the DPPH activity, thereby increasing the juice quality. Significant effects on the total colour difference (ΔE) and total phenolic content (TPC) were observed in proportion to the increases in ozone dose. The colour of the treated MJ was found to be positively correlated with the TPC, while a kinetic study was performed to investigate the proportionality of the colour and TPC degradation. The first-order reaction model fitted well with the degradation patterns of L^* and b^*, as well as the ΔE of the MJ samples. A significant difference was observed between the degradation rate constant (k-value) for the MJ samples, which suggested that the k-value could have been affected by not only the ozone dose, but also the juice matrix. The present work demonstrated that the composition of fruit juice was an essential intrinsic parameter that must be assessed before adopting ozone as a form of non-thermal pasteurisation to produce fruit juice which is stable in quality, and safe for consumption.

DOI
https://doi.org/10.47836/ifrj.29.5.04

Introduction

In propelling the economy of Malaysia to high-income status, 12 National Key Economic Areas (NKEAs) (PEMANDU, 2010) have been promulgated, in line with the government's effort to raise the gross national income (GNI) through the agricultural sector. These included the National Agrofood Policy (DAN) 2011-2020, which was specifically directed towards the agrofood industry (FAMA, 2011). Among its many approaches is the empowerment of the fruit juice industry. Therefore, farmers, producers, and manufacturers are strongly urged to vary its usage of mango fruit, and process it into valuable food products. Mango is recognised as a source of vitamins, minerals, and bioactive compounds including carotenoids (Ribeiro and Schieber, 2010; López-Cobo *et al*., 2017), which has led to an upsurge in mango cultivation in Malaysia. Mango production and hectarage have been reported as 15,307 mt and 6,373 ha, respectively across the country (DOA, 2019). In 2018, the total export value

*Corresponding author.
Email: nadiahkarim@upm.edu.my

of the main fruits was RM1.3 million, while the export volume of Chokanan mango (*Mangifera indic*a L. var. Chokanan) was 191,500 kg (DOA, 2019). Chokanan mango has tremendous commercial value, and is in high demand due to its distinctive taste, pleasant aroma, and highly palatable colour. Abdullah (2018) reported that Chokanan mango is frequently selected to be processed into juice due to its sweet taste and availability throughout the year.

Fruit juices are easily contaminated by pathogenic and spoilage microorganisms which shorten its shelf-life stability. The contamination of fruit juices arises from improper handling during the juice processing stage and poor storage conditions. The conventional heating method is widely used in the fruit juice industry to pasteurise and preserve fruit juices. Although thermal treatment has been proven to achieve the minimum 5-log reduction of relevant pathogens, the treatment may degrade the beneficial phenolic contents, including the bioaccessibility of carotenoids (Aschoff *et al*., 2015).

Over the last decade, academia and industry have explored new alternative technologies to destroy harmful pathogens while simultaneously improving or maintaining the quality and shelf-life of fruit juices, including ozone technology (Fu *et al*., 2020). The Food and Drug Administration (FDA) classified ozone as Generally Recognized as Safe (GRAS) (FDA, 2001), which means that ozone is safe for use in the food and beverage industry as an alternative processing method. Ozone does not involve a heating process; instead, it has a high oxidation potential (+2.07 eV) to effectively inactivate relevant pathogens. Various research has shown the ability of ozone to act as an antimicrobial agent as it has reduced a minimum 5-log reduction against Gram-negative and Gram-positive bacteria. This has been demonstrated in the treatment of cantaloupe juice (Fundo *et al*., 2018; Sroy *et al*., 2019), sugarcane juice (Garud *et al*., 2018), and peach juice (Garcia Loredo *et al.*, 2015). Therefore, understanding the reaction of ozone in fruit juices is imperative to ensure not only its efficacy to disinfect the fruit juices, but also its effects on the treated juices. Ozone behaviour in juices not only depends on the extrinsic parameters (processing time and ozone concentration), but also on the juices' composition (Cullen *et al*., 2010). Prabha *et al.* (2015) reported that the reaction of ozone molecules could be disturbed by organic or inorganic matter in the juices, and that these matters may create a protection against ozone molecule reactions. Therefore, it is necessary to understand the outcome of ozone to effectively gauge its applicability in the fruit juice industry.

Mango juice may be sold directly in its original concentration (100% fruit juice), reconstituted with water, or mixed with other fruit juices. Even though reconstituted mango juices may not offer nutritional quality as high as that of 100% juice, they can fill gaps in the juice market, and fulfil the demand for ready-to-drink products (Kuo *et al*., 2008). To date, however, the effects of ozone on the quality of reconstituted mango juice have not been widely reported. Therefore, the present work aimed to determine the influence of reconstituted juice matrix on the effects of aqueous ozone in terms of the resulting juice quality (physicochemical and antioxidant properties). Additionally, the present work also investigated the kinetic parameters of colour changes in ozone-treated reconstituted mango juices.

Materials and methods

Preparation of mango juices

Chokanan mango fruits were purchased from a local market in Serdang, Selangor, and stored in a chiller at 10 ± 1°C prior to sample preparation. The mango fruits were then washed and peeled, and the seeds removed. The fruits were then cut, and the juice extracted using a slow cold-pressed juicer (SJ-33, Russell Taylors, Malaysia). Mango juice samples were then filled into high-density polyethylene bottles, and stored in a freezer at -20°C for subsequent analyses. Prior to ozone treatment, the samples were thawed at room temperature for 2 h, and mixed with distilled water based on the set ratios (%, v/v) of mango juice (MJ) to distilled water (DW) of 100MJ:0DW, 75MJ:25DW, and 50MJ:50DW.

Ozone treatment

Ozone gas was generated using an ozone generator (Model GL-3189, China), producing an ozone output of 400 mg/h (6.667 mg/min). Ozone dosage rates at exposure times of 10, 20, 30, and 40 min were calculated based on the juice flow rate (GPM) and ozone generator output (mg/h) (Oxidation Technologies, 2017). The measured ozone doses were calculated as 0.33, 0.67, 1.00, and 1.33 mg/mL. The MJ samples (200 mL) were subjected to different ozone doses. Gaseous ozone was purged directly into beakers of MJ samples (200 mL) via a delivery tube.

The juice samples were stirred at 3,000 rpm using a magnetic stirrer to ensure homogenous dispersal of ozone.

Total soluble solids (TSS) and pH

The total soluble solids (TSS) (AOAC, 1996) and pH were measured using a digital refractometer (D Series, Graigar Technology, China) and pH meter (PB-10, Sartorius, Germany), respectively, without further dilution. The pH meter was calibrated with a buffer solution of pH 4.00 and 7.00, while the TSS instrument was calibrated using distilled water before the test. All measurements were conducted in triplicate.

Turbidity

The turbidity was measured using a turbidimeter (Model 2100P, Hach Company, Colorado, USA) following a method described by Shah (2015). The turbidimeter was calibrated using standard solution ranges of 0.02, 20, 100, and 800 NTU. MJ samples (2.5 mL) were diluted with 7.5 mL of distilled water, and placed in a vial for reading. Results were expressed in nephelometric turbidity units (NTU), and measured in triplicate. The actual turbidity value of the original sample was calculated using Eq. 1:

$$\text{Turbidity (NTU)} = \frac{S\,(B + C)}{C} \qquad \text{(Eq. 1)}$$

where, S = turbidity of the diluted sample (NTU); B = volume of sample used for dilution (mL); and C = volume of dilution water (mL).

Pectin methylesterase (PME) activity

The pectin methylesterase (PME) activity of the MJ samples was measured following a method described by Agcam *et al.* (2014) with slight modifications. Briefly, 10 mL of each MJ sample was mixed with 40 mL of 1% pectin-salt substrate (0.1 M NaCl), and incubated at 30°C. The solution was adjusted to pH 7.5 with 0.05 sodium hydroxide (NaOH). The amount of NaOH required to maintain the pH was recorded during the reaction time of 30 min. All measurements were conducted in triplicate. The PME activity was then calculated using Eq. 2:

$$\text{PME activity}\left(\frac{\text{PMEu}}{\text{mL}}\right) = \frac{(\text{mL NaOH})\,(\text{normality of NaOH})}{(\text{mL juice})\,(\text{reaction time})} \times 10^3 \qquad \text{(Eq. 2)}$$

Total phenolic content (TPC)

Ten millilitres of MJ were mixed with 80% (v/v) methanol before sonication for 15 min, and centrifuged at 9,000 rpm at 4°C for 10 min. The TPC was determined using the Folin-Ciocalteu assay as described by Guan *et al.* (2016) with slight modifications in volume. Next, 0.5 mL phenol extract and 0.5 mL of distilled water was added with 2 mL of Folin-Ciocalteau reagent solution. After 3 min, 10 mL of 7.5% (w/v) sodium carbonate (Na_2CO_3) was added. The mixture was shaken and allowed to rest for 30 min before the absorbance of mixtures was measured at 765 nm using a UV visible spectrophotometer (Ultraspec 3100 Pro, Amersham Pharmacia Biotech, UK). All measurements were conducted in triplicate. A standard curve was prepared with gallic acid solution at concentration range of 0 to 500 mg/L with $R^2 = 0.99$. The final result for the TPC was determined from the standard curve.

DPPH radical scavenging activity

The DPPH radical scavenging assay was performed following a method modified from that of Guan *et al.* (2016). Briefly, 24 mg of DPPH was dissolved in 80% (v/v) of methanol, and protected from the light by covering the beaker with aluminium foil. Next, 50 µL of MJ samples were mixed with 950 µL of DPPH-methanol solution. The mixture was shaken vigorously, and incubated in the dark at room temperature for 1 h. The absorbance of the samples was measured at 517 nm with 80% (v/v) methanol as a blank sample, using the UV spectrophotometer (Ultra Spec 3100 Pro, Amersham Pharmacia Biotech, UK). All measurements were conducted in triplicate. The percentage of the DPPH radical scavenging activity was calculated using Eq. 3:

$$\text{DPPH (\%)} = \frac{A_{\text{control}} - B_{\text{sample}}}{A_{\text{control}}} \times 100 \qquad \text{(Eq. 3)}$$

Colour

The colours of the MJ samples were measured using a Hunter Lab Ultra Scan Pro colour spectrophotometer (D65, 28 Hunter Lab Assoc. Lab Inc., Reston, VA, USA). The triplicate colour value was expressed in CIE Lab parameters, where L^* represent lightness/darkness, a^* represent redness/greenness, and b^* represent yellowness/blueness. The total colour difference (ΔE), chroma, and hue angle were also calculated, having been adapted and modified from the design of Aguilar *et al.* (2018) as follows:

The total colour difference (ΔE):

$$\Delta E = \sqrt{(L^* - L^*_o)^2 + (a^* - a^*_o)^2 + (b^* - b^*_o)^2} \quad \text{(Eq. 4)}$$

Chroma (C^*):

$$C^* = \sqrt{(a^{*2} + b^{*2})} \quad \text{(Eq. 5)}$$

Hue (h^*):

$$h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad \text{(Eq. 6)}$$

where, L^*_o, a^*_o, and b^*_o were the initial colorimetric values of MJ samples.

Kinetics modelling of colour

Colour mathematical models of the MJ samples were described by zero- and first-order kinetics (Wibowo *et al*., 2015) as follows:

$$[CIE\ lab] = [CIE\ lab]_o \exp(-k_o t) \quad \text{(Eq. 7)}$$

$$[CIE\ lab] = [CIE\ lab]_o \exp(-k_1 t) \quad \text{(Eq. 8)}$$

where, $CIE\ lab_o$ = initial *CIE Lab* parameters (L^*_o, a^*_o, b^*_o, and ΔE) at a time, t (min) respectively; k_o, k_1 = zero and first-order rate constants, respectively.

The goodness of fit of the model to the experimental data was assessed with the coefficient determination (R^2), the mean square error (MSE), the root mean square error (RSME), and the sum square error (SSE).

Statistical analysis

All analyses were conducted in triplicate, and the data were subjected to statistical analysis using Minitab software (Version 17, Minitab Inc., PA, USA). The experimental data for the physicochemical and antioxidant properties of the MJ were analysed using the General Linear Model (GLM). The correlation between the independent and dependent variables was performed using Pearson's correlation and full factorial design. Data were expressed as mean ± standard deviation (SD).

Results and discussion

Preliminary study of non-ozonated mango juice (MJ)

Table 1 shows the initial physicochemical and antioxidant properties of the various mango juice concentrations (reconstituted with distilled water) without ozone treatment. The total soluble solids (TSS) values in different MJ samples were observed to be substantially different ($p < 0.05$). The sugar components like the free amino acids, as well as the organic, protein, and vitamin contents in 100MJ:0DW were significantly higher ($p < 0.05$) than in the other MJ samples. The pH value of 100MJ:0DW (4.11 ± 0.01) showed a lower value as compared to 75MJ:25DW (4.23 ± 0.01) and 50MJ:50DW (4.31 ± 0.01). The 100% MJ was shown to have high hydrogen ion (H^+) concentration, and would readily give up its H^+ ions when added to distilled water (LibreTexts, 2020). In addition, the distilled water donated hydroxide ions (OH^-), and they combined with H^+, which had been donated by the MJ, thus raising the pH of the MJ. Although the pH values of the MJ samples showed significant changes, the pH phase did not change drastically from acidic to alkali. Fruit juices with pH below 4.0 are suitable for juice production as the enzyme activity is reduced (Ünal and Şener, 2013). However, pectin methylesterase (PME) and high pectin compounds may be synthesised and suspended in the juice during

Table 1. Physicochemical and antioxidant properties of non-ozonated mango juice (MJ).

Juice ratio (%)	100MJ:0DW	75MJ:25DW	50MJ:50DW
pH	4.11 ± 0.01^c	4.23 ± 0.01^b	4.31 ± 0.01^a
Total soluble solid (°Brix)	14.35 ± 0.06^a	11.10 ± 0.00^b	7.53 ± 0.06^c
Turbidity (NTU)	3908 ± 4.00^a	3281 ± 2.00^b	2895 ± 6.00^c
PME activity (× 10^{-3}) (PME u/mL)	3.1 ± 0.08^a	2.7 ± 0.10^b	2.1 ± 0.08^c
Colour (b^* value)	45.28 ± 0.01^a	41.19 ± 0.01^b	34.65 ± 0.02^c
DPPH (Free radical scavenging, %)	87.97 ± 0.42^a	85.20 ± 0.12^b	58.88 ± 0.62^c
Total phenolic content (mg GAE/L)	313 ± 0.15^a	275 ± 0.40^b	225 ± 0.70^c
Ascorbic acid (mg/100 mL)	16.35 ± 0.006^a	8.43 ± 0.006^b	2.85 ± 0.006^c

Values are mean ± standard deviation of triplicate (n = 3). Means that do not share a lowercase superscript in a row are significantly different ($p < 0.05$) among treatments.

extraction (Aghajanzadeh and Ziaiifar, 2018). If the PME enzyme is not inactivated or inhibited immediately after the juice is extracted, the pectin contributing to the turbidity will be de-esterified to form calcium pectate, causing the juice to separate into a clear supernatant and sediment. Therefore, once the MJ was diluted with distilled water, the increased pH caused the pectin methylesterase (PME) and polygalacturonase (PG) enzymes to become unstable. PME activity is at its optimum at a neutral pH (7.0 to 8.0) (Ünal and Şener, 2013), while the pH of all the MJ samples in the present work was in the mid-acidic range (4.0 to 4.3), which might have slowed down the PME activities.

Results also showed a positive correlation between the juice ratio and turbidity (R^2 = 0.991, p < 0.05), thus indicating that the turbidity of the non-ozonated MJ was proportional to the percentage of MJ concentrations. 100% of non-ozonated MJ contained a greater amount of mango pulp and suspended pectin, and also, the endocarp cells ruptured during juice extraction, thus contributing to the high turbidity of juice. Teleszko *et al.* (2016) stated that the factor that causes fruit juice to become cloudy is the presence of natural colloidal compounds such as the pectin, proteins, and free amino acids found in apple juice, which subsequently influences consumers acceptance. Furthermore, the turbidity of the non-ozonated MJ was highly correlated with the PME activity (R^2 = 0.955, p < 0.05). The turbidity of 50MJ:50DW was low as compared to the other samples, thus suggesting that (i) the natural colloidal compounds were initially low, and (ii) the particle size of the existing compounds was insufficient to aggregate; consequently, the juice became less turbid.

Based on the results of the various physicochemical and antioxidant parameters of non-ozonated MJ, it was observed that various MJ concentrations produced distinctive qualities. Generally, ideal juice conditions were of low pulp (Patil *et al*., 2009); total soluble solids/Brix of fruit juice of not less than 8 g in 100 mL; and a 25% (v/v) of minimum juice/puree content (FAO, 2005) to meet the regulatory requirements for fruit juice exports. At the same time, a high level of organic matter would affect the ozone dissolution rate (Patil *et al*., 2009) in juice, while the attraction of ozone to the double bond of the organic and inorganic compounds in juice may lead to the formation of unstable ozonides, which subsequently disintegrate (Cullen *et al*., 2010) and affect the juice quality. Hence, mango juice samples with different Brix content are required in order to evaluate how the effects of ozone treatment change the juice quality.

Physicochemical properties of ozonated mango juice (MJ)

Table 2 presents the physicochemical changes in the ozone-treated MJ samples. Ozone levels significantly affected the pH value of the MJ (p < 0.05) samples, with an increasing trend observed. This finding contradicts those of Fundo *et al.* (2018) which showed that ozone concentration and time (7.0 mg/mL for 30 and 60 min) decreased (p < 0.05) the pH values of *Cantaloupe* juice from 6.37 (untreated) to 6.17 (ozone-treated at 30 min) and 5.62 (ozone-treated at 60 min). The increases in pH of the ozone-treated MJ samples might have been due to the H^+ ions released (Shah, 2015) from the mango pulp organic matter by the ozone. The mango juice with a high concentration of hydrogen ions readily donates H^+ ions which combine with the hydroxide ions (OH^-) from the distilled water (LibreTexts, 2020) and increase the juice pH. In addition, the production of hydroxyl radicals (OH^-) from the ozone decomposition in the MJ samples (Brodowska *et al.*, 2018) might have also increased the juice pH from its original value. This is because this radical ion will bind with any free H^+ ion to form a water molecule (LibreTexts, 2020).

Total soluble solids (TSS) is a significant parameter to determine the sugar content that contributes to the organoleptic properties of MJ. The interactions between the TSS of the MJ and the ozone dose were found to be significant (p < 0.05), which indicated that the aqueous ozone treatments affected the TSS of the MJ samples. As compared to untreated MJ, the TSS values of the ozone-treated samples at an ozone dose of 1.33 mg/mL and an exposure time of 40 min showed a decreasing trend, decreasing by between 4 and 13%. The results are in agreement with the findings of Jaramillo-Sánchez *et al.* (2018) who reported that a slightly significant decrease (by 8.7%) in total soluble solids (TSS) was achieved at the highest ozone dose (2.48 mg/mL) for 12 min using ozone-treated peach juice. The change in TSS is generally caused by saccharification (Singh and Sharma, 2017), that is, the hydrolysis of

Table 2. Physicochemical properties of ozonated mango juice (MJ).

Analysis	Ozone dose (mg/mL)	Juice ratio (%)					
		100MJ:0DW	Percentage change	75MJ:25DW	Percentage change	50MJ:50DW	Percentage change
pH	Untreated	4.11 ± 0.01^{Cd}	-	4.23 ± 0.01^{Bd}	-	4.31 ± 0.01^{Aa}	-
	0.33	4.32 ± 0.02^{Cc}	↑5%	4.39 ± 0.01^{Bb}	↑4%	4.47 ± 0.02^{Abc}	↑4%
	0.67	4.44 ± 0.01^{Bb}	↑8%	4.48 ± 0.02^{ABc}	↑6%	4.58 ± 0.08^{Ab}	↑6%
	1.00	4.47 ± 0.00^{Ab}	↑9%	4.51 ± 0.01^{Ab}	↑7%	4.60 ± 0.10^{Ab}	↑7%
	1.33	4.65 ± 0.04^{Ba}	↑13%	4.76 ± 0.03^{Aba}	↑13%	4.87 ± 0.11^{Aa}	↑13%
TSS (°Brix)	Untreated	14.53 ± 0.06^{Aa}	-	11.10 ± 0.00^{Ba}	-	7.53 ± 0.06^{Ca}	-
	0.33	14.47 ± 0.15^{Aa}	↓0.4%	$10..90 \pm 0.10^{Bab}$	↓2%	7.40 ± 0.00^{Ca}	↓2%
	0.67	14.20 ± 0.00^{Ab}	↓2%	10.53 ± 0.06^{Bc}	↓5%	6.90 ± 0.17^{Cb}	↓8%
	1.00	14.30 ± 0.10^{Aab}	↓2%	10.77 ± 0.06^{Bb}	↓3%	7.10 ± 0.10^{Cb}	↓6%
	1.33	13.90 ± 0.10^{Ac}	↓4%	10.13 ± 0.12^{Bd}	↓9%	7.00 ± 0.10^{Cb}	↓13%
Turbidity (NTU)	Untreated	3908 ± 4.00^{Ab}	-	3281 ± 2.00^{Bb}	-	2895 ± 6.00^{Cb}	-
	0.33	3888 ± 4.00^{Ac}	↓0.5%	3268 ± 4.00^{Bb}	↓0.4%	2705 ± 2.31^{Cc}	↓7%
	0.67	3947 ± 6.11^{Aa}	↑1%	3431 ± 2.31^{Ba}	↑5%	3183 ± 29.5^{Ca}	↑10%
	1.00	3855 ± 6.11^{Ad}	↓1%	3229 ± 15.14^{Bc}	↓2%	2615 ± 27.2^{Cd}	↓10%
	1.33	3747 ± 6.11^{Ae}	↓4%	2928 ± 4.00^{Bd}	↓11%	2249 ± 15.1^{Ce}	↓22%
PME activity ($\times 10^{-3}$) (PME u/mL)	Untreated	3.1 ± 0.08^{Aab}	-	2.7 ± 0.10^{Bb}	-	2.1 ± 0.08^{Cb}	-
	0.33	3.5 ± 0.09^{Aa}	↑13%	2.9 ± 0.10^{Ba}	↑7.4%	2.3 ± 0.04^{Ca}	↑10%
	0.67	3.2 ± 0.37^{Aab}	↑3%	2.0 ± 0.05^{Bc}	↓26%	1.7 ± 0.06^{Bc}	↓19%
	1.00	3.0 ± 0.18^{Aab}	↓3%	1.7 ± 0.03^{Bd}	↓37%	1.4 ± 0.04^{Cd}	↓33%
	1.33	2.7 ± 0.10^{Ab}	↓13%	1.2 ± 0.10^{Be}	↓56%	1.2 ± 0.09^{Be}	↓43%

Values are mean ± standard deviation of triplicate (n = 3). Means that do not share an uppercase superscript in a row are significantly different ($p < 0.05$) among different MJ samples. Means that do not share a lowercase superscripts in a column are significantly different ($p < 0.05$) among different ozone doses. ↑ : increased in percentage changes, while ↓ : decreased in percentage changes. % changes $= \frac{A_2 - A_1}{A_1} \times 100$, where, A_1: untreated value, and A_2: treated value at different ozone doses. TSS: total soluble solid.

polysaccharides into soluble sugar compounds. Increasing the ozone dose might have reduced the breakdown process of long carbohydrates compounds into dissolved sugar compounds (Makroo *et al*., 2017), thus resulting in decreases in the sugar content of the MJ samples.

The effects of the ozone doses were found to be inversely proportional to the pectin methylesterase (PME) activity (R^2 = -0.530, $p < 0.05$). The decrease in the PME activity of ozone-treated MJ samples indicated the slow reaction of the PME enzyme combined with polygalacturonase (PG) and pectin lyase (PL) to de-esterify pectin (Kohli *et al*., 2015). The decreased PME activity may potentially produce ozone-pasteurised juice products that meet the dual goals of industrial needs and consumer preferences. Similar results were observed in a study by Rodoni *et al.* (2009) in which ozone treatment (10 µL/L for 10 min) was found to decrease the PME activity in tomatoes. Therefore, it was suggested that the decrease in PME activities in both studies might have been due to decreased solubilisation and modulated pectin polysaccharide depolymerisation. The results were also supported by the positive correlation coefficient (R^2 = 0.820, $p < 0.05$) that was obtained between the turbidity and the PME activities of the ozone-treated MJ samples, which showed that turbidity decreased as the PME activity decreased. However, it was also apparent that the low percentages of the changes in the turbidity of ozone-treated 100MJ:0DW (1 to 4%) in comparison to 50MJ:50DW (7 to 22%) indicated low PME inactivation and the reaction of PME with the ozone treatment in the naturally high TSS (°Brix = 14.53) and naturally low pH (4.11) value of 100MJ:0DW. The naturally high cloud particles present in 100MJ:0DW (°Brix = 14.53) might have been surrounded with a protective coat of negatively

charged pectin, thus resulting in an overall negative surface charge (Croak and Corredig, 2006). Therefore, the interaction between the cloud particles and the high surface charge in natural juice (pH 4.11) might have created electrostatic charge repulsion (Croak and Corredig, 2006), thus resulting in greater juice cloud stability in 100MJ:0DW as compared to the other MJ samples. In addition, PME activity is maximal when the juice pH is neutral at 7.5 (Ünal and Şener, 2013). Since the natural pH of 100MJ:0DW was very low (pH 4.11) as compared to the other MJ samples, the PME activity percentage changes were also low. Therefore, it can also be concluded that the PME can be inactivated when the ozone dose was sufficient to regulate the pectin chain from polymerising, and cause detrimental changes to the MJ. Even so, the results in Table 2 illustrate that sudden increases in PME activity occurred in 100MJ:0DW (+13%), 75MJ:25DW (+7.4%), and 50MJ:50DW (+10%) at the ozone dose of 0.33 mg/mL (20 min). These increases in PME activities might be ozone-induced oxidative stress (Shah *et al*., 2018), which might have triggered the PME reactions with pectin as the oxidation from the ozone dose increased in the sample.

Antioxidant properties of ozonated mango juice (MJ)

Table 3 shows that the exposure of the MJ to ozone doses up to 1.33 mg/mL (at 40 min) increased the antioxidant activity as compared to the untreated MJ. For the 75MJ:25DW and 50MJ:50DW samples, the DPPH activity increased from 7 to 14% and 60 to 67%, respectively, from their initial values (untreated). The increase in DPPH activity might have occurred due to the synthesis of antioxidant enzyme activities such as superoxide dismutase and phenylalanine ammonia-lyase (PAL), which work as scavengers of reactive oxygen species (ROS) derived from ozone exposure (Xu *et al*., 2014; Liu *et al*., 2020). The higher levels of these enzyme activities increased the scavenging activity of the superoxide anion free radicals and the hydrogen peroxide in the ozone-treated MJ. This complements previous findings by Alothman *et al*. (2010) who stated that ozone increased the antioxidant activity of fresh-cut papaya, banana, and pineapple. In their review, Sachadyn-Król and Agriopoulou (2020) also indicated that ozonation could be used as an abiotic elicitor of plant defence mechanisms in less-processed food products of plant origin. This can enhance the secondary metabolite content and antioxidant activity. However, at the ozone doses of 1.00 and 1.33 mg/mL, the DPPH scavenging activity in the ozone-treated 100MJ:0DW sample started to decrease, as compared to the value identified with the control sample. A possible reason is that the further accumulation of ROS in MJ samples due to the increased ozone dose which initiated the stress response. Therefore, this hyperactive ozone (ozone stress) might have started reducing the ability of PAL to activate the protective response (Ong *et al*., 2014). These results were also observed in the 100MJ:0DW sample, where the overall antioxidant activities were found to decrease in parallel with the increasing ozone doses.

The total phenolic content (TPC) of the non-ozonated MJ for 100MJ:0DW, 75MJ:25DW, and 50MJ:50DW were 313 ± 0.15, 275 ± 0.40, and 225 ± 0.70 mg GAE/L, respectively. Upon exposure to various ozone doses, the TPC for all the MJ samples decreased significantly ($p < 0.05$) from its control value (non-ozonated MJ). This finding corroborates a previous study by Torlak (2014) in which the degradation of TPC in apple juice at an ozone concentration of 0.0028 mg/L for 40 min significantly ($p < 0.05$) decreased from 571 ± 62 to 358 ± 68 mg GAE/L. As previously mentioned, the activation of PAL will contribute to higher level of antioxidant activity related to scavenging the reactive oxygen species (ROS). Therefore, the high TPC value in the MJ samples could be expected based on the decrease in ROS by PAL. Despite that, the hydroxylated aromatic ring of the phenolic compound is highly susceptible to ozone molecules, and a direct reaction to a high ozone dose alone could be enough to cause the formation of an aliphatic compound of phenol (Aghdam *et al*., 2020; Almeida *et al*., 2015). Furthermore, the inhibition of antioxidant defence activity such as peroxidase (POD) (Liu *et al*., 2020) might contribute to the oxidation of phenolic compounds. Lower TPC percentage losses were observed in 100MJ:0DW (< 50%) than the other MJ samples (Table 3), thus indicating that the reaction was highly dependent on the juice composition. The colloidal content of 100MJ:0DW might have caused a natural protective defence against the oxidative effects of ozone, which is supported by the study of Sachadyn-Król and Agriopoulou (2020). They suggested that the plant cells may become the walls of guard cells to protect the plant cells from the

Table 3. Antioxidant properties of ozonated mango juice (MJ).

Analysis	Ozone dose (mg/mL)	Juice ratio (%)					
		100MJ:0DW	Percentage changes	75MJ:25DW	Percentage changes	50MJ:50DW	Percentage changes
DPPH (Free radical scavenging, %)	Untreated	87.97 ± 0.42^{Ac}	-	85.20 ± 0.12^{Bd}	-	58.88 ± 0.62^{Cd}	-
	0.33	94.90 ± 0.77^{Ba}	↑8%	97.47 ± 0.12^{Aa}	↑14%	98.52 ± 0.18^{Aa}	↑67%
	0.67	89.89 ± 0.67^{Cb}	↑2%	94.50 ± 0.73^{Bb}	↑11%	96.39 ± 0.55^{Ab}	↑64%
	1.00	87.00 ± 0.32^{Cc}	↓1%	91.01 ± 0.49^{Bc}	↑7%	94.95 ± 0.97^{Abc}	↑61%
	1.33	85.28 ± 0.07^{Cd}	↓3%	90.77 ± 0.43^{Bc}	↑7%	94.18 ± 0.30^{Ac}	↑60%
TPC (mg GAE/L)	Untreated	313 ± 0.15^{Aa}	-	275 ± 0.40^{Ba}	-	225 ± 0.70^{Ca}	-
	0.33	212 ± 0.70^{Ac}	↓32%	167 ± 0.15^{Bb}	↓39%	110 ± 0.40^{Cb}	↓51%
	0.67	163 ± 0.70^{Ae}	↓48%	117 ± 0.26^{Be}	↓57%	75 ± 0.55^{Ce}	↓67%
	1.00	181 ± 0.30^{Ad}	↓42%	134 ± 0.61^{Bd}	↓51%	78 ± 0.26^{Cd}	↓65%
	1.33	214 ± 0.26^{Ab}	↓32%	152 ± 0.15^{Bc}	↓45%	97 ± 0.95^{Cc}	↓67%
Ascorbic acid (mg/100 mL)	Untreated	16.35 ± 0.006^{Aa}		8.43 ± 0.006^{Bb}		2.85 ± 0.006^{Cb}	
	0.33	3.13 ± 0.006^{Bb}	↓81%	5.27 ± 0.006^{Aa}	↓37%	2.89 ± 0.006^{Ca}	↑1%
	0.67	2.61 ± 0.006^{Bc}	↓85%	4.93 ± 0.006^{Ac}	↓42%	2.47 ± 0.006^{Cc}	↓13%
	1.00	1.58 ± 0.006^{Bd}	↓93%	5.01 ± 0.006^{Ad}	↓41%	1.17 ± 0.006^{Cd}	↓59%
	1.33	1.15 ± 0.006^{Be}	↓93%	4.49 ± 0.006^{Ae}	↓47%	1.12 ± 0.006^{Ce}	↓61%
Total colour difference (ΔE)	Untreated	-	-	-	-	-	-
	0.33	3.08 ± 0.04^{Aa}	-	2.61 ± 0.02^{Ba}	-	2.34 ± 0.03^{Ca}	-
	0.67	5.22 ± 0.04^{Ab}	-	3.87 ± 0.02^{Bb}	-	3.73 ± 0.04^{Cb}	-
	1.00	7.33 ± 0.05^{Ac}	-	5.95 ± 0.01^{Bc}	-	4.12 ± 0.01^{Cc}	-
	1.33	10.35 ± 0.03^{Ad}	-	9.92 ± 0.02^{Bd}	-	8.23 ± 0.01^{Cd}	-

Values are mean ± standard deviation of triplicate (n = 3). Means that do not share an uppercase superscript in a row are significantly different ($p < 0.05$) among different MJ samples. Means that do not share a lowercase superscripts in a column are significantly different ($p < 0.05$) among different ozone doses. ↑ : increased in percentage changes, while ↓ : decreased in percentage changes. % changes $= \frac{A_2 - A_1}{A_1} \times 100$, where, A_1: untreated value, and A_2: treated value at different ozone doses. TPC: total phenolic content.

reaction with ozone. The sensitivity of phenolic compounds and other antioxidants to ozone is most probably affected by the type of compound and its location in the cell (Karaca and Velioglu, 2014). Depending on the structure, phenolic compounds exhibit different tendencies to accumulate within the cell wall (Paissoni *et al*., 2017).

Table 3 shows that the ascorbic acid content in MJ samples also significantly decreased with the ozone dose increment ($p < 0.05$), in correlation with the TPC decreases in all the MJ samples. A potential reason for the ascorbic degradation is that the ozone affected the electrophilic and nucleophilic reactions of the ascorbic acid aromatic compounds (Cullen *et al*., 2009). A similar finding was reported by Fundo *et al.* (2018) who observed a decrease of almost 54% (7.0 mg/mL, 30 min) and 76% (7.0 mg/mL, 60 min) in the ascorbic acid content of ozone-treated *Cantaloupe* as compared to the control sample. The 100MJ:0DW sample demonstrated the highest percentage decrease in ascorbic acid (approximately above 80%) as compared to the other MJ samples, thus showing that the decrease was unacceptable (Polydera *et al*., 2003). Ozone is highly attracted to the double bond of organic compounds (ascorbic acid), and the direct reaction of ozone with ozonide formation can be expressed by the Criegee mechanism (Criegee, 1975). The electrophilic ozone becomes attached to the carbon-carbon double bond to form the molozonide intermediate (Patil and Bourke, 2012). This unstable primary ozonide cleaves to form carbonyl and carbonyl oxide. Then, the carbonyl compound further rearranges and reforms itself to structure the stable ozonide intermediate. However, the oxidative decomposition of ozonide due to the activation of the enzyme ascorbate oxidase (AO) initiates the formation of carbonyl compounds such as dehydroascorbic acid

(DHA) (Beltrán *et al*., 2005). The instability of DHA promoted further degradation, and decreased the total ascorbic acid content of the MJ samples.

Colour analysis on ozonated mango juice (MJ)

Colour is a key indicator of food quality and consumer preference. In the present work, the colour parameter (*b**) was described as the yellowness of the mango juice colour, which was correlated with carotenoid pigment content (α-carotene and β-carotene) present in the mango fruit. Based on the correlation study, the MJ colour (*b** value) showed a high correlation with TSS (R^2 = 0.912, $p < 0.05$), PME activity (R^2 = 0.825, $p < 0.05$) and TPC (R^2 = 0.823, $p < 0.05$). Therefore, it was suggested that the colour changes in treated mango juice (MJ) were sufficient to gauge the effects of aqueous ozone on its correlated characteristics in the various MJ samples.

The differences in perceivable colour, ΔE, can be analytically classified as not noticeable ($0 < \Delta E < 0.5$), slightly noticeable ($0.5 < \Delta E < 1.5$), noticeable ($1.5 < \Delta E < 3.0$), well visible ($3.0 < \Delta E < 6.0$), and greatly visible ($6.0 < \Delta E < 12$) (Santhirasegaram *et al*., 2015). Table 3 shows that the ozone-treated MJ samples demonstrated an increase in ΔE values with the ozone dose increments. All the MJ samples, 100MJ:0DW, 75MJ:25DW, and 50MJ:50DW showed obvious colour changes with values of 10.35, 9.92, and 8.23, respectively, at the highest ozone dose of 1.33 mg/mL for 40 min. *L** (lightness/darkness) values significantly decreased (1 to 15% decrement), *a** (redness/greenness) values increased (5 to 50% increment), and *b** (yellowness/blueness) values decreased (6 to 14% decrement) in all the MJ samples. This pattern supported the theory that colour considerably changes due to ozone treatment. Jaramillo-Sánchez *et al.* (2018) also observed decreases in *L** (7%) and *b** (1%) values, and increases in *a** (7%) values in peach juice when it was treated with an ozone dose of 2.48 g/L for 12 min. Ozone has a strong oxidising potential that reacts directly or indirectly with the carotenoid pigment, thus resulting in oxidative cleavage. Jaramillo-Sánchez *et al.* (2018) also described the potential for ozone molecules to break down the conjugated double bond of tetraterpene pigments (carotenoid). The disintegration of the doubled bond of this pigment caused colour changes in the ozone-treated MJ samples. Furthermore, perceivable (noticeable) colour of the 75MJ:25DW (ΔE = 2.61) and 50MJ:50DW (ΔE = 2.34) MJ samples was observed at the lowest ozone dose (0.33 mg/mL, 10 min), while well visible colour was observed for the sample 100MJ:0DW (ΔE = 3.08). Initially, the 75MJ:25DW and 50MJ:50DW non-ozonated MJ samples had lower colour values of *b** = 41.19 ± 0.01, *L** = 58.07 ± 0.03 and *b** = 34.65 ± 0.02, *L** = 53.66 ± 0.01, respectively, in contrast to 100MJ:0DW, which yielded values of *b** = 45.28 ± 0.01, and *L** = 58.07 ± 0.03. However, corresponding to the ascorbic acid content, the ozonated MJ sample of 100MJ:0DW might have lost the components that influenced the yellowness value (*b**) and lightness (*L**) of the juice, as ozone has affinity towards doubled bond of the organic and inorganic compounds present in the juice.

In terms of the hue of the treated MJ, this showed a slightly decreased value ($p < 0.05$) than those of the untreated MJ samples (Figure 1). The ozone dose increases contributed to the decreases in the hue value, which moves away from the pure yellow colour corresponding to 90°. Chroma, which represents the colour intensity, produced lower values for the treated MJ samples. The hue shifts and drops in chroma value could probably be due to the pigment profile changes during ozonation (Almeida *et al*., 2015). Therefore, it was suggested that the ozone dose levels of 0.33 and 0.67 mg/mL were able to minimise the effects of the colour quality changes.

Colour kinetic of ozonated mango juice (MJ)

Kinetic models can be used to make a simple and fast assessment of food safety. The concept of colour kinetics is vital as a non-destructive method of determining the degradation of MJ when it is subjected to continuous exposure to aqueous ozone. The experimental MJ colour degradation was elucidated using zero- and first-order kinetics. The best-fitted model was chosen based on mathematical validation and analysis of the determination of coefficient (R^2), the sum of square error (SSE), the mean square error (MSE), and the root mean square error (RSME), as shown in Table 4. The colour degradation of *L**, *b**, and ΔE followed the first-order kinetics model for the MJ samples, as the mathematical validation produced low SSE, MSE, and RSME values (Table 4). A significant difference was identified between the degradation rate constants (first-order model) for 100MJ:0DW, 75MJ:25DW, and 50MJ:50DW (Table 4), which implied that the *k*-value was affected not only by the ozone dose but also

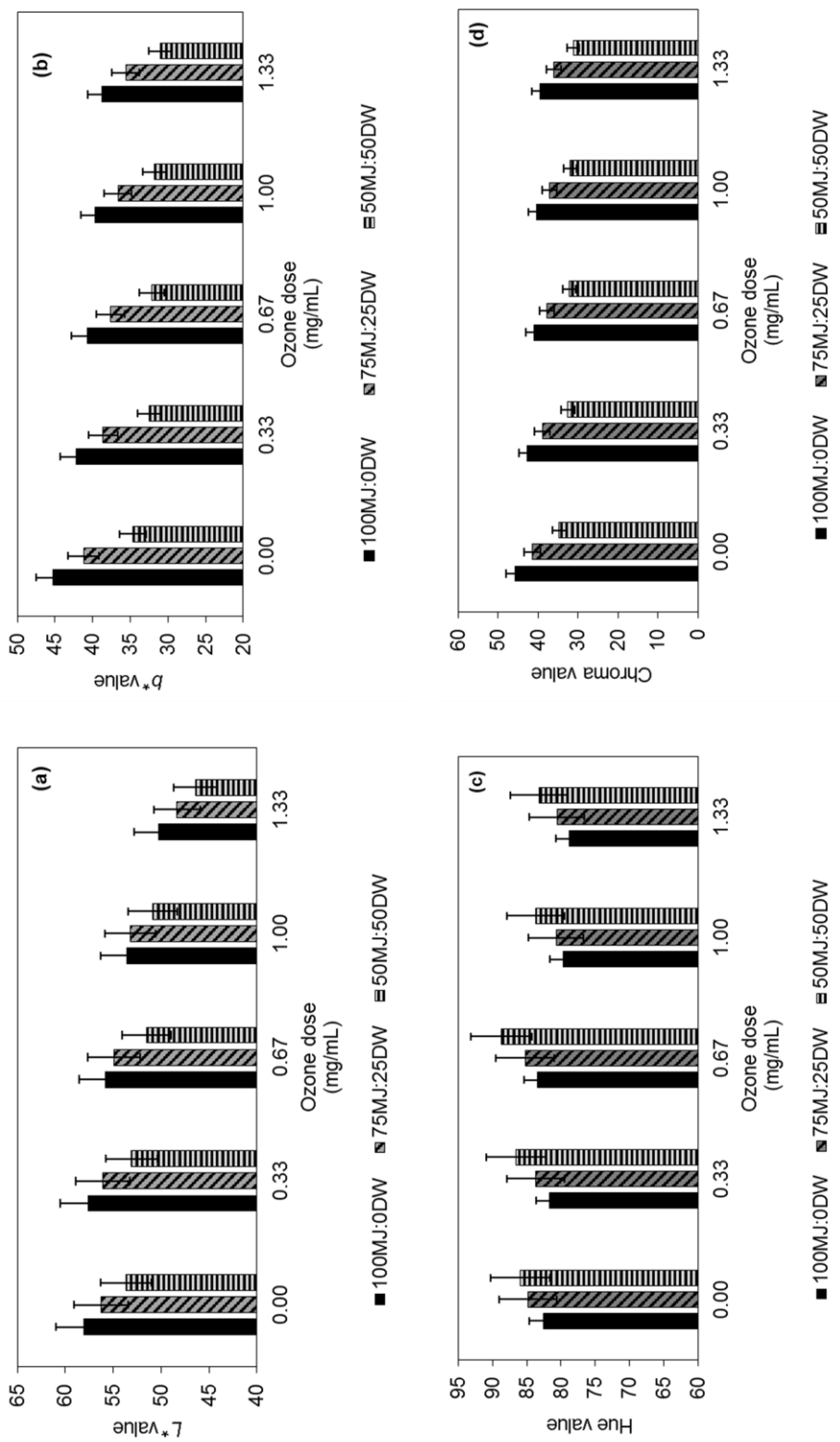

Figure 1. The colour *L** **(a)**, *b** **(b)**, hue **(c)**, and chroma value **(d)** of mango juice (MJ) samples.

Table 4. Kinetic parameters of colour properties for different mango juice (MJ) samples during ozone treatment.

Juice ratio (%)	Colour parameter	Kinetic model	*k*	SSE	MSE	RSME	R^2
100MJ:0DW	*L**	0th order	0.1967	11.0360	0.6130	0.7428	0.9334
		1st order	0.00362	0.0043	0.0002	0.0146	0.9248
	*b**	0th order	0.1573	6.9250	0.3847	0.5884	0.9346
		1st order	0.0038	0.0033	0.0002	0.0128	0.9456
	ΔE	0th order	0.2393	0.9680	0.0690	0.2460	0.9916
		1st order	0.0398	0.0418	0.0030	0.0511	0.9869
75MJ:25DW	*L**	0th order	0.1874	30.0810	1.6710	1.2264	0.8237
		1st order	0.00357	0.0119	0.0007	0.0244	0.8104
	*b**	0th order	0.1313	3.8547	0.2141	0.4390	0.9471
		1st order	0.0034	0.0022	0.0001	0.0104	0.9564
	ΔE	0th order	0.2402	7.5860	0.5420	0.6885	0.9383
		1st order	0.0444	0.0142	0.0010	0.0298	0.9964
50MJ:50DW	*L**	0th order	0.1671	19.648	1.092	0.9912	0.8505
		1st order	0.0033	0.0087	0.0005	0.0208	0.8368
	*b**	0th order	0.0800	4.4574	0.2476	0.4721	0.8518
		1st order	0.0024	0.0038	0.0002	0.0139	0.8617
	ΔE	0th order	0.1806	11.9210	0.8515	0.8632	0.8455
		1st order	0.0387	0.2386	0.0170	0.1221	0.9264

k: rate constant (min-1); *Δ*E: total colour difference; SSE: sum square error; MSE: mean square error; RSME: root square mean error; and R^2: coefficient determination.

other factors such as the juice condition. The *k*-values of *L** and *b** of the 100MJ:0DW and 75MJ:25DW samples were k_{L100MJ} = 0.00362 min^{-1}, k_{L75MJ} = 0.00357 min^{-1}, k_{L50MJ} = 0.0033 min^{-1} and k_{b100MJ} = 0.0038 min^{-1}, k_{b75MJ} = 0.0034 min^{-1}, k_{b50MJ} = 0.0024 min^{-1}, respectively. Wibowo *et al.* (2015) stated that different *k*-values indicated different reaction rates. It was observed from the results that the 100MJ:0DW sample had the highest k_L and k_b values, as compared to the other juice samples, as the ozone molecule reaction was faster towards the double bonds of the organic matter present in the juice, especially the carotenoid pigments, which were responsible for the yellow juice colour. Meanwhile, the $k_{\Delta E}$ value showed that 50MJ:50DW had the lowest rate constant value, thus indicating that the reaction of the total colour changes was low. Therefore, 50MJ:50DW could be considered the best ozone treatment as it produced higher colour retention than the other juice samples.

Conclusion

Based on the obtained data, the effects on the samples of 100MJ:0DW, 75MJ:25DW, and 50MJ:50DW treated with ozone doses at 0.67, 0.33, 1.00, and 1.33 mg/mL were found to depend highly on the processing conditions and the juice matrix. It was observed that aqueous ozone caused significant changes ($p < 0.05$) to the pH, total soluble solids (TSS), pectin methylesterase (PME) activity, antioxidant activity (DPPH), total phenolic content (TPC), ascorbic acid, and colour values of the MJ samples. The PME activity was shown to decrease at increased ozone dose, which was corroborated by the turbidity decrease in the MJ samples. The TPC decrease in the MJ samples could have been due to the increase ($p < 0.05$) in the antioxidant activity (DPPH). The direct reaction to the high ozone dose with the inhibition of peroxidase (POD) enzyme activity might have been sufficient to oxidise the compounds, further explaining the ascorbic acid decrease in all the MJ samples. Additionally, the colour parameters (*L**, *a**, and *b**) were significantly different ($p < 0.05$) after ozone treatment, while the total colour change (*ΔE*) values were much higher (perceived as greatly visible) for all the MJ samples at the highest ozone dose. Based on the SSE, MSE, and RSME values as an evaluation used to select the best models, the first-order reaction fitted well with the degradation kinetics of treated mango juice for *L**, *b**, and *ΔE* (R^2 > 90%). A significant difference ($p < 0.05$) was also identified between the degradation rate

constants for all the MJ samples, which implied that the *k*-value was affected by the ozone dose and juice matrix. The highest drop in ascorbic acid (above 80%) to an unacceptable level, which is an essential indicator of fruit juice quality, was observed in 100MJ:0DW. This is a high priority as it is a crucial decision to make before the implementation of ozone. Despite that, among all the MJ samples, 75MJ:25DW showed the lowest percentages of changes (below 60%) in terms of physicochemical characteristics (pH, TSS, and turbidity), PME activity, colour (*b** value), antioxidant properties (TPC and ascorbic acid), and antioxidant activity (DPPH). The implication of these findings is that it is important to acknowledge the influence of the fruit juice composition and the ozone processing parameters used (ozone dose or treatment time) on the effective pasteurisation of fruit juice while retaining the nutritional quality of the juice, and ensuring its safety for mass production and consumption.

Acknowledgement

The authors would like to acknowledge the financial support provided by Universiti Putra Malaysia through the GP-IPM grant scheme (grant no.: 9491300).

References

Abdullah, F. 2018. Variability in fruit characteristics of Chokanan mango (*Mangifera indica* L.). Journal of Tropical Agriculture and Food Science 46(1): 55-63.

Agcam, E., Akyıldız, A. and Evrendilek, G. A. 2014. Effects of PEF and heat pasteurization on PME activity in orange juice with regard to a new inactivation kinetic model. Food Chemistry 165: 70-76.

Aghajanzadeh, S. and Ziaiifar, A. M. 2018. A review of pectin methylesterase inactivation in citrus juice during pasteurization. Trends in Food Science and Technology 71: 1-12.

Aghdam, M. S., Luo, Z., Li, L., Jannatizadeh, A., Fard, J. R. and Pirzad, F. 2020. Melatonin treatment maintains nutraceutical properties of pomegranate fruits during cold storage. Food Chemistry 303: 125385.

Aguilar, D., Morales-Oyervides, L., Contreras-Esquivel, J. C., Méndez-Zavala, A., Raso, J. and Montañez, J. 2018. Effect of ozone processing conditions on stability of fungal pigments. Innovative Food Science and Emerging Technologies 45: 255-263.

Almeida, F. D. L., Cavalcante, R. S., Cullen, P. J., Frias, J. M., Bourke, P., Fernandes, F. A. N. and Rodrigues, S. 2015. Effects of atmospheric cold plasma and ozone on prebiotic orange juice. Innovative Food Science and Emerging Technologies 32: 127-135.

Alothman, M., Kaur, B., Fazilah, A., Bhat, R. and Karim, A. A. 2010. Ozone-induced changes of antioxidant capacity of fresh-cut tropical fruits. Innovative Food Science and Emerging Technologies 11(4): 666-671.

Aschoff, J. K., Kaufmann, S., Kalkan, O., Neidhart, S., Carle, R. and Schweiggert, R. M. 2015. *In vitro* bioaccessibility of carotenoids, flavonoids, and vitamin C from differently processed oranges and orange juices [*Citrus sinensis* (L.) Osbeck]. Journal of Agricultural and Food Chemistry 63(2): 578-587.

Association of Official Analytical Chemists (AOAC). 1996. Official methods of analysis of the AOAC International. 17th ed. United States: AOAC.

Beltrán, D., Selma, M. V., Marín, A. and Gil, M. I. 2005. Ozonated water extends the shelf life of fresh-cut lettuce. Journal of Agricultural and Food Chemistry 53(14): 5654-5663.

Brodowska, A. J., Nowak, A. and Śmigielski, K. 2018. Ozone in the food industry: Principles of ozone treatment, mechanisms of action, and applications: An overview. Critical Reviews in Food Science and Nutrition 58(13): 2176-2201.

Criegee, R. 1975. Mechanism of ozonolysis. Angewandte Chemie International 14(11): 745-752.

Croak, S. and Corredig, M. 2006. The role of pectin in orange juice stabilization: Effect of pectin methylesterase and pectinase activity on the size of cloud particles. Food Hydrocolloids 20(7): 961-965.

Cullen, P. J., Tiwari, B. K., O'Donnell, C. P. and Muthukumarappan, K. 2009. Modelling approaches to ozone processing of liquid foods. Trends in Food Science and Technology 20(3-4): 125-136.

Cullen, P., Valdramidis, V., Tiwari, B., Patil, S., Bourke, P. and O'Donnell, C. 2010. Ozone processing for food preservation: An overview

on fruit juice treatments. Ozone: Science and Engineering 32(3): 166-179.

Department of Agriculture Malaysia (DOA). 2019. Crop statistic (food crops sub-sector) 2019. Retrieved on March 18, 2020 from DOA website: http://www.doa.gov.my

Federal Agricultural Marketing Authority (FAMA). 2011. National agro-food policy (DAN) 2011-2020. Retrieved on March 18, 2020 from FAMA website: https://www.fama.gov.my/dasar-agromakanan-negara-2011-2020-dan-

Food and Agriculture Organization (FAO). 2005. CODEX STAN 247-2005 - Codex general standard for fruit juices and nectars. Retrieved on May 5, 2020 from FAO website: http://www.fao.org/fao-who-codexalimentarius/sh- proxy/en/?

Food and Drug Administration (FDA). 2001. Part 184 - Direct food substance affirmed as Generally Recognized as Safe: Subpart 184.1563 Ozone. Retrieved on September 8, 2018 from FDA website: https://www.accessdata.fda.gov

Fu, X., Belwal, T., Cravotto, G. and Luo, Z. 2020. Sono-physical and sono-chemical effects of ultrasound: Primary applications in extraction and freezing operations and influence on food components. Ultrasonics Sonochemistry 60: 104726.

Fundo, J. F., Miller, F. A., Tremarin, A., Garcia, E., Brandão, T. R. S. and Silva, C. L. M. 2018. Quality assessment of *Cantaloupe* melon juice under ozone processing. Innovative Food Science and Emerging Technologies 47: 461-466.

Garcia Loredo, A. B., Guerrero, S. N. and Alzamora, S. M. 2015. Inactivation kinetics and growth dynamics during cold storage of *Escherichia coli* ATCC 11229, *Listeria innocua* ATCC 33090 and *Saccharomyces cerevisiae* KE162 in peach juice using aqueous ozone. Innovative Food Science and Emerging Technologies 29: 271-279.

Garud, S. R., Priyanka, B. S., Rastogi, K. N., Prakash, M. and Negi, P. S. 2018. Efficacy of ozone and lactic acid as non-thermal hurdles for preservation of sugarcane. Ozone - Science and Engineering 40(3): 198-208.

Guan, Y., Zhou, L., Bi, J., Yi, J., Liu, X., Chen, Q., … and Zhou, M. 2016. Change of microbial and quality attributes of mango juice treated by high pressure homogenization combined with moderate inlet temperatures during storage. Innovative Food Science and Emerging Technologies 36: 320-329.

Jaramillo-Sánchez, G. M., Garcia Loredo, A. B., Gómez, P. L. and Alzamora, S. M. 2018. Ozone processing of peach juice: Impact on physicochemical parameters, color, and viscosity. Ozone - Science and Engineering 40(4): 305-312.

Karaca, H. and Velioglu, S. 2014. Effects of ozone treatments on microbial quality and some chemical properties of lettuce, spinach, and parsley. Postharvest Biology and Technology 88: 46-53.

Kohli, P., Kalia, M. and Gupta, R. 2015. Pectin methylesterases: A review. Journal of Bioprocessing and Biotechniques 5(5): 1-7.

Kuo, F.-J., Sheng, C.-T. and Ting, C.-H. 2008. Evaluation of ultrasonic propagation to measure sugar content and viscosity of reconstituted orange juice. Journal of Food Engineering 86(1): 84-90.

LibreTexts. 2020. 2.4 - acids and bases. Retrieved on September 8, 2021 from LibreTexts website: https://med.libretexts.org/Bookshelves/Anatomy_and_Physiology/Book%3A_Human_Anatomy_and_Physiology_Preparatory_Course_(Liachovitzky)/02%3A_Introduction_to_Anatomy_and_Physiology_Chemical_Building_Blocks/2.04%3A_Acids_and_Bases

Liu, J., Chang, M., Meng, J., Liu, J., Cheng, Y. F. and Feng, C. 2020. Effect of ozone treatment on the quality and enzyme activity of *Lentinus edodes* during cold storage. Journal of Food Processing and Preservation 44(8): 1-11.

López-Cobo, A., Verardo, V., Diaz-de-Cerio, E., Segura-Carretero, A., Fernández-Gutiérrez, A. and Gómez-Caravaca, A. M. 2017. Use of HPLC- and GC-QTOF to determine hydrophilic and lipophilic phenols in mango fruit (*Mangifera indica* L.) and its by-products. Food Research International 100: 423-434.

Makroo, H. A., Saxena, J., Rastogi, N. K. and Srivastava, B. 2017. Ohmic heating assisted polyphenol oxidase inactivation of watermelon juice: Effects of the treatment on pH, lycopene, total phenolic content, and color of the juice. Journal of Food Processing and Preservation 41(6): e13271.

Ong, M. K., Ali, A., Alderson, P. G. and Forney, C. F. 2014. Effect of different concentrations of ozone on physiological changes associated to gas exchange, fruit ripening, fruit surface quality and defence-related enzymes levels in papaya fruit during ambient storage. Scientia Horticulturae 179: 163-169.

Oxidation Technologies. 2017. Ozone dosage rate into water calculator. Retrieved on February 2, 2020 from Oxidation Technologies website: https://www.oxidationtech.com/ozone/ozone-calculations/o2-dosage-calculator.html

Paissoni, M. A., Segade, S. R., Giacosa, S., Torchio, F., Cravero, F., Englezos, V., … and Teissedre, P. L. 2017. Impact of post-harvest ozone treatments on the skin phenolic extractability of red wine grapes cv Barbera and Nebbiolo (*Vitis vinifera* L.). Food Research International 98: 68-78.

Patil, S. and Bourke, P. 2012. Ozone processing of fluid foods. In Cullen, P. J., Tiwari, B. K. and Valdramidis, V. P. (eds). Novel Thermal and Non-Thermal Technologies for Fluid Foods, p. 225-261. United States: Academic Press.

Patil, S., Bourke, P., Frias, J. M., Tiwari, B. K. and Cullen, P. J. 2009. Inactivation of *Escherichia coli* in orange juice using ozone. Innovative Food Science and Emerging Technologies 10(4): 551-557.

Performance Management and Delivery Unit (PEMANDU). 2010. Economic transformation programme - a road to Malaysia. Retrieved on February 2, 2020 from The Asia Pacific Energy website: https://policy.asiapacificenergy.org/sites/default/files/ETP.pdf

Polydera, A. C., Stoforos, N. G. and Taoukis, P. S. 2003. Comparative shelf life study and vitamin C loss kinetics in pasteurised and high pressure processed reconstituted orange juice. Journal of Food Engineering 60: 21-29.

Prabha, V., Barma, R. D., Singh, R. and Madan, A. 2015. Ozone technology in food processing: A review. Trends in Biosciences 16(8): 4031-4047.

Ribeiro, S. M. R. and Schieber, A. 2010. Bioactive compounds in mango (*Mangifera indica* L.). In Watson, R. R. and Preedy, V. R. (eds). Bioactive Foods in Promoting Health Fruits and Vegetables, p. 507-523. United States: Academic Press.

Rodoni, L., Casadei, N., Concellón, A., Chaves Alicia, A. R. and Vicente, A. R. 2009. Effect of short-term ozone treatments on tomato (*Solanum lycopersicum* L.) fruit quality and cell wall degradation. Journal of Agricultural and Food Chemistry 58(1): 594-599.

Sachadyn-Król, M. and Agriopoulou, S. 2020. Ozonation as a method of abiotic elicitation improving the health-promoting properties of plant products—A review. Molecules 25(10): 2416-2441.

Santhirasegaram, V., Razali, Z., George, D. S. and Somasundram, C. 2015. Comparison of UV-C treatment and thermal pasteurization on quality of Chokanan mango (*Mangifera indica* L.) juice. Food and Bioproducts Processing 94: 313-321.

Shah, N. N. A. K. 2015. Dean vortex ultraviolet light pasteurization of pummelo (*Citrus grandis* L. Osbeck) fruit juice. Malaysia: Universiti Putra Malaysia, PhD thesis.

Shah, N. N. A. K., Supian, N. A. M. and Hussein, N. A. 2018. Disinfectant of pummelo (*Citrus Grandis* L. Osbeck) fruit juice using gaseous ozone. Journal of Food Science and Technology 56(1): 262-272.

Singh, S. K. and Sharma, M. 2017. Review on biochemical changes associated with storage of fruit juice. International Journal of Current Microbiology and Applied Sciences 6(8): 236-245.

Sroy, S., Fundo, J. F., Miller, F. A., Brandão, T. R. S. and Silva, C. L. M. 2019. Impact of ozone processing on microbiological, physicochemical, and bioactive characteristics of refrigerated stored *Cantaloupe* melon juice. Journal of Food Processing and Preservation 43(12): e14276.

Teleszko, M. ł., Nowicka, P. and Wojdyło, A. 2016. Chemical, enzymatic and physical characteristic of cloudy apple juices. Agricultural and Food Science 25(1): 34-43.

Torlak, E. 2014. Efficacy of ozone against *Alicyclobacillus acidoterrestris* spores in apple juice. International Journal of Food Microbiology 172: 1-4.

Ünal, M. Ü. and Şener, A. 2013. Extraction and characterization of pectin methylesterase from Alyanak apricot (*Prunus armeniaca* L). Journal of Food Science and Technology 52(2): 1194-1199.

Wibowo, S., Grauwet, T., Santiago, J. S., Tomic, J., Vervoort, L., Hendrickx, M. and Van Loey, A. 2015. Quality changes of pasteurised orange juice during storage: A kinetic study of specific parameters and their relation to colour instability. Food Chemistry 187: 140-151

Xu, H., Hao, Q., Yuan, F. and Gao, Y. 2014. Nonenzymatic browning criteria to sea buckthorn juice during thermal processing. Journal of Food Process Engineering 38(1): 67-75.