International Food Research Journal 26(5): 1405-1415 (October 2019)

Journal homepage: *http://www.ifrj.upm.edu.my*

Quality assessment of ozone-treated citrus fruit juices

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Article history

Received: 29 January, 2018
Received in revised form: 12 September, 2018
Accepted: 3 July, 2019

Keywords

Gaseous ozone
Citrus fruit juice
Physicochemical
Antioxidant activity

Abstract

The aim of the present work was to analyse the impact of ozone treatment on the physicochemical parameters and antioxidant capacity of citrus fruit juices (orange, lemon and lime) with different juice components (total soluble solids). Each sample was ozonated at different ozone treatment time, between 0 to 30 min with fixed ozone concentration of 600 mg/h. The synergistic effects of ozone treatment and the different types of juice were found to significantly affect the pH, total colour difference (TCD), pectin methylesterase (PME) activity, ascorbic acid (AA) and total phenolic content (TPC) of treated juices, while total soluble solids, turbidity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay were found to be unchanged. It was observed that PME activity decreased with treatment time and this is related to the decrease of juice turbidity (R^2 = 0.86) and TCD (R^2 = 0.78). Ascorbic acid showed an abrupt decrease in all the juices especially in orange juice with percentage loss of 85%. TPC also showed decreasing trend for all juices with maximum loss of 84.4% in lemon juice after 30 min of ozonation time. The present work also found that lemon juice could retain most of its antioxidant activities (DPPH 98.9%, TPC 96%, AA 86.7%) after 10 min of ozone treatment time in comparison to orange and lime juices.

Introduction

Consumers are now demanding for safe and natural products with palatable appearance. This can be achieved by controlling the process that changes nutritional and quality characteristics, particularly pasteurisation process. Fruit juices are usually flash-pasteurised, a type of high-temperature-short-time (HTST) pasteurisation method that uses rapid heating and cooling steps. This traditional pasteurisation process is designed to inactivate pectin methylesterase (PME) and destroy spoilage microorganisms. In fact, 98% of all juices are flash-pasteurised (NFPA, 1999). Pasteurisation effectively produces products that are safe with longer shelf-life for consumers, but the synergistic effect of treatment time and temperature is also proportional to the amount of quality and nutritional losses (Dolatowski *et al*., 2007). This has led to increasing interest in non-thermal technologies. Non-thermal liquid food pasteurisation technologies such as ozone, ultraviolet (UV-C), pulsed electric field (PEF) and high-pressure processing (HPP) have been extensively researched and applied in order to comply with USFDA. USFDA (FDA, 2004) has regulated that liquid food processors must achieve a minimum of 5-log reduction of pertinent microorganisms to market their products to the masses. Ozone, however, has been unpopular choice of pasteurisation technology due to the negative image projected by ozone. Few studies have reported on the effect of ozone treatment on quality attributes on tropical fruit juices, even though the capital cost of ozone is considered the lowest among non-thermal technologies.

Ozone is a triatomic (O_3) molecule, consisting of three oxygen atoms. It is an allotrope of oxygen and is much less stable than the diatomic O_2. Ozone rapidly decomposes into oxygen and numerous free radicals, predominantly hydroxyl free radicals, leaving no toxic by-products, making it environmentally friendly (Tiwari and Muthukumarappan, 2012). The usage of ozone as an antimicrobial agent in food processing was reviewed by various researchers (Cullen *et al*., 2010; Perry and Yousef, 2011; Patil and Bourke, 2012; Tiwari and Muthukumarappan, 2012; Nath *et al*., 2014; Pandiselvam *et al*., 2017). Ozone oxidation

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efficacy against Gram-positive and Gram-negative bacteria, fungi and viruses has been reported in detail by Restaino *et al*. (1995). In his study, the author revealed that microbial inactivation by ozone is mainly due to the rupture of cellular membranes and dispersion of the cytoplasm. Ozone oxidises the organic matter of bacterial membranes thus weakening cellular walls and causing cell damage, consequently leading to cell death. Such advantages make ozone pasteurisation an attractive option for the food industry.

The effectiveness of ozone against microorganisms depends not only on the amount used, but also on the residual ozone in the medium, medium pH, temperature, humidity and the amount of organic matter surrounding the cells (Patil and Bourke, 2012). Furthermore, Kim *et al*. (1999) stated that the rate of inactivation of the microorganisms is greater in ozone demand-free system than when the medium contains oxidisable organic substances. Miller *et al*. (2013) stated in her review that the half-life of aqueous ozone is only 20 min at 20°C, which is considered highly unstable and quickly degrades into oxygen. Given the limited available data on the high reactivity and instability of ozone, it is difficult to predict its reaction in the presence of different organic matters; for example, orange from different cultivars possess different organic matters. It may oxidise or ionise a substrate or spontaneously decompose to oxygen and free radicals. Thus, in order, to optimise its application for sterilising liquid food products, it is necessary to understand the key parameters. Williams *et al*. (2004) reported a low efficiency of ozone to inactivate *Escherichia coli* in orange juice in the presence of organic matter and ascorbic acid. The impact of different compounds such as sugars, fibres, ascorbic acid and other organic matters in the dissolution rate and availability of ozone have created a protective effect in some components.

Medium pH is also an important intrinsic factor of ozone efficacy in disinfection process. Cullen *et al*. (2010) stated that decreasing pH is able to increase ozone efficiency, and ozone decomposition rate changes drastically with changes in pH. Citrus fruit juice with its low pH could be the best candidate to be disinfected using ozone. Additionally, the conventional pasteurisation methods plus relying on the acidity of their product to ensure microbiological safety is irrelevant as several incidents of food-borne illnesses have been associated with spoiled acidic fruit juices.

Usage of ozone has been reported for processing of orange juice and its effect on inactivation of pertinent microorganisms and juice quality parameters (Angelino *et al*., 2003; Williams *et al*., 2004; Tiwari *et al*., 2008a; 2008b; Patil *et al*., 2009). However, to date, the effect of ozonation on the quality of other citrus fruit juices has not been widely reported. There are still knowledge gaps in the literature with respect to the effect of ozone on juice quality changes and the limitation posed, particularly in lime and lemon fruit juices. Thus, the objective of the present work was to investigate and to compare the effect of ozonation on the physicochemical and antioxidant properties on various citrus fruit juices (orange, lemon and lime juice) of Malaysian varieties.

Materials and methods

Sample preparation

Orange (*Citrus* × *sinensis* (L.) Osbeck), lemon (*Citrus limon* (L.) Osbeck) and lime (*Citrus* × *aurantiifolia* (Christm.) Swingle) fruits were bought from a local supermarket in Selangor, Malaysia. The fruit juices were extracted using juice extractor (Model HR1811, Philips, Malaysia) and stored in a refrigerator at 4 ± 1°C until further analyses.

Ozone treatment

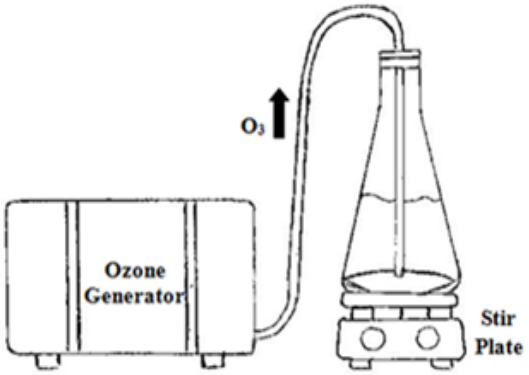
Ozone gas was generated in a closed system using water ozoniser (Model SY- 004, Taiwan) by corona discharge method in a 200 mL beaker (Figure 1). The fixed ozone output concentration at 600 mg/h was measured using an ozone sensor (Model 200 Series, Aeroqual, New Zealand). Ozone gas was directly pumped into the juice for up to 30 min through the food-grade silicone tube into the beaker and stirred using magnetic stirrer (100 rpm) to ensure the ozone molecules were completely mixed with the samples. The gas flow rate was fixed at 0.2 L/min and treatment temperature was fixed at 20°C. Untreated fruit juices (control = 0 min of ozone exposure time) and treated fruit juice were stored at 4 ± 1°C in sterile dark glass bottles to protect from light. All experiments were carried out in triplicate and analyses were immediately performed after processing (within an hour).

Physicochemical and antioxidant properties

Physicochemical parameters such as total colour difference (TCD), total soluble solids (TSS), pH, and turbidity of the orange, lemon and lime juices were performed as described by AOAC (1996).

Chemicals and reagents

Sodium chloride (NaCl), pectin from citrus peel, Folin-Ciocalteu reagent, pure methanol, metaphosphoric acid, and L-ascorbic acid were



Ozone fixed concentration	600 mg/h
Ozone generator time limit	50 minutes
Ozone generator power and frequency	8 W, 60 Hz
Pipe type, diameter and length	Food-grade silicone, 0.5 cm, 0.5 m

Figure 1: Schematic diagram of ozone treatment system and the dimensions of its apparatus.

obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), gallic acid standard, 2,-6 dichlorophenol-indophenol and 2,2-diphenyl-1-picrylhydrazyl were purchased from Merck Millipore KGaA (Darmstadt, Germany).

Determination of Total Colour Difference (TCD)

The triplicate values of *L** (whiteness), *a** (redness/greenness), and *b** (yellowness/blueness) of the orange, lemon and lime juices were determined using Hunter Lab Ultra Scan Pro (D65, 28 Hunter Lab Assoc Lab Inc., Reston, VA, USA) colour spectrophotometer. It was calibrated using white reference tiles (*L* = 97.55; *a* = -0.11; *b* = -0.04) and light trap. The total colour differences were calculated using Equation 1 adapted from Tiwari *et al*. (2008b):

$$TCD = \sqrt{(L-L_o)^2+(a-a_o)^2+(b-b_o)^2} \quad \text{(Equation 1)}$$

where L_o, a_o, b_o = initial values obtained from untreated juice.

The calculated TCD indicated the magnitude of the colour difference between juices at initial time and after ozone treatment time (10, 20 and 30 min). Measurements were made in triplicate, before and after the ozone treatment.

Determination of Total Soluble Solids (TSS)

The total soluble solid of the orange, lemon and lime juices were measured using a digital refractometer (LR-01 Digital Refractometer, Maselli, Stockton, CA, USA). The instrument was first calibrated using distilled water prior to the test. Then, a substantial amount of extracted juice was dropped onto the refractometer. The resultant readings were expressed as Brix°. Measurements were made in triplicate, before and after the ozone treatment.

Determination of pH

A pH meter (Model 860031, Sper Scientific Direct, Scottdale, AZ, USA) was utilised to measure pH of orange, lemon and lime juice samples. The pH meter was first calibrated with commercial buffer solution at pH 7.0 and pH 4.0. Samples (40 mL) were placed in a beaker with a magnetic stirrer and measured at 27 ± 0.5°C. Measurements were made in triplicate, before and after the ozone treatment.

Determination of turbidity

The turbidity of the orange, lemon and lime juices was determined using a portable Turbidimeter (Model 2100P, Hach Company, Loveland, CO, USA). The treated juice was homogenised prior to the measurements. The sample was then filled into a sample cell, filling up to the horizontal mark (~10 mL). The results were reported in nephelometric turbidity units (NTU) by measuring the scattered light from the sample at a 90-degree angle from the incident light. Measurements were made in triplicate, before and after the ozone treatment.

Determination of Pectin Methylesterase (PME) activity

The PME activity of the orange, lemon and lime juices was measured using the Uçan *et al*. (2014) method. PME activity was expressed as PMEu/mL which represents µ-equivalent of acid liberated per min per mL of test sample at pH 7.7 and 30°C. Pectin solution (1%) was prepared using 0.1 mol/L sodium chloride (NaCl) solution at the temperature of 50°C. Then, 10 mL of juice sample was added to 40 mL aliquot substrate solution at 30°C. The pH of the solution was adjusted to pH 7.0 by adding 2 mol/L sodium hydroxide (NaOH) solution and was re-adjusted again until 7.7 with 0.05 mol/L sodium hydroxide (NaOH) solution, during which the time was measured. Pectin methylesterase (PME) activity was calculated using Equation 2:

$$\text{Activity}\left(\frac{\text{PMEu}}{\text{mL}}\right) = \frac{(\text{ml NaoH})(\text{Normality of NaOH})}{(\text{mL juice})(\text{reaction time})} \times 10^3$$

(Equation 2)

Measurements were made in triplicate, before and after the ozone treatment.

Determination of Total Phenolic Content (TPC)

The methods for determination of total phenolic content of the orange, lemon and lime juices were referenced from Alothman *et al*. (2010). Samples were centrifuged at 5,000 rpm for 5 min at 4°C. Then, 1 mL of juice sample was mixed with 1 mL of Folin-Ciocalteu reagent and 10 mL of 20% sodium carbonate (Na_2CO_3) before being diluted to 100 mL with distilled water. The mixture was thoroughly mixed and incubated for 1 h at room temperature in the dark prior to the measurement. UV Visible Spectrophotometer (Ultraspec 3100 Pro, Amersham Pharmacia Biotech, UK) was then used to measure the absorbance of mixture at 765 nm. Data for total phenolic content were obtained from the calibration curve prepared with gallic acid at concentrations of 0 to 500 mg/L, and were expressed as gallic acid equivalents (GAE). Measurements were made in triplicate, before and after the ozone treatment.

Determination of Ascorbic Acid (AA) content

Determination of ascorbic acid content in the orange, lemon and lime juices using 2,6-dichlorophenol-indophenol titration method was adopted from JBT FoodTech Citrus Systems (2011). Briefly, 0.5% sodium 2,6-dichlorophenol-indophenol dye and 3% acid stabilisation solution were prepared prior to the test. Then, 2 mL of juice sample, blank (distilled water), or standard of 1 mg/mL of ascorbic acid solution was added into 5 mL of acid stabilisation solution. The mixture was rapidly titrated with the dye solution until a light but distinct rose pink colour appeared for at least 5 s. The vitamin C or ascorbic acid content was calculated using Equation 3 and expressed as mg/100 mL:

$$\text{Ascobic Acid} = \frac{(\text{mL of titrant for sample})-(\text{mL titrant for blank})}{(\text{mL titrant for standard})-(\text{mL titrant for blank})} \times 100$$

(Equation 3)

Measurements for ascorbic acid were made in triplicate, before and after the ozone treatment.

Determination of 2,2-Diphenyl-1-Picrylhydrazyl radical scavenging (DPPH)

Radical scavenging activity of the orange, lemon and lime juices against stable 2,2-diphenyl-1-picrylhydrazyl was determined by the method of Brand-Williams *et al*. (1995). Briefly, 4 mg of DPPH and 100 mL of pure methanol were dissolved to get 0.1 mM stock solution. Then, it was stored at 20 ± 1°C until further use. Next, 5 mL of sample was mixed with 5 mL of 80% methanol and incubated for 30 min. The mixture was then centrifuged at 3,000 rpm for 10 min. Then, 200 µL of juice samples were vortexed prior to reaction with 1 mL of the DPPH-methanol solution at room temperature in the dark after 30 min. An UV spectrophotometer (Model UV-1700, Shimadzu, Japan) was then used to measure the absorbance at 515 nm. The standard curve for DPPH radicals and methanol of 10 to 200 µg/mL was established, and results were expressed as the percentage of decline of the absorbance using Equation 4:

$$\text{DPPH\%} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad \text{(Equation 4)}$$

Measurements were made in triplicate, before and after the ozone treatment.

Statistical analysis

The statistical data were analysed via General Full Factorial methodology utilising Design Expert Software (Version 6, Stat-Ease Inc., MN, USA). Two independent variables: type of fruit juice (lemon, lime and orange juice) and ozone treatment times (0, 10, 20, and 30 min) were used to investigate their influences on the physicochemical properties and antioxidant activity of treated fruit juices. The experiments were performed in triplicate and the data were analysed using Analysis of Variance (ANOVA).

Results and discussion

Table 1 shows the initial characteristics (means ± standard deviation) of orange, lime and lemon fruit juices prior to ozone treatment. These values are imperative to establish the significant changes influenced by the type of juice (its juice components) and ozone treatment time.

The total soluble solid (TSS) is an important index which indicates the fruit juice quality. TSS generally refers to the amount of sugars, with smaller amount of organic acids, vitamins, proteins, free amino acids, essential oils and glucosides. Approximately 85% of

Table 1: Physico-chemical parameters of untreated orange, lime and lemon juices.

Characteristics	Orange juice	Lime juice	Lemon juice
Total Soluble Solids (^{0}Brix)	11 ± 0.00	10.7 ± 0.10	6.87 ± 0.15
pH	2.37 ± 0.06	1.4 ± 0.00	1.23 ± 0.153
Turbidity (NTU)	972 ± 0.93	322 ± 0.58	47.7 ± 0.15
Pectin Methylesterase Activity (PMEu/mL)	0.07 ± 0.005	0.07 ± 0.005	0.08 ± 0.005
Ascorbic Acid (mg/100 mL)	33.63 ± 0.24	12.8 ± 0.20	22.4 ± 0.00
Total Phenolic Content (GAE mg/mL)	180.2 ± 12.12	183.3 ± 15.07	119.6 ± 8.64
DPPH (%)	91.88 ± 0.86	75.66 ± 0.45	75.34 ± 0.38
Colour, L*	73.59 ± 1.40	13.75 ± 1.56	66.98 ± 2.88
Colour, a*	62.17 ± 1.56	17.47 ± 2.42	68.78 ± 1.15
Colour, b*	78.47 ± 1.24	12.14 ± 1.02	60.14 ± 1.82

the TSS of citrus fruit juice are sugars (Magwaza and Opara, 2015). Figure 2(a) shows the range of TSS after the effect of ozone treatment. Orange juice yielded an average value of 10.9 °Brix, while lemon and lime juices 6.8 °Brix and 10.8 °Brix, respectively. The model and the interaction between two independent factors (type of juice and ozone treatment time) were found to be insignificant ($p > 0.05$) which indicates the ozone treatment did not affect the juice samples, regardless of types. This result is consistent with the study of Tiwari *et al*. (2008a) where TSS of ozonated orange juice was found to be unchanged.

Meanwhile, the range of pH value of ozonated orange juice, lemon juice and lime juice ranged from 2.4 to 3.3, 1.1 to 3.1, 1.4 to 3.0, respectively (Figure 2(b)). A significant increasing trend can be seen over ozone treatment time. For lemon and lime juices, the pH value gradually increased from minute 0 to 10 and minute 20 to 30, and it dramatically increased in the minute 10 to 20. Whereas for orange juice; the pH gradually increased over ozone treatment time (0.36 min). The interaction of two factors (type of juice and ozonation time) was significant ($p < 0.05$). This result is inconsistent with the result of Tiwari *et al*. (2008a), where pH and titratable acidity was recorded to be unchanged. The changes observed in the present work could possibly be the result of an overdose of ozone concentration in the juices. When ozone decomposes, it will produce free radicals, mainly hydroxyl acids which substantially increase with increasing temperature and low pH (Cullen *et al*., 2010). Hydroxyl radicals (OH-) produced after ozone treatment and remained in the aqueous solution may have led to the pH changes. From the changes of pH in the citrus juices, it is believed that the colour of the citrus fruit juice would also vary from the initial value, as reported by Tiwari *et al*. (2008b), that degradation of colour may have also been caused by secondary oxidators that the ozone treatment produces (•OH, HO^2, $•O_2$- and $•O_3$-).

The result obtained in the present work further proves that the theories reported by Tiwari *et al*. (2008a) are factual. The authors further reported that the differences in perceivable colour can be analytically classified as very distinct (TCD > 3), distinct (1.5 < TCD < 3), and small differences (TCD < 1.5). The average of total colour difference (TCD) for orange juice in the present work was observed to be distinct (1.5, $p < 0.05$), whereas, lemon and lime juices were shown to have small differences (1.25 and 1.18, $p < 0.05$) (Figure 2(c)). It can be summarised that the colour degradation of ozone-treated citrus fruit juices was significantly different ($p < 0.05$) for both model and interaction factors. Ozone-treated orange juice has also been reported to have colour degradation (Angelino *et al*., 2003; Tiwari *et al*., 2008b). These changes were reported to be a function of ozone concentration, gas flow rate and treatment time, consistent with the result obtained in the present work. Ozone treatment has been reported to break conjugated double bonds of carotenoid pigments resulting in oxidative cleavage of chromophore responsible for the colour of fruit juices. Carotenoid pigments, which contributed to yellow, orange or red colour in citrus fruit juices, contain one or more aromatic rings (Meléndez-Martínez *et al*., 2007). Thus, the ozone and hydroxyl radicals produced may open these aromatic rings, facilitating the interaction with ozone and leading to partial oxidation of products such as organic acids, aldehydes, and ketones, in addition to the oxidative cleavage that was reported as the main effects of ozone treatment. This mechanism is described as a Criegee mechanism where ozone molecules undergo 1-3 dipolar cyclo addition with double bond present, leading to the formation of ozonides from alkenes and ozone with aldehyde or ketone oxides as decisive intermediates, all of which has finite lifetimes (Criegee, 1975). This leads to the oxidative disintegration of ozonide and formation of carbonyl compounds, while oxidative work-up leads to carboxylic acids or ketones. Ozone attacks OH radicals, preferentially to the double bonds in organic compounds. This not only affected

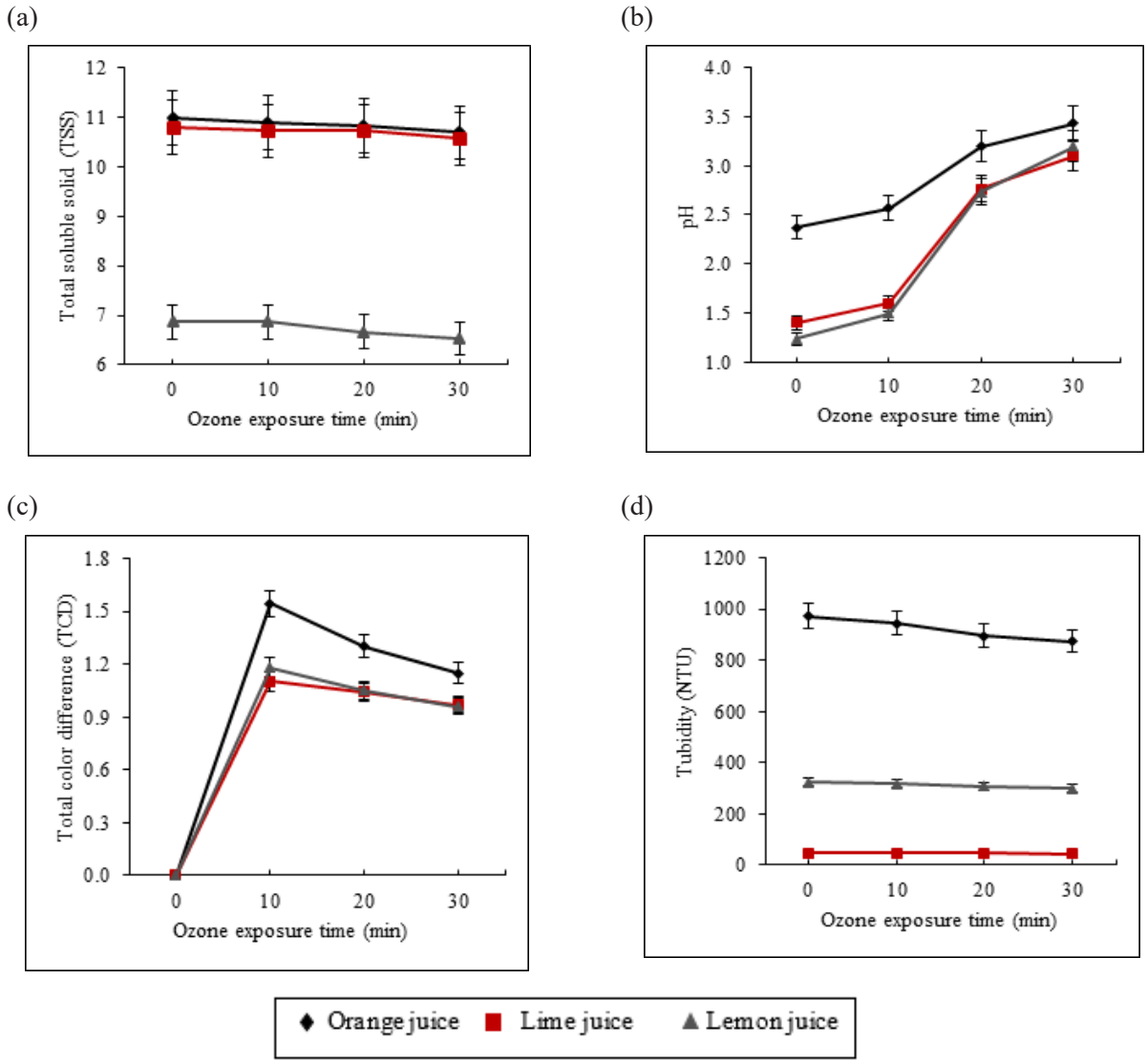


Figure 2: Variation of (a) TSS, (b) pH, (c) TCD, and (d) turbidity at different ozone exposure times.

the colour of treated juice, but also in ascorbic acid and total phenolic content. Meanwhile, in a study done by Jaramillo Sánchez *et al*. (2017), it was reported that colour changes were significant, which were reasoned by browning development. Browning effect could be associated not only with the enzyme action at the beginning of ozone exposure, but also with non-enzymatic browning. Cullen *et al*. (2010) have reported that ozone could induce non-enzymatic browning by the oxidation of the phenolic compound. Nevertheless, the colour changes induced by ozone treatment observed in the present work were too slight to greatly impair the citrus fruit juice quality.

The turbidity of a fruit juice is a measure of the light-scattering properties of the solution, consequently providing the most direct measure of the concentration of polydisperse particles in a solution (Vaillant *et al*., 2008). Turbidity of dispersions depends mainly on the shape, size, and mineral composition of particles. It also depends on their relative refractive index and to some extent, on the colour of the liquid phase (Vaillant *et al*., 2008). Furthermore, turbidity values could be used to discriminate between juices from the same fruit species but with different concentrations, shapes, and sizes of the suspended particles (Vaillant *et al*., 2008). Figure 2(d) shows that a significant ($p < 0.05$) decreasing trend for orange juice was observed upon ozonation time from 0 - 30 min with the range between 982 - 865 NTU. In contrast, lemon and lime juices had a slight decrement of 277 - 275 NTU and 40 - 38 NTU, respectively. The p-value ($p < 0.05$) indicates that both parameters; type of juice and ozonation time, significantly affected the turbidity. It was also observed that orange juice has shown the highest degradation in turbidity with 11.9% loss followed by lemon (6.8%) and lime juice (6.47%). These decreasing trends can be related to the decrease

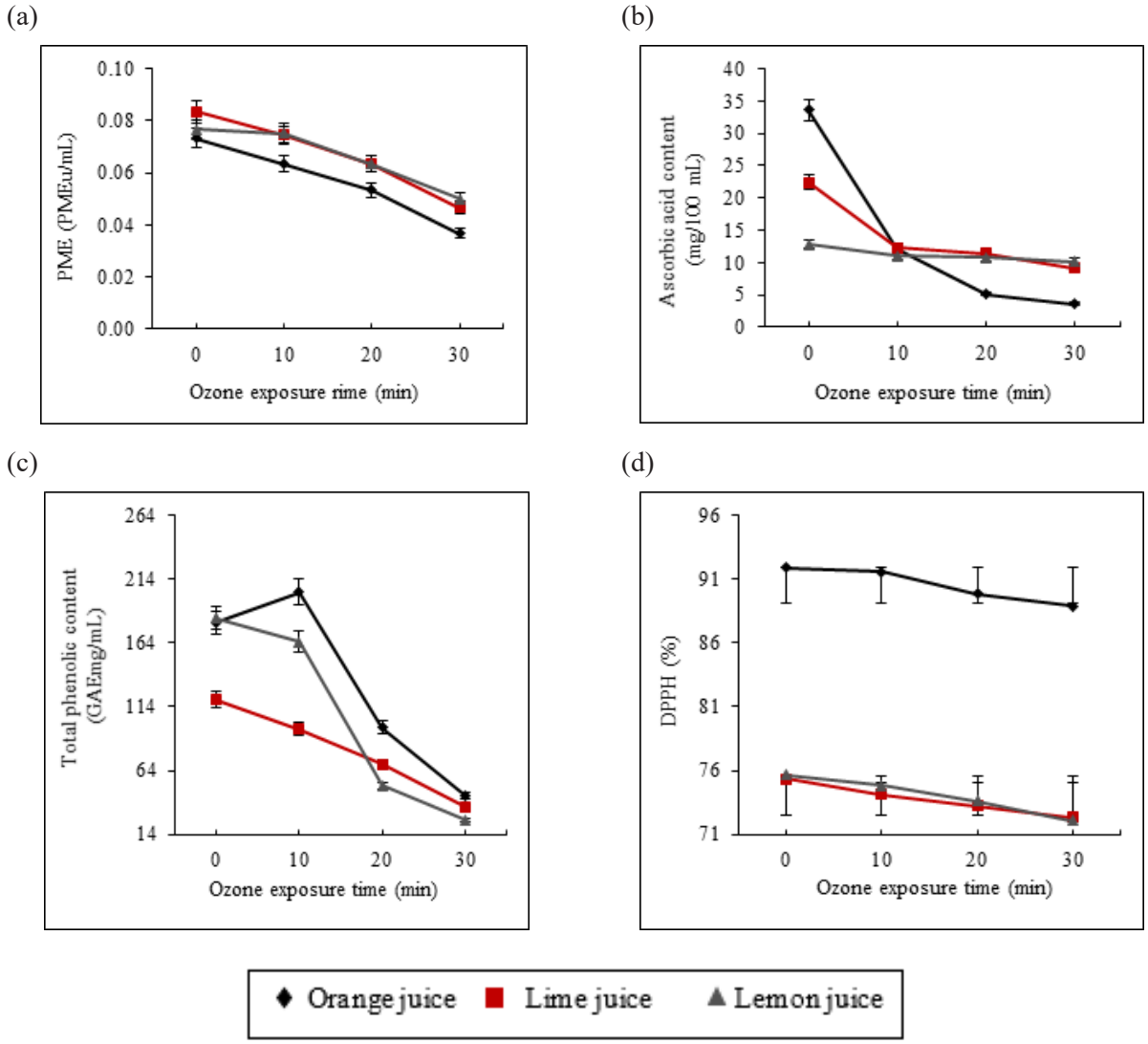


Figure 3: Variation of (a) PME, (b) Ascorbic acid, (c) TPC, and (d) DPPH at different ozone exposure times.

of PME, ascorbic acid and TPC. Correlation of PME and turbidity also showed that PME contributes the highest percentage of turbidity loss for orange (R^2 = 0.87), lemon (R^2 = 0.91) and lime juice (R^2 = 0.90).

Pectin, a major component of the cloud of fruit juice, plays a factor in juice destabilisation in the presence of the active enzyme pectin methylesterase (PME), where pectin forms calcium pectate complexes and causes the precipitation of cloud particles (Croak and Corredig, 2006). Based on Figure 3(a), PME degraded over time for all juices, where the value of PME varied from 0.03 - 0.08 PMEu/mL for orange, lemon and lime juices. A zero-order kinetics model fitted well to PME values with all coefficients of determination R^2 > 0.90, 0.87 and 0.87 for orange, lemon and lime juices, respectively. The reaction rate constants were evaluated as a function of treatment time where orange, lemon and lime juices' constant were 0.0113, 0.007 and 0.011 min-1, respectively. PME activity is optimum in neutral pH rather than acidic condition (Croak and Corredig, 2006), hence, the value of PME activity in the present work was found to be low, since the natural pH of the citrus fruit juices were low (Table 1). The p-value of PME activity was < 0.05, indicating that the effect of two parameters were significant. The result also shows that PME activity of orange, lemon and lime juices decreased by 50%. This is in line with the result of Rodoni *et al*. (2010) who stated a reduction of 50% in PME activity in ozone-treated tomato juice. Thus, it is justified that with the decrement of PME, the turbidity of fruit juice also decreases.

The nutritional quality of orange juice is primarily related to the ascorbic acid content. Ascorbic acid is thermolabile and highly sensitive to various processing and storage conditions (Tiwari *et al*., 2008a). Results from Figure 3(b) show that the ascorbic acid content decreased in the three fruit juices due to the scavenging of free radicals formed during the decomposition of ozone (Rabie *et al*.,

2015). The Figure also shows an abrupt decrease in orange juice at 0 - 10 min from 33 - 11.2 mg/100 mL and a gradual decrease from 10 - 30 min of ozone treatment time with the range of 11.2 - 3.4 mg/100 mL. The rate of reaction was calculated at 1.002 min-1. The rapid decrease of ascorbic acid concentration at the beginning of ozone can be attributed to the immediate reaction of an amount of ascorbic acid with the dissolved oxygen molecule of ozone (Polydera *et al*., 2003). Lemon juice gradually decreased throughout the ozone treatment at 0 - 30 min with ascorbic acid content of 11.4 – 11 mg/100 mL, with the rate of reaction as a function of treatment time of 0.41 min-1. Lime juice with the lowest rate of reaction, 0.122 min-1 steadily decreased from 0 - 30 min from the initial value of 22.4 to 9 mg/100 mL.

According to analysis of variance, the model and the effects between type of juice and ozonation time had a significant p-value of < 0.0001. From Figure 3(b), orange juice had the highest loss of ascorbic acid (85%) followed by lime (60%) and lemon juice (21%). The decrease of ascorbic acid concentration to an unacceptable level (less than 50%) by legislation or industrial practice often defines fruit juice shelf life, rendering ascorbic acid an important indicator of fruit juice quality (Polydera *et al*., 2003). From the data, only lemon juice had an acceptable loss of ascorbic acid (21%) even after 30 min which was below 50%. These reductions were expected as ozone strong oxidation potential (+ 2.07 eV) could cause the loss of antioxidant bioactive compounds (Rawson *et al*., 2011). Ozone oxidises organic matter via two different pathways: by direct oxidation with ozone molecules or by the generation of free radical intermediates such as OH•, which is considered a powerful, effective and non-selective oxidising agent (Staehelin and Hoigne, 1985). A study by García-Viguera and Bridle (1999) indicated that the degradation of ascorbic acid by ozone is more likely to be due to the free radical mechanism.

Another factor that could have contributed to the degradation of ascorbic acid in the three ozone-treated fruit juices is the activation of ascorbate oxidase (Lee and Kader, 2000). This enzyme is activated under stress conditions, such as chemical exposure. Ascorbate oxidase has been reported to promote the degradation of ascorbic acid to dehydroascorbic acid. Based on the study of Vines and Oberbacher (1963), orange had the highest enzyme activity of ascorbate oxidase, while lime and lemon had the lowest. This explains the loss of ascorbic acid in all the juices, especially in orange juice.

The combined odour–flavour characteristics in fruit products are due in part to phenolic compounds. Phenols are also used as indicators of physiological state and potential damage in quality of fruit products (Gomis *et al*., 2001). From Figure 3(c), orange juice showed an increasing trend during 0 to 10 min (180.45 - 184.32 mg GAE/mL) and started to drop dramatically from 10 to 20 min (184.32 - 95.55 mg GAE/mL). The decreasing trend gradually continued from 20 to 30 min (95.55 - 60.32 mg GAE/mL). The result shows the same pattern found by Alothman *et al*. (2010) and Rabie *et al*. (2015), where total phenol and flavonoid contents of pineapple and banana fruits significantly increased when exposed to ozone for up to 20 min. The increase in the phenolic and flavonoid contents of the fruit juice also might have been caused by modification that occurred during ozone exposure; this modification may have increased the phenolic compounds. The increase in the phenolic content might have been caused by cell wall modification that occurred during ozone exposure, which might free some of the conjugated phenolic compounds in the cell wall. The auto-decomposition of ozone is accompanied by the production of numerous free radicals. Therefore, the by-products of ozone decomposition were scavenged by the phenolic compounds in the fruits, to different extents, which might have led to the reduction of phenolic contents after 30 min of ozone exposure. Phenolic compounds are particularly susceptible to ozone attack. According to Yeoh *et al*. (2014), ozone reacts very efficiently on degradation of aromatic compounds of phenolic content. The molecular ozone action on the aromatic compound favours the formation of hydroxylated and quinone compounds because the formation of aliphatic compounds originates from rupture of the aromatic ring.

Lime and lemon juices showed similar trend but differed from orange juice. Both lime and lemon juices gradually decreased from 0 - 10 min with value ranging from 115.5 - 84.67 and 180.82 – 166 mg GAE/mL, respectively. Lime juice continued to gradually decrease from 10 - 30 min resulted in 84.67 - 54.45 mg GAE/mL. A different pattern was shown by lemon juice as it started to decrease abruptly at 10 - 20 min and steadily at 20 - 30 min with a decreasing value of 166 to 60 mg GAE/mL and 60 to 20 mg GAE/mL, respectively.

According to Hoigné and Bader (1983), the auto-decomposition of ozone is accompanied by the production of numerous free radical species, such as hydroperoxyl (H2O), hydroxyl (OH), and superoxide (O2 −) radicals. Therefore, the by-products of ozone decomposition were scavenged by the phenolic compounds in the fruit juice, to different extents, which might have led to the reduction of phenolic

and flavonoid contents of the fruit juices (guava and papaya). Analysis of variance has shown that this model and the effects between type of juice and ozonation time were significant ($p < 0.05$). Figure 3(c) also shows that the highest percentage loss of TPC was found in lemon juice (84.36%) followed by lime juice (78.7%) and orange juice (76.15%). Ozone treatment has decreased the phenolic content of each juice by 50% after 20 min of ozonation time. This is coherent with Torres *et al*. (2011) which claimed that during ozonation at processing conditions (15 ppm ozone concentration for 10 min processing time), a decrease of 99.1%, 96.6%, 99.8% and 49.7% was observed for chlorogenic acid, caffeic acid, cinnamic acid and total phenol content, respectively. The degradation of polyphenols during ozonation may result from a variety of possible chemical reactions. These reactions may be direct reactions of ozone with the target compound or its intermediates and radical reactions between hydroxyl radicals produced through ozone decomposition catalysed mainly by the hydroxide ion (OH-) (Cullen *et al*., 2010).

Based on Figure 3(d), it is clear that ozone treatment and type of juice had a significant effect on all citrus juice tested. Orange juice had the highest reduction of DPPH (10%; $p < 0.05$), followed by lemon and lime juices of 7% each. All juices degraded upon ozonation time with steady decreasing trend. Ozone is known to induce the production of reactive oxygen species (ROS) in the juice via ozone decomposition, and causes the scavenging activity to increase. The absorbance decreases when the radical is reduced by antioxidants, as proven in the present work. However, the diminutive result may have been caused by the scavenging activity of the total phenolic content within the juice (Tiwari *et al*., 2008a), which as shown in the present work, have caused a reduction of TPC in lemon juice (84.36%), followed by lime juice (78.7%) and orange juice (76.15%).

In summary, the efficacy of ozone depends upon its composition and the composition of the medium. Furthermore, it is difficult to produce pure ozone due to technical limitation and the maximum ozone concentration that can be achieved is about 10-14% w/w of oxygen. In addition, ozone is sparingly soluble in water, which is ten times less than chlorine (Tizaoui *et al*., 2008). The oxygen in ozone treatment gas mixture will not play a role in microbial inactivation; however, it will significantly influence the degradation of organic compound present in fruit juice (Cullen *et al*., 2010). Subsequently, applying doses that are large enough for effective decontamination may change the sensory qualities of treated fruit juice (Patil and Bourke, 2012). Thus, further study should be done with consideration of extrinsic and intrinsic parameters in order to pursue an optimum condition for ozone processing for citrus fruit juices.

Conclusion

The effects of orange, lemon and lime fruit juices treated with ozone for 0, 10, 20 and 30 min with a fixed ozone concentration and gas flow rate of 600 mg/h and 0.2 L/min were non-linear and highly dependent on processing conditions and the food matrix. In summary, it was found that ozone treatment had no significant effect towards juice pH, total soluble solid (TSS) and 2,2-diphenyl-1-picrylhydrazyl assay (DPPH) on all fruit juice samples. The synergistic effect of ozone treatment and the type of fruit juice also showed that the interaction effect of the two parameters affected the total colour difference (TCD), pectin methylesterase (PME) activity, ascorbic acid (AA) and total phenolic content (TPC). These dependent parameters were shown to decrease with ozone treatment time which can be related in the decrement of juice turbidity. The drastic decrement of ascorbic acid to an unacceptable level for orange and lime juices was an important indicator of fruit juice quality, rendering an important decision prior to industrial adoption. Thus, further validation studies are still needed to determine the critical limits (ozone concentration and time parameters) for effective treatment in terms of bactericidal activity and ultimately, to retain bioactive compounds of citrus fruit juice products.

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