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Decontamination of pineapple (*Ananas comosus*) juice using ozone as a non-thermal sterilisation method

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Ozone is a non-thermal preservation technology used for enhancing shelf life and the safety of food products. The main objective of this study was to evaluate the effect of ozone on microbial decontamination and some physicochemical characteristics (soluble solids content, pH, titratable acidity, and colour), bioactive compounds and total antioxidant activity of pineapple juice. Ozone treatments were applied to pineapple juice at 4 different exposure times; at 15 minutes, 30 minutes, 45 minutes, and 60 minutes at 25°C and the overall impact on microbial load were evaluated. The reduction of aerobic plate count was recorded as 1.99 log cycles, and the reduction of yeast and mould count was recorded as 1.92 log cycles after exposure to 60 minutes of ozone at 200mg/hr dose concentration. However, some quality parameters were significantly affected by ozone treatments. The most prominent changes were observed for colour and total phenolic content. Colour parameters including Chroma, Hue angle, and b* value has been significantly reduced through the ozone treatment, but the total phenolics content has been increased significantly in ozonized juices. In this context, and particularly for the pineapple beverage, ozone has been exploited due to its potential for inactivating spoilage microorganisms while being moderately effective in the overall quality retention of the products.

1. Introduction

The consumption of soft drinks, such as colas and flavoured sodas, is reducing globally as they have high sugar content, artificial colouring agents, sweeteners, and other harmful additives which can cause negative effects on the human body. Because of this, a higher number of consumers are shifting towards natural fruit juices (Ruanpeng *et al.*,2017). Fruit juice is an extract, or an extractable fluid content of cells or tissues made by mechanically squeezing or pressing out the natural liquid in ripe fruits. Fruit juices are rich in macronutrients and micronutrients. They also provide a rich source of nutraceutical compounds that can provide better immunity and various other health benefits such as the prevention of heart diseases, cancer, and diabetes (Abeysinghe *et al.*,2007).

The perishable nature of fruit juices poses significant challenges associated with the production and pres-

ervation of fruit juices. Unless the juice is consumed fresh, storage at chilling or freezing temperatures is the only alternative to protect the organoleptic properties of juice (Kaur & Kumar, 2019). Considering the extension of shelf life and preservation of fruit juice, the most widely adopted technology remains the conventional thermal processing which ensures prevention of microbiological deterioration, elimination of oxygen and prevention of enzymatic action. Thus, thermal inactivation maintains product safety. In thermal processing, heat is generated by a heating source and transferred into the product through conduction and convection mechanisms. This heat processing ensures the microbial safety of the food product (Petruzzi *et al.*,2017).

However, thermal processing may cause changes in bioactive compounds as well as organoleptic changes. Alternative non-thermal technologies have been studied to obtain ready-to-drink "fresh-like" juices with minimum nutritional, physicochemical, functional, or organoleptic changes (Esteve & Frigola, 2007). These emerging non-thermal technologies include high hydrostatic pressure, pulsed electric field, ozone processing, membrane filtration and ultraviolet light (Yildiz *et al.*,2019).

Among these non-thermal processing technologies, ozone processing is used in various processing systems today. In 2001, the US FDA approved ozone in the gaseous and aqueous phases (21CFR173.368) as an effective antimicrobial agent for foods (Khadre, Yousef & Kim, 2001). The food industry has started utilising ozone processing methods for pathogen inactivation. They have conducted research involving the application of ozone to preserve various fruit juices, such as apple cider, orange juice, strawberry juice, and apple juice (Patil *et al.*,2009).

Ozone is widely used in the food industry because it has many advantages over other treatments. Ozone is a triatomic allotrope of oxygen that decomposes rapidly to nontoxic oxygen, thereby leaving no harmful residues in food materials (Burleson, Murray, and Pollard, 1975). Ozone shares a higher oxidation potential of 2.07V; therefore, it is used as an effective antimicrobial agent (Fisher *et al.*,2000; Kim, Yousef & Dave, 1999). Due to its higher oxidation potential, it can destroy most microorganisms at relatively low concentrations. Ozone effects various cellular components like proteins, peptidoglycans in cell envelopes, enzymes, and nucleic acids in the cytoplasm of bacteria. When microbes get contacted with ozone, it initiates oxidation of the cell envelope, and that will cause cell lysis (Daş, Gürakan & Bayindirli, 2006; Khadre, Yousef & Kim, 2001).

Studies on the efficiency of ozone application in fruit juices have been done, mainly in apple and orange juices (Miller and Silva, 2013), focusing on quality and safety characteristics. Color, pH, soluble solids content, and phenolic compounds are the most studied characteristics for apple juices, while ascorbic acid is also often reported for orange juice. The majority of works reported significant colour alterations and ascorbic acid decay in ozonised juices (Patil *et al.*,2010; Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2008; Torres *et al.*,2011), which was highly dependent on the fruit, treatment time and ozone concentration applied. The efficacy of ozone on bacteria inactivation has been mainly studied for Salmonella and Escherichia coli with observed reductions of up to 5 log-cycles (Patil *et al.*,2010; Patil *et al.*,2009; Williams, Sumner, & Golden, 2005).

However, there are some knowledge gaps concerning the effect of ozonation on the quality of tropical fruit juices. Therefore, the main objective of this work was to evaluate the efficacy of Ozone as a non-thermal sterilisation technique for pineapple juice (variety Mauritius) compared to thermal pasteurisation.

2. Materials and methods

2.1. Sample preparation

The matured pineapples purchased from the local market (Malabe, Sri Lanka) were cleaned by washing three times with potable water, with 100 ppm chlorine solution and again with potable water. The outer scales, eyes and inner core of pineapples were removed, and the fruit was cut into pieces. The pieces were blended using a domestic juice extractor, and the obtained juice was filtered through a clean muslin cloth. Then the filtered juice was mixed with water and food-grade sugar according to a formula of 18% filtered fruit juice, 73% water and 9% sugar and stored in a refrigerator at 4 ± 1°C until further analyses.

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2.2. Hot Filling / Pasteurisation

The prepared juice was heated in a water bath until the temperature reached 80oC and then it was maintained at 80oC for 15 minutes. The heated juice was then filled into sterile dark glass bottles and capped. Then the bottles were allowed to cool, labelled, and stored in the refrigerator (4ºC).

2.3. Ozone treatment

Ozone treatment was performed according to the method described in Czekalski *et al.,*. (2016) with some modifications. Ozone gas was generated in a closed system using water ozoniser (Model No. 11022 Kent RO systems India) using a corona discharge method in a 500 ml conical flask (Figure 1). The fixed ozone output concentration at 200 mg/h was directly pumped into the juice through a food-grade silicone tube into the beaker and stirred using a magnetic stirrer (100 rpm) to ensure the ozone molecules were completely mixed with the sample. Treatment was provided at four different exposure times: 15 minutes, 30 minutes, 45 minutes and 60 minutes and treatment temperature were fixed at 25°C (Room Temperature). Untreated fruit juices (control = 0 minutes of ozone exposure time) and treated fruit juice were stored at 4 ± 1°C in sterile dark glass bottles to protect from light. All the described experiments were performed in triplicate, and all analyses were immediately performed after processing (within an hour).

2.4. Microbiological analysis

All beverage samples obtained as untreated, hot-filled / pasteurisation (which will be referred to as thermally processed, from here on), and ozone processed were tested for microbial safety by subjecting them to aerobic plate count test and yeast and mould count test (Keyser *et al.*,2008). For each ozone treatment time, 1mL of juice was removed, and aerobic bacteria were determined by pour plating the diluted samples onto Plate count agar. The plates were incubated at 30°C (Sanyo MIR-262) for 72 hours. Microbial counts were performed in triplicate and expressed as CFU/mL.

Yeast and mould were enumerated by spread plating the diluted samples onto the Dichloran Glycerol Media base. The plates were incubated at 25°C (Sanyo MIR-262) for 5 days. Microbial counts were performed in triplicate and expressed as CFU/mL.

2.5. Physicochemical analysis

2.5.1. Soluble solids content, pH, and titratable acidity

Soluble solids content (SSC), pH and titratable acidity (TA) of juice samples were directly analysed: pH was measured using a pH meter (EUTECH Instruments, PH510, pH/ mv/ºC meter); SSC was measured with a Palette PR-32 digital refractometer (Atago, Tokyo, Japan) and was expressed as °Brix; titration was conducted with a 0.1M NaOH and TA was expressed as a

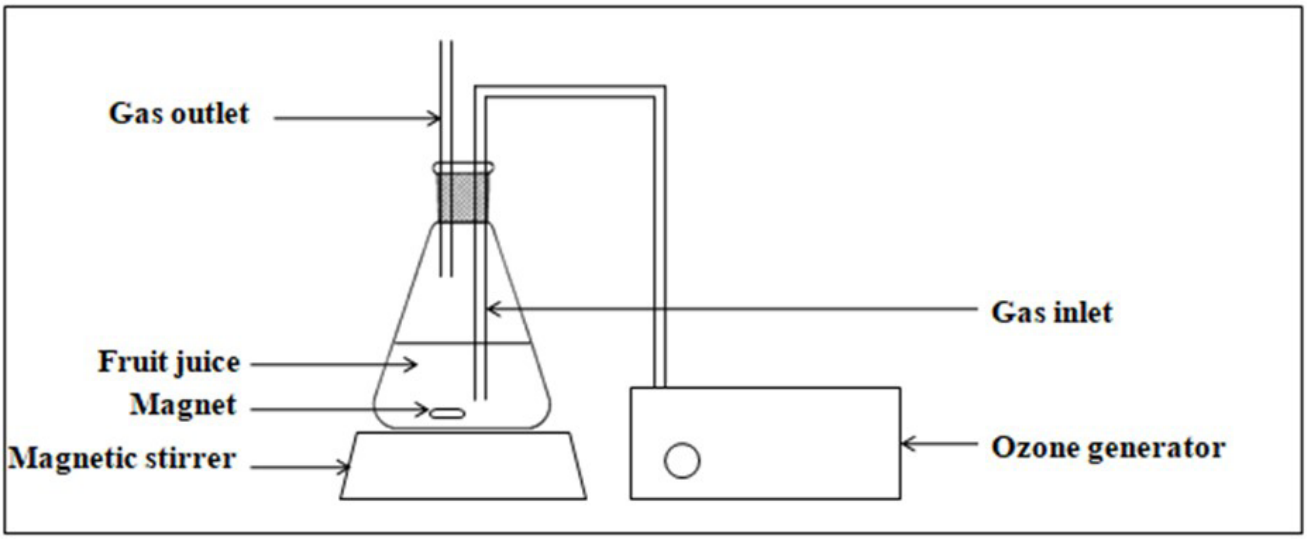

Figure 1. Schematic diagram of the ozone treatment system

percentage by mass of sample according to the following equation (1) (Sadler and Murphy, 2010).

$$\text{Titratable Acidity (\% by mass)} = \frac{6.404 \times V \times M}{m} \quad (1)$$

In the above equation, V is the Volume (in ml) of standard NaOH required for titration, M is the Molarity of the standard NaOH solution, and m is the Mass (in g) of the sample taken for the test.

2.5.2 Colour measurement

The colour coordinates (L*, a*, b*) of juice samples were measured using a Minolta CR-400 colourimeter (Konica-Minolta, Osaka, Japan). For each sample, three readings were carried out. Chroma (colour intensity) and hue angle (red at 0°, yellow at 90° green 150° and blue at 270°) (Hammond, 2016) were determined, according to the following equations:

$$\text{Hue Angle } (H^0) = \tan^{-1}(b^* / a^*) \quad (2)$$

$$\text{Chroma value} = \sqrt{(a*)^2 + (b*)^2} \quad (3)$$

2.5.3 Oxidation-reduction potential and active hydrogen score

The digital oxidation-reduction potential meter (EUTECH Instruments, Cyberscan pH110, pH/ mv/°C meter with RS232) was calibrated against standard solutions. The prepared juice samples were mixed well to homogenise, and the ORP values were measured using the calibrated ORP meter at room temperature. The active hydrogen score (rH) was calculated from the oxidation-reduction potential (ORP) and the pH of the juice, using the following formula (Holmes & Farley, 2008).

$$rH = ((ORP + 200) / 30) + (2 \times pH) \quad (4)$$

2.5.4 Viscosity

Rheological properties of pineapple juice were measured using ROTAVISC Rotational viscometer with No.2 spindle head. 200ml of each sample was placed in a glass beaker, and the spindle was set at 60rpm and 100rpm at 30 ± 2°C and the viscometer reading was taken (Jaramillo-Sánchez *et al.*,2018).

2.6 Bioactive compounds determination

2.6.1 Total phenolic content

The total phenolic content of juice samples was determined by the Folin-Ciocalteu reagent using 96-well microplates. 20µl from each juice sample was mixed with 110µl of Folin-Ciocalteu reagent and 70µl of 10% sodium carbonate solution. It was left for incubation at room temperature (25 ± 2 °C) for 30 minutes. The absorbance was read at 765nm, and Gallic acids with different concentrations were used as the standard antioxidant to construct the standard curve (Margraf *et al.*,2015).

2.6.2 (DPPH) 2, 2-Diphenyl-1-Picrylhydrazyl radical scavenging activity

Radical scavenging activity of the pineapple juices against stable 2,2-diphenyl1-picrylhydrazyl was determined by the method of Cuvelier and Berset, 1995. Briefly, 3 mg of DPPH and 50 mL of pure methanol were dissolved to get the stock solution. Then, it was stored at 20 ± 1°C until further use. Next, 2.5g of sample was mixed with 25 mL of 50% methanol and incubated for 30 min. The mixture was then centrifuged at 2,000 rpm for 5 min. Then, 2 mL of juice samples were vortexed before reaction with 2 mL of the DPPH-methanol solution at room temperature in the dark after 30 min. A UV spectrophotometer (Model UV-1700, Shimadzu, Japan) was then used to measure the absorbance at 517 nm. Standard ascorbic acid was used for the preparation of the standard solution series.

2.7 Statistical analysis

The statistical data analysis was performed for all experiments using ANOVA to test the significance of each variable (α = 0.05) and followed by a comparison performed using the Tukey test by the statistical software MINITAB 17. One way ANOVA was used to determine the effect of treatments on different juice parameters for each juice type.

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3. Results and Discussion

3.1 Microbial survival

According to the results shown in Figure 2, the microbial count was reduced significantly when ozone treatment time increased. Results showed in Table 4.2 shows a log reduction of 0.94, 1.36, 1.87 and 1.99 of Aerobic bacteria (APC) at 15 minutes, 30 minutes, 45 minutes, and 60 minutes exposure to ozone gas, respectively. The initial yeast and mould counts were decreased by 0.61, 0.96, 1.39 and 1.92 log cycles after exposing ozone gas for 15 minutes, 30 minutes, 45 minutes, and 60 minutes. No microbes were recorded in the thermally processed juice sample.

In the food industry, a minimum reduction of 2 log cycles is required to consider an agent as antimicrobial (Tiwari and Mason, 2012). Since the results obtained showed a reduction of around 2 log cycles at 60 minutes ozone treatment, it can be considered a promising non-thermal technology for preserving pineapple juice. Log survival of the microbial population at each ozone processing time shows a significant difference ($p < 0.05$).

The ozone microbicide action can be related to the damage of several cellular constituents, including proteins, unsaturated lipids and respiratory enzymes in cell membranes, peptidoglycans in cell envelopes, enzymes and nucleic acids in the cytoplasm, and proteins and peptidoglycan in spore coats and virus capsids (Khadre, Yousef, & Kim, 2001).

3.2 Physicochemical characterisation

Soluble solids content describes the balance of sugars and acids present in a matrix, which mainly impact juice flavour. As can be observed in Table 1, ozone-treated samples have shown no significant difference in soluble solids content when compared to untreated samples ($p > 0.05$). This agrees with most published results of ozone processed juices (Miller and Silva, 2013). However, thermally processed juice sample shows a significant increment in Total Soluble Solids (TSS) content (TSS=14.00 ± 0.10). The concentration of solids during the thermal processing will lead to a higher TSS content than other treatments (Maskan, 2006).

Titratable acidity and pH describe the acidic nature of the food materials, giving insights into food quality. Titratable acidity describes the effect of organic acids on the flavour of the food. Most food acids are par-

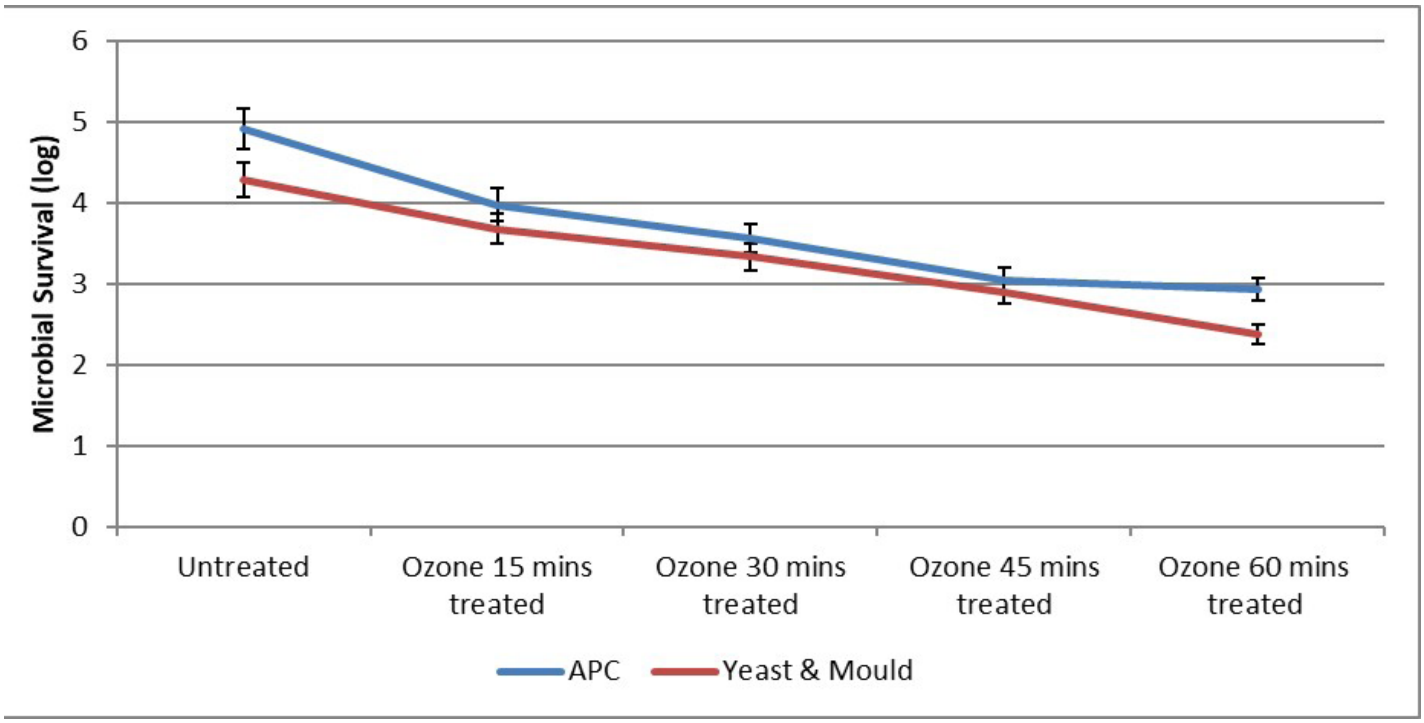

Figure 2. Microbial log survivals in Pineapple juice at different ozone processing times

tially ionised as they are weak in nature. Thus, some properties of foods are based on this ionised fraction of acids, whereas other properties are based on the total acid content. However, ozone processing did not significantly affect pH values and titratable acidity values of the juice. But thermal processing shows the highest mean value for titratable acidity (3.76 ± 0.005) due to the concentration of organic acids during the thermal processing. Therefore, it shows a significant difference in comparison to the untreated and ozone processed juices.

The colour of the untreated, thermally processed and ozone-treated juices are shown in Table 2. Here L, a* and b* values represent lightness, redness, and yellowness, respectively. All the colour parameters have shown a significant difference ($p < 0.05$). L* represents the lightness that has shown a significant increment over the ozone processing time. As shown in Table 3, the Chroma parameter showed a significant reduction in ozonised juices, indicating that ozone affected their colour intensity; however, no differences were observed between samples treated for 30 and 45 minutes. Related to hue angle, significant differences were obtained in untreated, thermally processed and ozone processed juices. The thermally processed juice had the most yellowness and vivid colour (b*=4.66 ± 0.09a and Chroma=4.67 ± 0.09).

Colour degradation of fruit juices due to ozone processing has also been reported for apple (Patil *et al.*,2010; Torres *et al.*,2011), grape (Tiwari *et al.*,2009a), orange (Tiwari *et al.*,2008), strawberry (Tiwari *et al.*,2009b), and tomato juices (Brijesh K. Tiwari *et al.*,2009). Different compounds in fruit juices are responsible for different colours. Changes in colour of the juice are associated with the degradation of colour pigments in particular juice (Tiwari *et al.*,2009).

Oxidation-reduction potential (ORP) indicates the dissolved oxygen concentration in water/liquid. As shown in Table 3, there is a significant difference ($p < 0.05$) in the ORP values between the untreated, thermally processed and ozone processed pineapple juices. 60 minutes ozone-treated juice shows the highest ORP mean value (275.33 ± 1.52), where thermally processed juice shows the lowest ORP mean value (249.67 ± 2.52). The reason for this is the elimination of dissolved oxygen during thermal processing.

Active Hydrogen score (rH) is the absolute indicator of the reductive potential of a substance which shows the number of ions in active hydrogen in solutions, of either organic or inorganic origin. This is a direct indicator of Total antioxidant content in the juice sample (Amorati and Valgimigli, 2015). However, the rH values calculated using the respective ORP values showed a significant difference between treatments

Table 1. TSS, pH and Titratable acidity values for different treatment conditions

Sample	Total Soluble Solids	pH	Titratable acidity
Untreated	11.07 ± 0.11^{b}	3.70 ± 0.005^{b}	1.36 ± 0.04^{b}
Hot Filled	14.00 ± 0.10^{a}	3.76 ± 0.005^{a}	1.81 ± 0.04^{a}
O_3 15 min treated	11.07 ± 0.06^{b}	3.70 ± 0.005^{b}	1.39 ± 0.05^{b}
O_3 30 min treated	11.03 ± 0.06^{b}	3.69 ± 0.005^{b}	1.36 ± 0.10^{b}
O_3 45 min treated	11.07 ± 0.12^{b}	3.69 ± 0.005^{b}	1.30 ± 0.00^{b}
O_3 60 min treated	11.00 ± 0.10^{b}	3.70 ± 0.005^{b}	1.41 ± 0.03^{b}

*Data presented as mean values for three replicates ± S.D. (n=3). Mean in the same column that does not share a letter significantly different at 5% significance level (Tukey HSD test).

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Table 2. Colour parameters of Pineapple juice under different treatment conditions

Sample	*L**	*a**	*b**	Chroma	Hue Angle
Untreated	14.59 ± 0.18^{d}	0.24 ± 0.03^{c}	3.55 ± 0.13^{b}	3.56 ± 0.13^{b}	86.03 ± 0.35^{a}
Hot Filled	14.10 ± 0.13^{e}	$0.35 \pm 0.05^{b,c}$	4.66 ± 0.09^{a}	4.67 ± 0.09^{a}	85.69 ± 0.71^{a}
O_3 15 min treated	$14.74 \pm 0.17^{c,d}$	0.55 ± 0.05^{a}	3.06 ± 0.05^{c}	3.11 ± 0.04^{c}	79.75 ± 0.06^{b}
O_3 30 min treated	$14.87 \pm 0.02^{b,c}$	0.48 ± 0.03^{a}	2.80 ± 0.06^{d}	2.84 ± 0.06^{d}	80.20 ± 0.55^{b}
O_3 45 min treated	15.00 ± 0.05^{b}	0.50 ± 0.03^{a}	2.76 ± 0.01^{d}	2.80 ± 0.01^{d}	79.58 ± 0.64^{b}
O_3 60 min treated	15.29 ± 0.03^{a}	$0.45 \pm 0.03^{a,b}$	2.41 ± 0.04^{e}	2.45 ± 0.03^{e}	79.39 ± 0.85^{b}

*Data presented as mean values for three replicates ± S.D. (n=3). Mean in the same column that does not share a letter significantly different at 5% significance level (Tukey HSD test).

Table 3. ORP and rH values for Pineapple juice under different treatments

Sample	ORP	Active H score
Untreated	267.67 ± 2.31^{b}	22.99 ± 0.06^{b}
Hot Filled	249.67 ± 2.52^{c}	22.52 ± 0.08^{c}
O_3 15 min treated	267.33 ± 1.52^{b}	22.99 ± 0.06^{b}
O_3 30 min treated	269.33 ± 0.57^{b}	23.03 ± 0.01^{b}
O_3 45 min treated	270.66 ± 0.57^{b}	23.08 ± 0.03^{b}
O_3 60 min treated	275.33 ± 1.52^{a}	23.25 ± 0.06^{a}

*Data presented as mean values for three replicates ± S.D. (n=3). Mean in the same column that does not share a letter significantly different at 5% significance level (Tukey HSD test).

($p < 0.05$). The highest score was reported in 60 minutes ozone-treated sample, implying that it contains the highest total antioxidant content. At 15 minutes, 30 minutes and 45 minutes, ozone-treated samples share no significant difference in rH score with the untreated sample.

Considering the viscosity of pineapple juice, there is a significant difference at 60 rpm and 100 rpm for the untreated, thermally processed and ozone-treated juice samples (Table 4). The thermally processed juice sample shows the highest mean viscosity value, 11.86

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± 0.30 and 13.60 ± 0.10 at 60 rpm and 100 rpm, respectively. At 60 minutes, ozone processing viscosity decreased significantly, showing the least mean viscosity of all treatments.

Obtained results agree with the findings of prior research which reported a decrease in apparent viscosity in ozonised apple juice (1–4.8% w/w ozone) during 12 minutes of exposure (Torres *et al.*,2011). Similar results were obtained in the ozone processing of peach juice (Jaramillo-Sánchez *et al.*,2018).

Fruit juices exhibit two different phases as the pulp and the serum. The fruit pulp comprises fruit tissue cells, whereas the serum is a mixture of sugars, acids, salts, and soluble polysaccharides. Therefore, the rheology of fruit juices is defined by the interactions inside and between each phase. Because of its strong oxidising activity, ozone exposure has been reported to decrease the molecular weight of food polymers as pectins, resulting in a decrease in viscosity (Muthukumarappan *et al.*,. 2016).

3.3 Bioactive compounds and antioxidant activity

According to Table 5, total phenolic content in untreated and thermally processed juice samples have no significant difference ($p < 0.05$). Considering the ozone-treated samples, the highest polyphenolic content was recorded in 60 minutes ozone-treated sample, which is 866.67 ± 15.28 mg of gallic acid equivalents per 200ml of sample on a dry weight basis. Results showed that TPC significantly increased when exposure time increased.

This reaction to ozone treatment could be attributable to the activation of phenylalanine ammonia-lyase (PAL; EC 4.3.1.5). PAL is one of the key enzymes used in the synthesis of phenolic compounds in plant tissues. Recent researchers found that the activation of PAL in mango 'Haden' fruits were strongly correlated with the increase in the phenolic content of the fruits (González-Aguilar, Zavaleta-Gatica and Tiznado-Hernández, 2007). According to some research studies PAL can be stimulated by different stimulants rather than wounding (Camm and Towers, 1973). The increase in the phenolic contents of the juice might have also been caused by cell wall modification that occurred during ozone exposure; this modification may have increased the extractability and freed some of the conjugated phenolic compounds in the cell wall. Studies have suggested that the walls of guard and subsidiary cells of ^{o}3-treated spruce needles became de-lignified through the reaction with ozone (ozonolysis), which resulted in the accumulation of non-lignin phenolic compounds (Maier-Maercker and Koch, 1992).

DPPH radical scavenging activity of untreated thermally processed and ozone-treated samples show a significant difference ($p < 0.05$). According to Table 6, the highest mean value for DPPH radical scavenging activity was observed in the untreated sample. However, during ozonation DPPH radical scavenging activity decreased compared to the untreated sample. But there is no such pattern in the reduction of DPPH radical scavenging activity with ozone exposure time.

Table 4. Viscosity values for juice under different treatments

Sample	Viscosity at 60 rpm (cP)	Viscosity at 100 rpm (cP)
Untreated	10.10 ± 0.10^{b}	12.23 ± 0.15^{b}
Hot Filled	11.86 ± 0.30^{a}	13.60 ± 0.10^{a}
O_3 15 min treated	10.10 ± 0.10^{b}	12.20 ± 0.20^{b}
O_3 30 min treated	10.03 ± 0.15^{b}	12.13 ± 0.15^{b}
O_3 45 min treated	10.03 ± 0.20^{b}	12.10 ± 0.10^{b}
O_3 60 min treated	9.46 ± 0.15^{c}	11.60 ± 0.10^{c}

*Data presented as mean values for three replicates ± S.D. (n=3). Mean in the same column that does not share a letter significantly different at 5% significance level (Tukey HSD test).

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Table 5. Total Polyphenolic content of juices under different treatments

Sample	mg of Gallic acid equivalents/ ml of sample	mg of Gallic acid equivalents/ 200ml of Sample (1 Bottle)
Untreated	1.81 ± 0.05^{d}	363.33 ± 11.55^{d}
Hot Filled	1.65 ± 0.00^{d}	330.00 ± 00.00^{d}
O_3 15 min treated	2.58 ± 0.07^{c}	516.67 ± 15.28^{c}
O_3 30 min treated	3.15 ± 0.10^{b}	630.00 ± 20.00^{b}
O_3 45 min treated	4.18 ± 0.11^{a}	836.70 ± 23.10^{a}
O_3 60 min treated	4.33 ± 0.07^{a}	866.67 ± 15.28^{a}

*Data presented as mean values for three replicates ± S.D. (n=3). Mean in the same column that does not share a letter significantly different at 5% significance level (Tukey HSD test).

Table 6. DPPH radical scavenging activity of juices under different treatments

Sample	mg of β ascorbic acid equivalents/ml of sample	mg of ascorbic acid equivalents / 200ml of Sample (1 Bottle)
Untreated	0.04788 ± 0.00^{a}	9.576 ± 0.009^{a}
Hot Filled	0.04488 ± 0.00^{c}	8.976 ± 0.009^{c}
O_3 15 min treated	$0.04552 \pm 0.00^{b,c}$	$9.105 \pm 0.019^{b,c}$
O_3 30 min treated	$0.04551 \pm 0.00^{b,c}$	$9.102 \pm 0.038^{b,c}$
O_3 45 min treated	0.04611 ± 0.00^{b}	9.222 ± 0.039^{b}
O_3 60 min treated	0.04500 ± 0.00^{c}	9.000 ± 0.057^{c}

*Data presented as mean values for three replicates ± S.D. (n=3). Mean in the same column that does not share a letter significantly different at 5% significance level (Tukey HSD test).

Considering the results obtained in Total Polyphenolic content (Table 5), and DPPH radical scavenging activity (Table 6), there is some contradiction, as ozone treatment has induced the antioxidant content in TPC, wherein DPPH radical scavenging activity shows a reduction in antioxidant content after ozonation. One explanation is 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 samples to different extents, which might have led to the reduction of DPPH radical scavenging activity of the samples after ozone exposure (Hoigné and Bader, 1983).

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4. Conclusion

It can be concluded that ozone can be a helpful process in low contaminated juices since a reduction of around 2 log cycles of aerobic bacteria and yeast and mould were obtained after 60min. However, ozone treatments significantly affected some of the quality characteristics analysed in the pineapple juice. The most prominent changes were observed for colour and antioxidant activity. Despite these negative impacts of ozone on the juice, the total phenolics content increased as the exposure time increased, with 68% of the variation. In conclusion, the effect of ozone on the nutritional and organoleptic quality of pineapple juice must be considered before adopting it as a preservation technique. Different ozone concentrations and exposing times should be tested, aiming at minimising overall quality losses.

Conflict of interest

The authors declare no conflict of interest

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