Current Science International
Volume: 12 | Issue: 04| Oct. – Dec.| 2023

EISSN:2706-7920 ISSN: 2077-4435
DOI: 10.36632/csi/2023.12.4.49
Journal homepage: www.curresweb.com
Pages: 655-667

Effect of Ozone Treatment on the Physiochemical Microbiological and Sensory Properties of Guava and Pomegranate Juices Packed in Two Types of Packaging

Ginat El-Sherif [1], Aziza T. Gamal[1] and Abeer M. F. Elbaz [2]

[1]Horticultural Crops Res. Dept., Food Tech. Res. Inst., Agric. Res. Center, Giza, Egypt
[2]Food Engineering & Packaging Res. Dept., Food Tech. Res. Inst., Agric. Res. Center, Giza, Egypt

Received: 25 Oct. 2023 **Accepted:** 30 Nov. 2023 **Published:** 10 Dec. 2023

ABSTRACT

Samples of guava and pomegranate juice were treated with ozone, with a processing variable of 8 ppm and a 5-minute treatment duration. The impact of processing variables was assessed during 14 days of cold storage (4℃) on the intrinsic microflora inactivation, physio-chemical characteristics, nutritional quality, and color values L, a*, and b*. However, compared to the pasteurizing process, the study's results show that ozonated samples were able to retain the majority of their antioxidant activities (DPPH was about 85% and total phenolic compounds was about 90%), as well as lower their intrinsic microflora loads. On the other hand, the ascorbic acid content of the guava juice samples (35%) and the anthocyanins and ascorbic acid of the pomegranate samples (37% and 86%, respectively) were significantly reduced by the same results. Results also showed that ozone processing has a greater impact on visual color. So, it must be taken into consideration the impact of ozonation on the nutritional qualities of fruit juices before using it as a preservation method.

Keywords: Guava and pomegranate juice. ozone treatment. physicochemical properties, microbial inactivation, nutritional quality

1. Introduction

Due to their nutritional and organoleptic qualities, guavas (*Psidium guajava* L.) and pomegranates (*Punica granatum*) are two of the most significant fruits that humans have consumed since ancient times. They are known to be excellent sources of vitamins, minerals, and bioactive compounds with a pleasant flavor and low-calorie content.

In many communities today, fruits and their juices are starting to play a significant role in the diet. Because of their delicious flavor and the range of naturally occurring nutrients they contain, they are nutrient-dense and play a big part in a balanced diet. While pomegranates contain large amounts of antioxidant components like anthocyanins, vitamin B6 and K, and polyphenols that contribute to weight loss slimming as a healthy diet to human body (Nyarko *et al.,* 2016). Guavas contain five times more vitamin C than citrus fruits and a large amount of potassium and magnesium (Shah *et al.,* 2022).

Owing to their high nutrient content and healthful attributes, pomegranate and guava are widely used as raw materials in the fruit juice industry.

According to Ukuku *et al.* (2016), the majority of fresh juices have a population of 4–6 log microorganisms per gram due to their composition, which promotes the growth of pathogenic and spoilage microorganisms. Juice consumption linked to 34 foodborne disease outbreaks reported by the Centers for Disease Control and Prevention between 1973 and 2011 (Nyarko *et al.,* 2016). As a result, fruit juices are typically preserved through a process called pasteurization, which involves quick heating and cooling and gives the juices a long shelf life. However, the highest ratio of nutrients and physicochemical characteristics of fruit juices were damaged by the heat treatment. It also had an impact on the flavor, color, and taste. As a result, interest in non-thermal treatments is growing. Food products can be preserved using a variety of non-thermal methods, including ozone exposure, high-

Corresponding Author: Ginat El-Sherif, Horticultural Crops Res. Dept., Food Tech. Res. Inst., Agric. Res. Center, Giza, Egypt.

pressure processing (HPP), pulsed electric field (PEF), and ultraviolet (UV) treatment. As an allotrope of oxygen, ozone is a triatomic molecule (O3) that is far less stable than diatomic oxygen (O2). It breaks down quickly into oxygen and a lot of free radicals, with no harmful byproducts remaining (Tiwari *et al.,* 2009). Ozone was classified as "Generally Recognized as Safe" (GRAS) in 2001, which allowed the FDA to approve its use as a direct food additive for food processing, storage, and treatment (Khadre *et al.,* 2001). As a result, many commercial fruit juice processors in the US and Europe announced that they would begin using ozone as a substitute non-thermal fruit juice pasteurization treatment for citrus and apple juices. Ozone is safe for consumption because it breaks down quickly and leaves no residue inside food products. Products that have been treated with ozone are categorized as organic food worldwide (Shah *et al.,* 2022).

According to Tiwari *et al.* (2009) the FDA mandated that the fruit juice industry implement treatments that would enable a reduction of 5 log10 of pathogenic microorganisms and/or target spoilage. Singly *et al.* (2021) found that ozone efficiently killed gram-positive bacteria like Listeria monocytogenes, staphylococcus aureus, and gram-negative bacteria like Pseudomonas aeruginosa and Yersinia enterocolitica. Additionally, they assessed how well it preserved a range of food items, such as milk, meat products, gelatin, albumin, and casein.

The authors proved that the primary mechanisms of ozone-induced microbial inactivation are the rupturing of cellular membranes and the dispersion of cytoplasm. Bacterial membranes' organic matter is oxidized by ozone, weakening cellular walls and resulting in cell damage and eventual cell death. Ozone pasteurization is a desirable alternative for the food Although there are several ways to produce ozone, including electrical discharge, electro-chemical method, UV method, and radio-chemical method, electrical discharge is the method most frequently used to apply ozone. According to Toprak, (2021), this technique used oxygen-sourced plasma to transfer electron energy in dominant O_2 gas molecules during the collision process, producing the primary radical O*. industry because of these benefits.

The residual ozone in the medium, temperature, humidity, medium pH, and the amount of organic matter surrounding the cells all affect how effective ozone is against microorganisms (Patil and Bourke, 2012).

Given the limited data available on the high reactivity and instability of ozone, the rate of inactivation of the microorganisms is greater in the ozone demand-free system than when the medium contains oxidizable organic substances. Aqueous ozone has a half-life of only 20 minutes at 20 °C, making it highly unstable and rapidly degrading into oxygen (Miller *et al.,* 2013).

It is therefore important to comprehend the key parameters that determine the impact of various compounds, including sugars, fibers, ascorbic acid, and other organic matters in the dissolved medium, in order to optimize its application for sterilizing liquid food products, as it is difficult to predict its reaction in the presence of different organic matters. Furthermore, ozone's availability and rate of dissolution have given some components a protective effect (Patil and Bourke, 2012).

Juice's medium pH is another crucial intrinsic component of ozone efficacy in the disinfection process; lowering juice pH can accelerate the rate at which ozone breaks down and increases ozone efficiency. Ozone disinfection may be most effective on low pH surfaces (Cullen *et al.,* 2010).

Thus, fruit juice ozonation is still in its infancy, as ozone is only now being approved as a direct food additive for the treatment. Foods were stored and processed in both the gaseous and aqueous phases in 2001 (Khadre *et al.,* 2001). Based on the scant information in the literature regarding the high reactivity and instability of ozone, it is challenging to forecast how the gas will react when organic materials are present (Cho *et al.,* 2003). It could break down spontaneously into oxygen and free radicals, or it could oxidize or ionize a substrate. Juices that contain organic compounds, such as carotenoids, ascorbic acid (AA), or other organic acids, may degrade in the presence of ozone due to direct reactions with the gas or indirect reactions caused by secondary oxidators (Tiwari *et al.,* 2009). For this reason, processors should think twice before ozonating juices as a preservation method. At an ozone concentration of 7.8% (w/w) and a treatment time of 10 minutes, Tiwari *et al.* (2009) observed a significant reduction in the anthocyanin content (98.2%) of strawberry juice and in ascorbic acid (85.8%) of orange juice. However, ozone had minor effects at small concentrations (4–8 ppm) and treatment times of 5–10 min.

Water or soft drink bottles were typically packed in glasses and polymeric plastic materials, particularly polyester (PET) and polyethylene terephthalate (PET), to preserve the natural taste, color, and flavor of the juices and beverages.

The effectiveness of these containers has been the subject of numerous studies in various contexts, but not much research has been done on the microbial and sensory quality of these containers under various heating conditions inside the selling market.

The aim of this study was to examine and contrast the impact of ozonation on the physiochemical, antioxidant, sensory, and microbial properties of pomegranate and guava juices. These juices differ in terms of their organic matter content and microbial intrinsic numbers. These findings are compared to the quality characteristics of thermally pasteurized samples, and two different kinds of juice-packing containers glass and polyester are assessed. Before and after treatments, as well as during 14 days of cold storage at 4oC, the evaluation was completed.

2. Material and methods

2.1. Sample preparation

Guava and Pomegranate were purchased after harvest from particular orchard (Wadi El-Natron). Juices were obtained using a domestic centrifuge (Centrifugal juicer Excel JE 850 UK). Polyester and glass bottles were purchased from El-Shark Co., October city.

2.2. Chemicals and reagents

All chemical and reagents were of analytical grade and were obtained from Sigma-Aldrich Chemical Co. (St. Louis MO. USA).

2.3. Ozone treatments

Utilizing corona discharge apparatus (Model SY004, Taiwan), ozone gas was produced. 200 ml of juice was constantly stirred in a glass beaker that was directly pumped with ozone gas. The ozone output concentration was determined using a New Zealand Model 200 series ozone sensor. The juice was directly pumped with a regulated flow of ozone gas. In order to make sure the ozone molecules were thoroughly combined with the samples, up to 8 ppm were added to the beaker and stirred for 5 minutes at 100 rpm using a magnetic stirrer. The treatment temperature of the gas was set at 20℃, and the gas flow rate was fixed at 0.12 L/min, producing an ozone concentration of 4.8% w/w of oxygen. Fruit juices without treatment (Control = 0 min. of ozone exposure) of the samples, half were stored at 4 ±℃ in sterile dark containers for a maximum of 14 days.

One group filled glass bottles, and the other half-filled polyester bottles. Throughout the storage period, physiochemical and microbiological characterization analyses were conducted both before and after treatments.

2.4. Thermal pasteurization:

A thermostatic water bath with temperature control and stirring capability (Julabo FP40, Seel Bach, Germany) was used to carry out the treatment. The juices were directly cooled to 4oC after being heated to 72^{o}C for 15 seconds.

2.5. Microbiological analysis

Buffer-treated peptone water (BPW) was used for juice decimal dilutions in order to quantify total mesophylls, yeast, and molds. Plate Count Agar – PCA was used to measure total mesophylls in duplicate (Lab M, Lancashire, UK). The samples were incubated for 48 hours at 37°C. Rose Bengal Agar, or RBA, was used to identify yeasts and molds in duplicate (Lab M. Lancashire, UK). The samples were incubated for 120 hours at 25 oC. Both treated and untreated samples were analyzed, as well as samples that were in storage.

2.6. Physiochemical analysis

2.6.1. Total soluble solid content and pH

A Palette PR-32 digital refractometer (Atago, Tokyo, Japan) was used to measure the juice's TSS content, which was expressed as

Brix. A pH meter (GLP 22, Cris on instruments) was used to measure the juice's pH and total soluble solids (TSS) content. For every replicate, two measurements were made.

2.6.2. Color properties

The Minolta CR-400 colorimeter (Konica-Minolta, Osaka, Japan) was utilized to measure the juice color coordinates, which are L*, a*, and b*. For every sample, three separate replicates were measured twice .

To assess how samples' colors changed, total color difference (TCD) was computed using Equation 1 (Ihns *et al.,* 2011). Greater TCD values signify a more noticeable decline in color.

$$\text{TCD} = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)}$$

Where the "0" indicates the reference sample.

2.7. Bioactive compounds

2.7.1. Total phenolic content

According to Voung *et al.,* (2015), total phenolic content (TPC) was measured spectrophotometrically using Folin Ciocalteu's reagent assay with gallic acid as standard. Folin-ciocalteu's reagent (0.5 ml) was combined with the sample (0.1 ml) and swirled. Following three minutes, 1.5 ml of a 7% sodium carbonate solution was added, stirred, and topped off with 10 ml of distilled water. The absorbance was determined at 750 nm using spectrophotometer (Unicum UV 300). Total phenolics in samples were expressed as mg Gallic Acid Equivalents / 100 g (using a calibration curve).

2.7.2. Determination of ascorbic acid

The ascorbic acid analysis HPLC method (Chromatography system) was adapted from Gird *et al.,* (2018). In short, 10 ml of distilled water, 4 ml of methanol, and 4 ml of sample were mixed, and the mixture was filtered through a 0.45 µm membrane filter (Whatman Int. Ltd, UK). 1 ml of the filtrate was saved for analysis after the first three milliliters were thrown away. The sample was injected into the HPLC system in 20 µl increments. The column effluents were observed at 254 nm, which is the maximum UV absorbance wavelength for ascorbic acid. The findings were given as mg of juice per 100 ml.

2.7.3. Total anthocyanins

Juice sample total anthocyanin content was assessed using a Zabetakis *et al.,* (2000) method. In a nutshell, the juice was diluted with 20/30/ 1: v/v/v of ethanol, water, and HCl (0.12 m(20/30/ 1:v/v/v), and the absorbance was assessed at 540 nm wavelength. The results were expressed as mg of malvidin-3-o-glucoside equivalents/100g (mg ME/100g), with malvidin-3-o-glucoside serving as the calibration standard.

2.7.4. Total antioxidant activity (TAA)

TAA was calculated using the following procedure: Brand-William *et al.,* (1995). To obtain 0.1 mM stock solution, 100 mL of pure methanol and 4 mg of DPPH were briefly dissolved. After that, it was kept at 20±1oC until needed again. The sample was combined with 5 milliliters of 80% methanol and allowed to incubate for 30 minutes. After that, the mixture was centrifuged for 10 minutes at 3,000 rpm. After 30 minutes at room temperature in the dark, 200 µL of juice samples were vortexed before being reacted with 1 ml of the DPPH_ Methanol solution. The absorbance at 515 nm was then measured using a UV spectrophotometer (Model UV-1700, Shimadzu, Japan). The standard curve for methanol and DPPH radicals of 10 to 200 µg/ml was established and results were expressed as the percentage of decline of the absorbance using Equation:

$$\text{DPPH\%} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100.$$

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

2.7.5. Sensory properties

Eight experienced employees made up the sensory panel. After being introduced to the guava and pomegranate juice samples during storage, the panelists were asked to assess them based on color, taste, texture, and overall acceptability. Five points were assigned to each sample; one point denotes the greatest dislike and five, the greatest like (Macfie and Bratchell, 1989).

2.8. Statistical analysis

The analysis was replicated three times, SPPS version 16 statistical software (SPSS, Inc., United States) was utilized to analyze the results. Means of the three replicates were compared using one-way analysis of variance (ANOVA). The differences between DBD plasma-treatment means were evaluated using Duncan's test ($p<0.05$).

3. Results and Discussion

3.1. Physio-chemical characteristics

The fresh Guava and pomegranate fruit juices' initial characteristics (means ± standard deviation) are displayed in Table (1) before they are pasteurized or ozonized. In order to determine the significant changes that are influenced by the type of juice and type of treatment, these values are essential .TSS, turbidity, and color are the primary factors used to define fruit juice quality (Anam *et al.,* 2019).

TSS is a crucial metric that evaluates the quality of fruit juice. TSS primarily describes the quantity of sugars; organic acids, vitamins, proteins, free amine acids, essential oils, and glucosides are included in smaller amounts.

The second crucial physical factor affecting the quality of fruit juices is turbidity. The most accurate way to determine the concentration of polydisperse particles in a solution is to measure the light scattering properties of the solution (Vaillant *et al.,* 2008). The shape, size, and mineral composition of the particles have a major role in the turbidity of the dispersions. Additionally, their relative refractive index and, to a lesser extent, the liquid phase's color play a role (Vaillant *et al.*, 2008)

Fruit juices' actual color is also a very significant factor. Hunter's measurements served as its definition. Hunter's a and b values change two times in tandem with L's value changes, or vice versa. As a result, combinations of L, a, and b values should be taken into consideration to characterize any visual color degradation. These numbers could be regarded as the physical characteristics that characterize the deterioration of visual color.

Table 1: Physio-chemical parameters fresh Guava and Pomegranate

Parameters	Guava juice	Pomegranate juice
Total soluble solids (ºBrix)	12.50 ± 0.43^{a}	9.87 ± 0.30^{a}
pH	4.21 ± 0.06^{a}	3.11 ± 0.09^{c}
Turbidity (NTU)	516 ± 0.9^{a}	395.00 ± 0.04^{b}
Ascorbic acid (mg/ 100 ml)	208 ± 0.13^{b}	45.30 ± 0.20^{cd}
T. phenolic content (GAE mg / 100 ml)	380.20 ± 0.04^{c}	194.43 ± 0.08^{c}
Anthocyanins (mg ME/ 100 ml)	--	52.50 ± 5.7^{ad}
DPPH %	95.48 ± 0.09^{cd}	81.24 ± 0.4^{cb}
Lightness (L*)	46.90 ± 0.09^{a}	39.85 ± 0.15^{c}
Redness (a*)	4.80 ± 0.11^{d}	34.60 ± 0.26^{cd}
Yellowness (b*)	20.20 ± 0.07^{b}	33.07 ± 0.13^{c}
Total plate count (log CFU/ml)	5.40 ± 0.03^{b}	5.13 ± 0.01^{a}
Yeasts & molds (log CFU/ml)	4.46 ± 0.41^{cd}	4.29 ± 0.19^{d}

Values followed by same letter in a row are not significant ($p>0.05$)

3.2. Bioactive parameters and total antioxidant activity

One of the bioactive markers used to evaluate the effects of ozone and heat treatments on fruit juices was total phenolic content. This class of substances contributes to the flavor and color

characteristics of fruits and has the potential to be antioxidants with health-promoting qualities (Rabie *et al.,* 2015). The findings, which are presented in Tables (2, 3), show that after 14 days of cold storage (4oC), there were no discernible changes between the two treatments or between the fresh and treated samples.

Table 2: Characteristics of immediately pasteurized (72ºC/15 sec.) and ozonized (8 ppm/ 5min.) of pomegranate juice and during 14 days of storage at 4ºC in glass bottles.

Parameters	Pasteurized juice			Ozonated juice		
	Zero time	7 days	14 days	Zero time	7 days	14 days
TSS (ºBrix)	9.87 ± 0.08^{a}	9.41 ± 0.0	9.27 ± 0.05^{a}	9.87 ± 0.03^{c}	9.50 ± 0.14^{d}	9.32 ± 0.06^{a}
pH	3.11 ± 0.01^{a}	3.07 ± 0.42^{c}	3.02 ± 0.09^{b}	3.11 ± 0.17^{b}	3.09 ± 0.08^{b}	3.06 ± 0.02^{b}
Turbidity(NTU)	395.00 ± 0.11^{b}	381.0 ± 0.21^{d}	374.0 ± 0.21^{c}	395.0 ± 0.10^{cd}	388.00 ± 0.02^{a}	365.0 ± 0.11^{a}
Ascorbic acid (mg/100 ml)	17.12 ± 0.31^{a}	15.07 ± 0.09^{a}	13.10 ± 0.10^{cd}	29.03 ± 0.06^{c}	26.00 ± 0.07^{b}	22.22 ± 0.25^{cd}
Anthocyanins (mg/100 ml)	19.42 ± 0.21^{d}	16.47 ± 0.11^{b}	12.52 ± 0.31^{c}	7.35 ± 0.08^{c}	6.15 ± 0.11^{a}	5.82 ± 0.28^{c}
Total phenols (GAE mg/100 ml)	192.43 ± 0.14^{cd}	190.47 ± 0.7^{cb}	186.83 ± 0.25^{ab}	192.43 ± 0.14^{d}	191.56 ± 0.34^{c}	187.00 ± 0.01^{b}
DPPH (%)	74.00 ± 0.14^{ab}	72.09 ± 0.04^{b}	69.26 ± 0.09^{d}	72.50 ± 0.20^{b}	69.46 ± 0.25^{c}	66.58 ± 0.09^{d}
Total plate count (log CFU/ml)	0.75 ± 0.01^{d}	0.79 ± 0.04^{c}	0.90 ± 0.08^{ab}	0.48 ± 0.32^{ab}	0.48 ± 0.16^{b}	0.48 ± 0.04^{d}
Yeasts & Molds (log CFU/ml)	0.45 ± 0.06^{cd}	0.48 ± 0.01^{d}	0.53 ± 0.03^{c}	3.43 ± 0.19 ^{ac}	3.43 ± 0.32^{cd}	3.43 ± 0.07^{ab}

The slight reduction of phenolic contents due to ozone decomposition were scavenged by phenolic compounds or due to the oxidation of some aromatic phenolic compounds by heat treatment (Wibowo *et al.,* 2015b).

Table 3: Characteristics of immediately pasteurized (72ºC/15 sec.) and ozonized (8 ppm/ 5min.) of Guava juice and during 14 days of storage at 4ºC in glass bottles.

Parameters	Pasteurized juice			Ozonated juice		
	Zero time	7 days	14 days	Zero time	7 days	14 days
TSS (ºBrix)	12.50 ± 0.02a	11.83 ± 0.07c	11.38 ± 0.10a	12.50 ± 0.03a	12.08 ± 0.04b	11.47 ± 0.06a
pH	4.21 ± 0.07c	4.09 ± 0.01d	4.02 ± 008a	4.21 ± 0.21cd	4.14 ± 0.03a	4.06 ± 0.01a
Turbidity(NTU)	516.0 ± 0.18c	507.0 ± 0.09a	480.0 ± 0.06b	516.0 ± 0.02a	509.0 ± 0.02cd	500.0 ± 0.05c
Ascorbic acid (mg/100 ml)	79.16 ± 0.05	67.29 ± 0.12a	52.15 ± 0.04b	135.41 ± 0.05c	124.88 ± 0.14 ab	114.94 ± 0.12
Anthocyanins (mg/100 ml)	--	--	--	--	--	--
Total phenols (GAE mg/100 ml)	378.20 ± 0.21ab	376.00 ± 0.24b	370.09 ± 0.19ab	378.20 ± 0.17d	376.91 ± 0.03b	373.55 ±0.09cd
DPPH (%)	87.93 ± 0.06cd	84.15 ± 0.05cd	80.82 ± 0.09c	84.97 ± 0.03d	82.07 ± 0.19 ab	79.50 ± 0.09cd
Total plate count (log CFU/ml)	0.77 ± 0.04c	0.82 ± 0.19ab	0.93 ± 0.15cd	0.54 ± 0.08c	0.54 ± 0.24b	0.54 ± 0.32c
Yeasts & Molds (log CFU/ml)	0.98 ± 0.09a	1.03 ± 0.04b	1.15± 0.18c	3.56 ± 0.11b	3.56 ± 0.05c	3.56 ± 0.40d

Guava and pomegranate juices' ascorbic acid and anthocyanin pigments, respectively, are also thought to serve as indicators of their nutritional value. Following the application of both treatments, the current study's tables (2, 3) and Figure (2a) showed a significant decrease in these parameters.

Only about 38% of the vitamin C was preserved in pasteurized guava and pomegranate juices, whereas ozonated juices retained roughly 65%. Due to its instability during processing, ascorbic acid is degraded by exposure to high temperatures and oxygen (Wibowo *et al.,* 2015b). It degrades less when exposed to ozone gas; this may be because ozone exposure can induce oxidative stress in cells, which likely triggers defense mechanisms and preserves some antioxidant compounds, like vitamin C (Miller *et al.,* 2013). After both treatments, Table (2,3) and Figure 2, B demonstrate a significant reduction in anthocyanin contents and degradation. Ozonated pomegranate juice exhibits an abrupt 86% decrease in content after exposure, whereas pasteurized pomegranate juice only experiences a 63% decrease after heat treatment. Because anthocyanins are unstable, processing conditions such as pH, temperature, light, oxygen, and enzymes can have a significant impact on their stability (Rein and Henonen, 2004). In the meantime, the strong oxidizing potential of ozone, which is produced from the nascent oxygen atom, may be the cause of the anthocyanin degradation in pomegranate juice caused by ozone processing (Tiwari *et al.,* 2009) .

Ozonated and/or pasteurized pomegranate samples did not significantly decrease over the course of 15 days of cold storage ($p \leq 0.05$).

3.3. Comparison of the impact of thermal pasteurization and ozone treatment

3.3.1. Physio-chemical parameters

Table (2,3) displays the impact of thermal pasteurization and a 5-min. 8 ppm ozone treatment on a few physio-chemical parameters of pomegranate and guava juices. Cloud, pH, and total soluble solids (TSS) are three crucial indicators of fruit juice quality. With a smaller amount of organic acid, vitamins, proteins, free amino acids, essential oils, and glucoside, TSS typically refers to the amount of sugars.
The range of TSS, pH, and turbidity in guava and pomegranate juices following ozone and heat treatment is depicted in Figure 1.a, b, and c. Neither of the two treatments had an impact on these parameters. No significant differences ($p \leq 0.05$) were observed in the values of TSS, pH, or clouds. This is consistent with the findings of multiple authors who also reported that thermally treated orange juice (Rabie *et al.,* 2015) and grape, orange, and tomato ozonized juices (Tiwari *et al.,* 2009) did not exhibit any appreciable changes in TSS, pH, or turbidity.

The current investigation also showed that flash pasteurization and/or the use of short-term gaseous ozone were maintained.

During the 14 days of cold storage (4℃), TSS, pH, and turbidity were nearly constant. Inconsistent findings were discovered in the literature. (Wibowo *et al.,* 2015) state that pasteurization had no effect on TSS when orange and lemon juices were stored. Nonetheless, (Zahid *et al.,* 2008) found that apple juice's TSS significantly increased over storage.

3.3.2. Microbial indicators

The intrinsic total mesophylls bacteria were not found in the juices of guava and pomegranates after 5 minutes of ozone exposure (4.8% w/w) (Table 2, 3). The log-reduction in guava and pomegranate juices was approximately 4.75 log cycles decay. Only in both juices was a log-reduction of roughly 0.88 log cycles decayed in relation to yeasts and molds. More exposure to ozone concentrations might be necessary to completely eradicate these microbes and cause significant inactivation.

In contrast, after 15 seconds of thermal pasteurization at 72°C, the intrinsic total mesophylls bacteria in guava and pomegranate juices showed decays of 4.63 and 4.38 log cycles, respectively, while the counts of yeast and molds showed reductions of 3.48 and 3.84 log cycles, respectively.
These outcomes aligned with (Sengly *et al.,* 2021) findings regarding cantaloupe melon juice.

Ozone allowed intrinsic microflora loads to be maintained for 15 days at 4℃ (Table 2, 3). On the other hand, in guava and pomegranate juices, thermal pasteurization did not stop the remaining living microflora from growing during the course of the 14 days of cold storage. After treatment, this microflora increased to roughly 5-6% after one week and about 20% after two weeks at 4oC.

Fruits have antioxidant properties because they contain vitamin C, carotenoids, and polyphenols. This parameter was significantly impacted by both processes (Table 2, 3). DPPH is the

reduction of guava and pomegranate juices by pasteurized and ozonated treatments, respectively, by 8% and 11% ($p<0.05$). (Wibowo *et al.,* 2015b) speculate that pasteurization may have oxidized anthocyanins, which have the potential to function as antioxidant compounds, and vitamin C. Because of its potent oxidizing properties, ozone also contributes to the loss of antioxidant compounds. Through ozone decomposition, ozone is known to increase scavenging activity and induce the production of reactive oxygen species (ROS) in juice. As a result of the juice's total phenolic content's scavenging activity, the absorbance decreases (Tiwari *et al.,* 2009).

Antioxidant activity in both ozonated and/or pasteurized treatments of guava and pomegranate juices showed the same trend of decreasing polyphenol contents during cold storage (Table 2, 3).

3.3.3. Color degradation

In (Table 4) results showed that, when compared to the reference (untreated samples), pasteurized and/or ozonized guava and pomegranate juice samples were lighter in color, or had an increased L* value, while their a* and b* values were lower. The discernible variations found in samples treated with ozone were easier to see than those found in samples treated with heat. The chromophore responsible for the color of fruit juices underwent oxidative cleavage as a result of the breakage of conjugated double bonds in the carotenoid and anthocyanin pigments. As a result, the hydroxyl radicals and ozone that are created may open these aromatic rings, allowing the ozone to interact with them more easily and partially oxidizing products like organic acids, aldehydes, and ketones (Melendez-Martinez *et al.,* 2007; Garcia and Bridle 1999).

There have been reports of other fruits, including cantaloupe (Sengly *et al.,* 2017), apple (Patil and Bourke 2012), and grape (Tiwari *et al.,* 2008), having their fruit juices negatively affected by ozone exposure.

The breaking of double bonds in organic compounds present in the juices that were impacted by heat temperature was the cause of the color degradation caused by heat treatment (Jaramillo Sanchez *et al.,* 2017).

Table 4: Effect of ozonized and pasteurized treatments on reaction rate constants for L*, a*, b* and TCD of Guava and Pomegranate juices.

Color Parameters	Guava juice			Pomegranate juice		
	Untreated	**Ozonated**	**Pasteurized**	**Untreated**	**Ozonated**	**Pasteurized**
L*	47.90 ± 0.01^{a}	70.65 ± 0.01^{b}	65.47 ± 0.08^{a}	39.85 ± 0.02^{a}	58.77 ± 0.08^{a}	54.39 ± 0.01^{b}
a*	4.85 ± 0.11^{c}	1.21 ± 0.04^{c}	2.33 ± 0.02^{c}	34.60 ± 0.12^{a}	8.94 ± 0.12^{c}	16.67 ± 0.06^{a}
b*	20.20 ± 0.03^{c}	10.10 ± 0.07^{a}	12.52 ± 0.01^{b}	33.07 ± 0.07^{d}	8.33 ± 0.03^{b}	20.60 ± 0.04^{a}
TCD	0.00 ± 0.11^{b}	6.03 ± 0.03^{a}	5.26 ± 0.13^{b}	0.0 ± 0.10^{c}	8.32 ± 0.02^{b}	6.70 ± 0.05^{d}

3.3.4. Sensory attributes

Overall, both the Guava and Pomegranate samples (Table 5) showed a slight change in all the sensory attributes (color, taste, texture, and overall acceptability) over the course of the two treatments and the entire storage period at 4℃, with the exception of the color scores of the ozonated samples, which differed significantly ($p<0.05$) from the first day scores of the untreated samples.

3.5. Packaging materials

Juices from guavas and pomegranates were packaged in two different materials and kept for a period of 14 days at 4℃ to determine how the material of the container affected the microbial quality and shelf life of the product. Glass is preferable to polyester plastic containers, according to Table 6 results; however, after 14 days of storage, no discernible changes were found in the counts of mesophilic bacteria, yeasts, or molds in the guava or pomegranate juices.

Curr. Sci. Int., 12(4): 655-667, 2023
EISSN: 2706-7920 ISSN: 2077-4435 **DOI: 10.36632/csi/2023.12.4.49**

Table 5: Sensory attributes of ozonized and pasteurized Guava and Pomegranate juice samples during storage at 4°C (mean*±SE)

Samples	Kind of juice	Storage time (days)	Color	Taste	Texture	General acceptance
Ozonated	Guava	Zero	4.80 ± 0.02^{a}	5.00 ± 0.04^{d}	4.93 ± 0.01^{c}	4.95 ± 0.02^{a}
		7	2.90 ± 0.04^{a}	4.80 ± 0.07^{a}	4.11 ± 0.06^{c}	4.20 ± 0.02^{b}
		14	2.72 ± 0.03^{a}	4.72 ± 0.03^{b}	3.85 ± 0.01^{a}	4.00 ± 0.04^{b}
	Pomegranate	Zero	5.00 ± 0.01^{b}	5.00 ± 0.02^{ab}	4.91 ± 0.10^{ab}	4.89 ± 0.07^{c}
		7	2.65 ± 0.01^{b}	4.62 ± 0.02^{ab}	4.83 ± 0.03^{abc}	4.78 ± 0.08^{c}
		14	2.41 ± 0.02^{c}	4.50 ± 0.05^{cd}	4.64 ± 0.09^{abc}	3.60 ± 0.10^{cd}
Pasteurized	Guava	Zero	4.82 ± 0.00^{a}	5.00 ± 0.02^{b}	4.90 ± 0.02^{ab}	4.92 ± 0.01^{a}
		7	4.73 ± 0.01^{a}	4.73 ± 0.01^{b}	4.00 ± 0.05^{ab}	4.30 ± 0.06^{b}
		14	3.69 ± 0.03^{a}	4.68 ± 0.02^{c}	3.72 ± 0.03^{ab}	3.93 ± 0.01^{b}
	Pomegranate	Zero	5.00 ± 0.10^{c}	5.00 ± 0.02^{c}	4.90 ± 0.02^{abc}	4.88 ± 0.04^{c}
		7	3.00 ± 0.05^{b}	4.56 ± 0.03^{ab}	4.79 ± 0.01^{abc}	4.70 ± 0.02^{cd}
		14	4.34 ± 0.08^{cd}	4.44 ± 0.04^{ab}	4.50 ± 0.10^{abc}	3.55 ± 0.03^{cd}

*Means with different superscript in a raw differ significantly ($p<0.05$)

Table 6: Effect of packaging material on the shelf-life of microbial load of juice samples during 14 days of storage at 4°C.

Microbes (log CFU/ml)		Guava juice				Pomegranate juice			
		Ozonated		Pasteurized		Ozonated		Pasteurized	
		7 days	14 days	7 days	14 days	7 days	14 days	7 days	14 days
Glass	Total plate count	0.54	0.54	0.82	0.93	0.48	0.48	0.79	0.90
	Yeasts & molds	3.56	3.56	1.03	1.15	3.43	3.43	0.48	0.53
Polyester	Total plate count	0.74	0.75	0.98	1.11	0.56	0.56	0.94	1.06
	Yeasts & molds	3.60	3.63	1.12	1.17	3.67	3.69	0.60	0.89

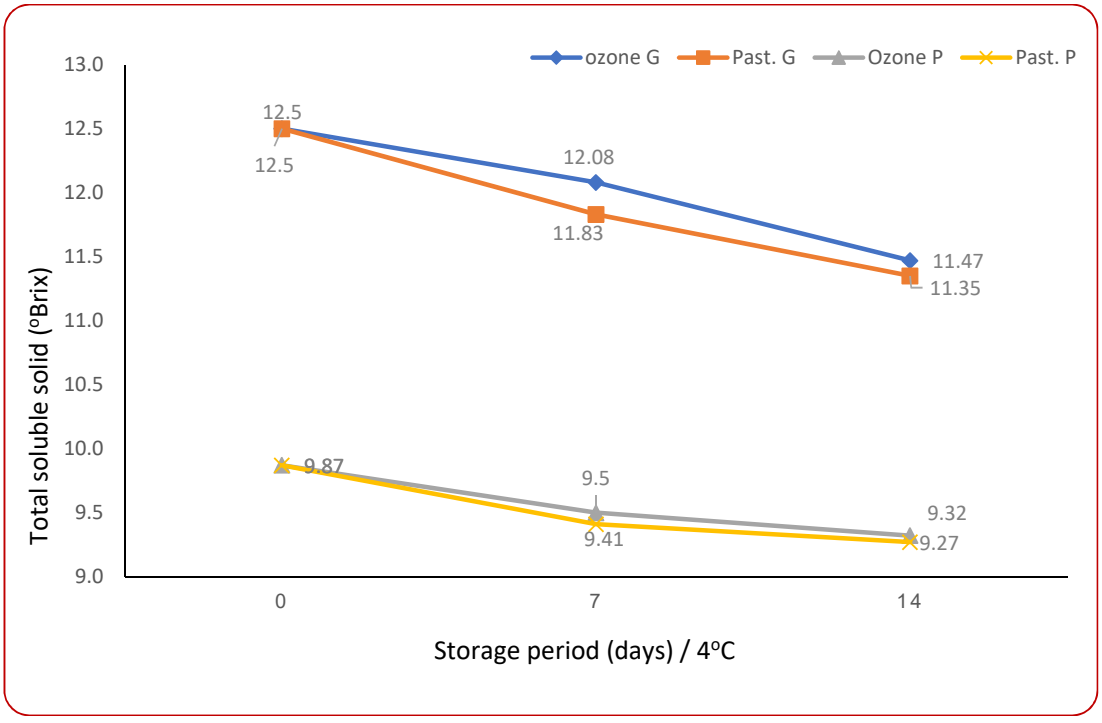

Fig. 1a: TSS for pasteurized and ozonated Guava and pomegranate during storage period/ 4°C

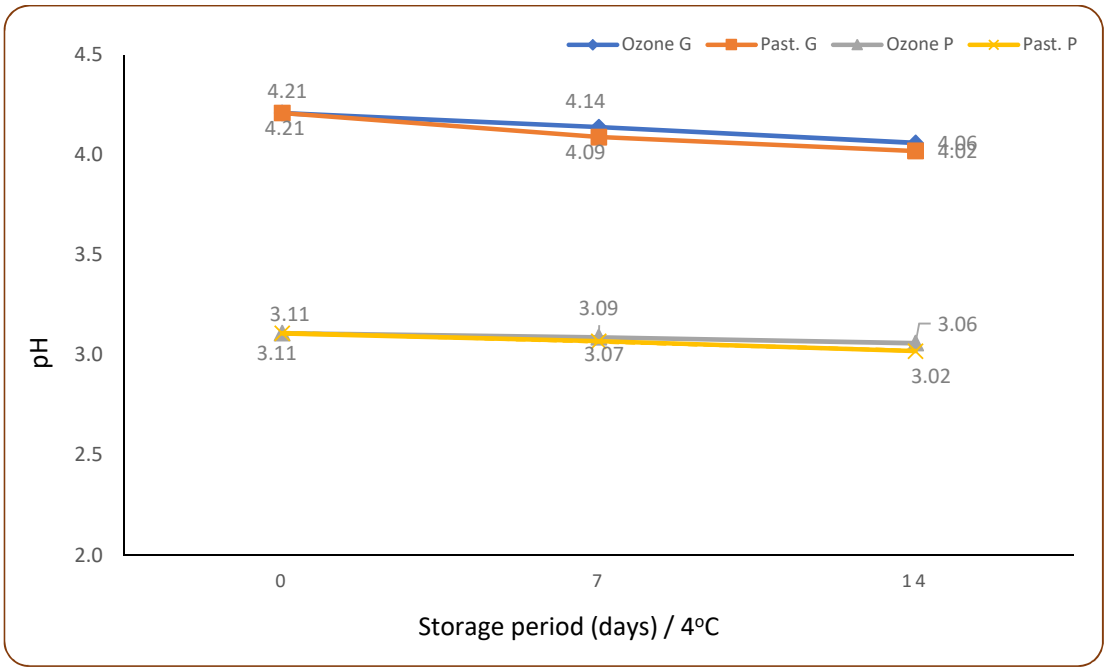

Fig. 1b: pH for pasteurized and ozonated Guava and pomegranate during storage period/ 4°C

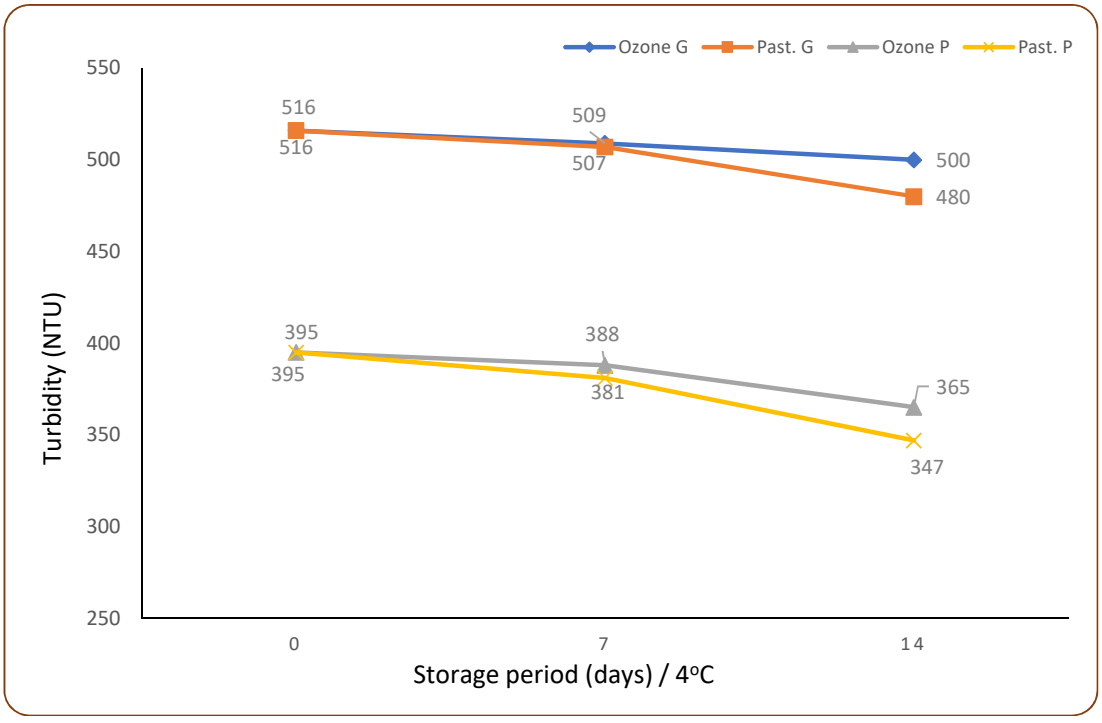

Fig. 1c: Turbidity for pasteurized and ozonated Guava and pomegranate during storage period/ 4°C

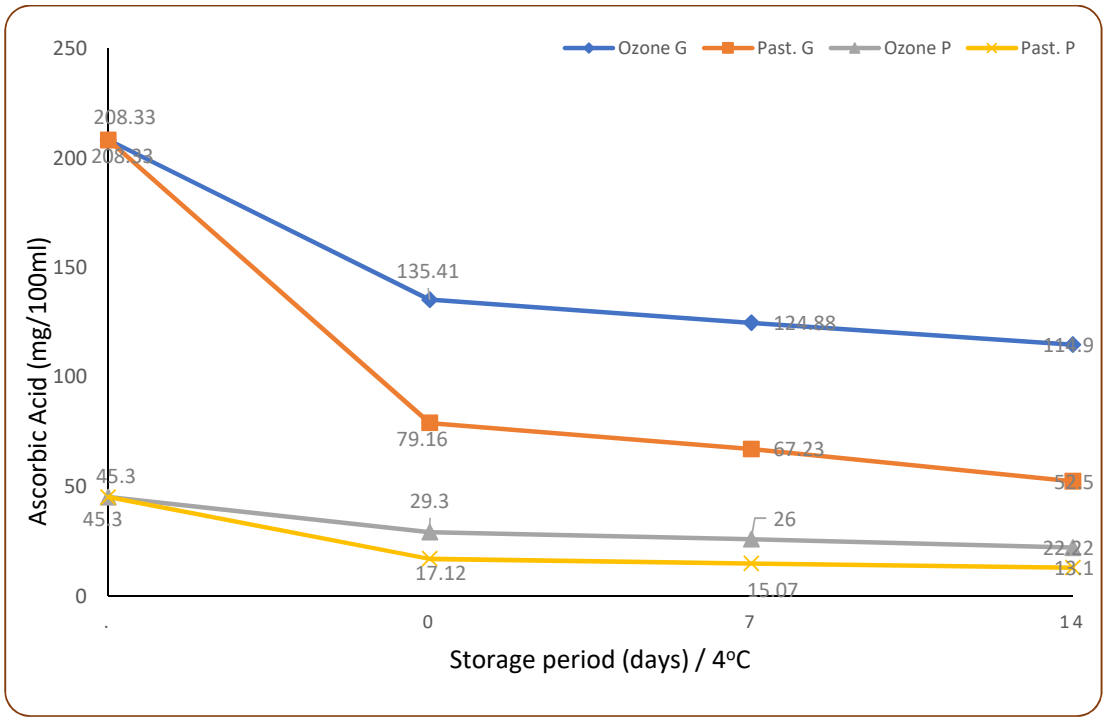

Fig. 2a: Ascorbic Acid (mg/ml) for pasteurized and ozonated Guava and pomegranate during storage period/ 4ºC

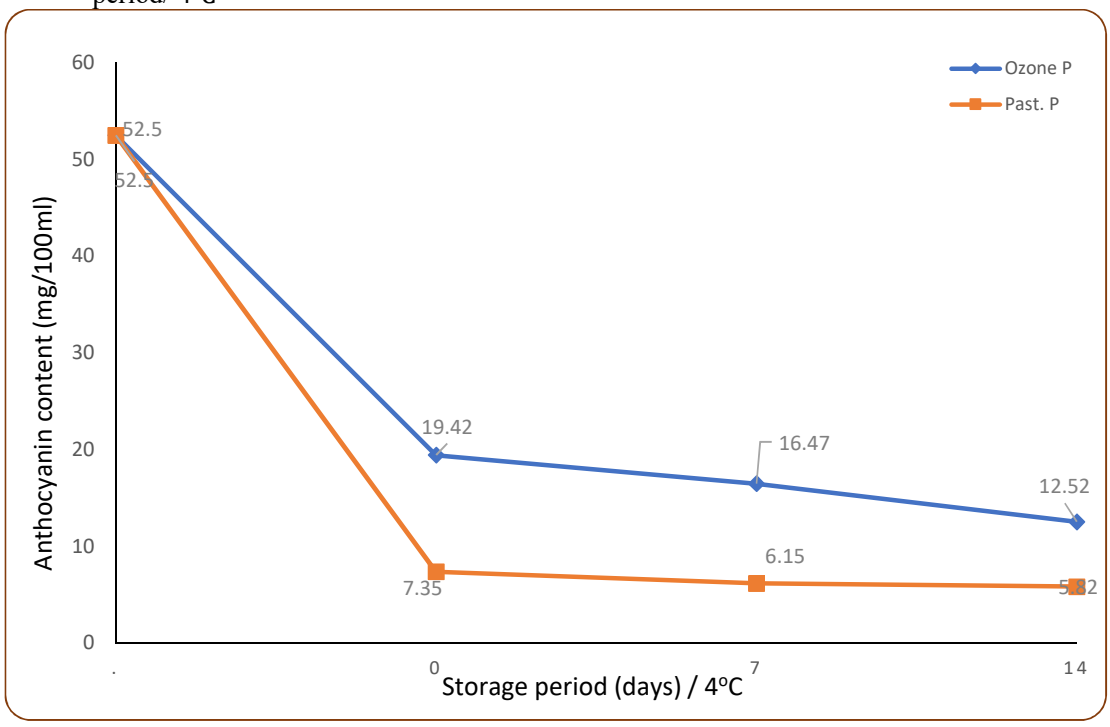

Fig. 2b: Anthocyanin content for pasteurized and ozonated Guava and pomegranate during storage period/ 4ºC

3. Conclusions

The majority of quality parameters analyzed did not show any significant differences between ozonized and pasteurized Guava and Pomegranate juices. While ozone can be successfully used to decontaminate juice intrinsic microflora and maintain its load during storage periods, the nutritional compounds (vitamin C and anthocyanin pigment) in Guava and Pomegranate juices have drastically declined to an unacceptable level, in addition to a significant degradation of their color a crucial indicator of fruit juice quality—making this decision crucial before being adopted industrially and requiring careful consideration of the characteristics of the juice products.

In order to retain the bioactive components of fruit juices and to identify the critical limits (concentration and time parameters) for effective treatment in terms of bactericidal activity, more validation research is still required.

References

Anam, C., A.A. Perwitasari, and H.N. Jannah, 2019. Effect of ozone treatment on microbiological and physicochemical properties of soy milk beverage. Earth and Env. Sci. J. 443: 1-8.

Brand-Williams, W., M.E. Cuvelien and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. Food Sci. and Tech. 28(1): 25-30.

Cullen, P.J., V.P. Valdramidis, B.K. Tiwari, S. Patil, P. Bourke and C.P. O'Donell, 2010. Ozone processing for food preservation an overview on fruit juice treatments. Ozone Sci. & Engineering, 32(3): 166-179.

Garcia-Viguera, C. and P. Bridle, 1999. Influence of structure on color stability of anthocyanins flavylium salt with ascorbic acid. Food Chem. J. 64(1): 21-26.

Gird, C.E., I. Nencu, L.E. Duţu, L. Popescu, T. Costea, and A.F. Neagu, 2018. HPLC evaluation of the ascorbic acid content of Romanian fruits and vegetables from retail markets. *system*, 11, 25.

Ihns, R., L.M. Diamante, G.P. Savage, and L. Vanhanen, 2011. Effect of temperature on the drying characteristics, color, antioxidant and beta-carotenes of two apricot varieties. Int. J. of Food Sci. & Tech., 46: 275-283.

Jaramillo Sanchez, G.M., A.B. Garcia Loredo, E.V. Contigiani, P.L. Gomez and S.M. Alzamora, 2017. Inactivation Knetico of Peroxidase and polyphenol oxidase in peach juice treated with gaseous ozone. Int. J. of Food Sci. & Tech., 53(2): 347-355.

Khadre, M.A., A.E. Yousef, and J. Kim, 2001. Microbiological aspects on ozone applications in food. J. Food Sci., 6: 1242-52.

Macfie, H.J., N. Bratchelt, K. Greenhoff, and L.V. Vaillis, 1989. Designs to balance the effect of order of presentation and firs-order carry-over effects in hall testes. J. Sens, Stud., 4:129-148.

Melendez-Martinez, A.J., I.M. Vicario, and F.J. Heredia, 2007. Analysis of carotenoids in orange juice. J. of Food Comp. and Analysis. 20(7): 638-640.

Miller, F.A., C.L.M. Silva, and T.R.S. Brandao, 2013. A review on ozone-based treatments for fruit and vegetables preservation. Food Eng. Reviews. 5:77-106.

Nyarko, E., K.E. Kniel, P.D. Millner, Y. Luo, E.T. Handy, R. Reynnells, and M. Sharma, 2016. Survival and growth of listeria monocytogenes on whole cantaloupes is dependent on site of contamination and storage temperature. Int. J. of Food Micro. 223: 65-70.

Patils, S. and P. Bourke, 2012. Ozone processing of fluid foods. Novel Thermal Technologies for fluid foods, 225-262.

Rabie, M.A., A.Z. Soliman, Z.S. Diaconeasa, and B. Constantin, 2015. Effect of pasteurization and shelf life on the physicochemical properties of physalis juice. J. of Food pross. & Preser. 39(6): 1051-1060.

Shah, N.N.A.K., A. Sulaiman, N.S.M. Didek, and N.A.M. Supian, 2022. Quality assessment of ozone treated citrus fruit juices. Int. food Res. J. 36(4): 1405-1415.

Singly, S., F.F. Joan, A.M. Fatima, R.S.B. Teresa, and L.M.S. Cristina, 2021. Impact of ozone processing on microbiological, physicochemical, and bioactive characteristics of refrigerated stored cantaloupe melon juice. J. of Processing and preservation. 25(1):1-9.

Tiwari, B.K., K. Muthukumarappan, C.P. O'Donell, and P.J. Cullen, 2008a. Kinetico of breshly squeezed orange juice quality changes during ozone processing. J. of Agri. & Food Chem. 56(15):6418-6422.

Tiwari, B.K., C.P. O'Donell, A. Patras, N. Brunton, and P.J. Cullen, 2009. Anthocyanins and color degradation in ozonated grape juice. J. of Food & Chemical Toxicology. 47: 2824-2829.

Toprak, S., 2021. Ozone Generator and Ozone Generation. Amasya University, Technical Sciences Vocational School, Amasya, Turkey, https://doi.org/10.54559/jauist.981674

Ukuku, D.O., D.J. Geveke, L. Chau, and B.A. Niemira, 2016. Microbial safety and overall quality of cantaloupe. Int. J. of Food Microbiology, 231: 86-92.

Vaillant, F., A.M. Perez, O. Acosta, and M. Dornier, 2008. Turbidity of pulpy fruit juice. A key factor for predicting cross-flow microfiltration performance. J. of Membrane Sci. 325(1): 404-412.

Vuong, Q.V., N. Zammit, B.R. Murchie, M.C. Bowyer and J. Seariett, 2015. Effect of drying conditions on physicochemical and antioxidant properties of Vitex aguns-castus leaves. J. Food Pross & Preservation. 39: 2562-2571.

Wibowo, S., T. Grauwet, G.B. Gedefa, M. Hendrickx, 2015b. Quality changes of pacterurised orange juice during storage. Food Chem. J. 187: 140-151.

Wimalasir, P., and R.B.H. Wills, 2010. Simultaneous analysis of ascorbic acid in fruit and vegetables, by high performance liquid chromatography. J. Chromatography, 15: 418-421.

Zabetakis, I., D. Lecierc, and P. Kajda, 2000. The effect of high hydrostatic pressure on the strawberry anthocyanins. J. of Agri. & food Chem. 48: 2749-2754. http://dx.doi.org/10.1016/S0308-8146(98)00107-1.