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Effect of Ozone and Combined Ozone -Freezing Treatments on The Physico-Chemical Properties and Enzymatic Activity of Sugarcane Juice

*[1]Yara, I. EL-Geddawy, [2]Shaymaa, A.A. Badr & [3]Dalia, I. EL-Geddawy

[1]Department of Sugar Technology, Sugar Crops Institute, Agricultural Research Center, Egypt

[2]Central Laboratory of Agriculture Climate, (Clac), Agricultural Research Center, Egypt

[3]Department of Agricultural Practices, Sugar Crops Institute, Agricultural Research Center, Egypt

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ABSTRACT

Sugarcane juice is a primary raw material for the sugar industry in Egypt and many other countries. It is also consumed fresh as a refreshing beverage rich in nutrients. This study, conducted at the Central Laboratory of Agriculture Climate (CLAC), Dokki, Egypt, during December/January 2023/2024, investigated the impact of ozonation on the biochemical and physiochemical properties of sugarcane raw juice. Sugarcane stalks were crushed to extract the juice, and three volumes (250, 500, and 1000 ml) were subjected to ozone (O_3) treatment for three durations (5, 10, and 15 minutes). Results revealed that exposure time, volume, and their interaction significantly influenced various characteristics, including °Brix, total soluble sugars (TSS), reducing sugars, invertase activity, total phenols (TP), total flavonoids (TF), polyphenol oxidase (PPO), and color coordinates. Fresh ozonized juice (250 ml/5 min) exhibited favorable industrial properties compared to 1000 ml. TP content was significantly affected by exposure time and the combination of ozonation with freezing. Correlation analysis highlighted strong, significant negative correlations between reducing sugars and sucrose, reducing sugars and pH, sucrose and TPC, and pH and TPC.

1. Introduction

Sugar beet and sugarcane are the primary sources of sugar production in Egypt, accounting for 91.1% of the total, with sugarcane contributing 27.3% (Sugar Crops Council-Egypt, 2023). Cane juice, extracted from sugarcane stalks, serves as the raw material for sugar production. It is also widely consumed as a refreshing beverage, particularly in sugarcane-growing regions during hot summer months. Renowned for its numerous health benefits, sugarcane juice is rich in phenolic acids, flavonoids, and various glycosides (Hewawansa et al., 2024). Regular consumption is linked to improved kidney function (Khare, 2007 and Singh et al., 2015) and hepatoprotective effects (Khan et al., 2015) due to its antioxidant properties. Its carbohydrate and electrolyte content make it a popular choice among athletes (Kalpana et al., 2013). Wijayanti et al. (2021) reported that sugarcane juice contains 0.55% total phenols and 0.44% total flavonoids. These compounds, including gallic acid, 4-Hydroxybenzoic acid, and vanillic acid, exhibit potential anti-COVID-19 and alpha-amylase inhibitory properties. However, the lack of proper hygiene and sanitary conditions during the extraction and sale of fresh juice poses significant quality and safety concerns (Nisha et al., 2017). A major challenge in the beverage industry is the rapid deterioration of fresh sugarcane juice after extraction. Its short shelf life, limited to around six hours, is attributed to high sugar, polyphenol, and organic acid content. Polyphenol oxidase activity leads to fermentation and browning, rendering the juice unmarketable (Qudsieh et al., 2001, Özoğlu and Bayındırlı, 2002). To extend the shelf life of sugarcane juice, various techniques have been explored, including hot water blanching, antioxidant addition, antimicrobial agents, spray drying, enzyme inactivation, gamma irradiation, low-temperature storage, and freeze concentration (Taylor et al., 2005, Nishad et al., 2017, Yusof et al., 2000, Alcarde et al., 2001,

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*Corresponding Author

Email: Yara_Elgeddawy@yahoo.com

Songsermpong and Jittanit, 2010). For the production of high-quality white refined sugar, sulfur dioxide is commonly used as a clarifying agent in the sulfitation or sulfo-defecation process (Morilla et al., 2015). However, this process has been criticized due to environmental concerns, potential health risks, and the formation of residual sulfur compounds in sugar crystals (Morilla et al., 2015, Sartori et al., 2015; Favero et al., 2011; Ferreira, 2015). To mitigate these issues, thermal and non-thermal processing techniques have been explored. While thermal processing can reduce microbial load and inactivate enzymes, it can also lead to nutrient loss, color degradation, and the need for artificial additives. Non-thermal techniques, such as ozonation, offer a more environmentally friendly and safer alternative. Ozonation has been used to inhibit browning and maintain microbial stability in fruit juices. However, limited research is available on its impact on the physiochemical quality of sugarcane juice. Garud et al. (2018) combined ozone treatment (1.2g/h, 10 min) with lactic acid addition to reduce polyphenol oxidase (PPO) activity by 60% in sugarcane juice. Panigrahi et al. (2023) evaluated the effects of ultrafiltration and ozonation on phenolic acid and flavonoid concentrations, finding that the combined treatment (UF-OZ) inactivated PPO by 85%. HPLC analysis revealed that ozonation directly affected phenolic acids and flavonoids, particularly caffeic acid and vitexin derivatives. Di-Tanno et al. (2021) concluded that both ozonation time and sugarcane variety influenced the physiochemical characteristics of the juice. While ozonation affected pH, acidity, and reducing sugar content, it also led to a significant reduction in polyphenol and flavonoid content. Panigrahi et al. (2020) reported a 67.8% inactivation of PPO enzyme through ozonation treatment. In conclusion, ozonation shows promise as a non-thermal technique for improving the quality and shelf life of sugarcane juice. Further research is needed to optimize treatment conditions and assess the long-term effects on the nutritional and sensory properties of the juice. So, problems of sugar cane industry can be summarized in a) Enzymatic activity that led to its short preservation period and lower sensory acceptability; b) health aspects associated with clarifying agents used for the clarification of the sugarcane juice. Therefore, this study aims to investigate the extent to which ozonation treatment can impact the biochemical and physiochemical properties of raw sugarcane juice. The study will explore the effectiveness of ozonation as a standalone clarifying agent and in combination with freezing to optimize the process and achieve the best possible quality outcomes.

2. Materials and Methods

This study was conducted at the Central Laboratory of Agricultural Climate (CLAC), Dokki, Egypt, during December and January 2023-2024, to investigate the effect of ozonation on the biochemical and physicochemical properties of sugarcane juice subjected to ozone and combined ozone -freezing treatments.

Chemicals and reagents

All the chemicals and reagents used were of analytical grade. The chemicals and reagents included: Methanol (98%), Folin-Ciocalteu reagent, gallic acid, sodium hydroxide, anhydrous sodium carbonate and anhydrous aluminum chloride, quercetin hydrate, sodium nitrite, which were purchased from Loba Chemie (Mumbai, India); whereas, catechol, phenol solution(5%), sulphuric acid, sodium phosphate buffer, glucose, Picric crystals, sodium carbonate, acetate buffer and sucrose was purchased from Sigma-Aldrich Chemie GmbH (DE), (Steinheim- Germany) purchased from modern lab and technogene lab for chemicals and lab equipment's, Dokki, Egypt.

Raw Material and ozonation process

The juice of sugarcane commercial variety (GT 54-9) was purchased from local market (about 13 L) in Dokki, Egypt. Three different volumes of juice (250, 500, and 1000 ml) were then subjected to ozone (O_3) treatment at intervals of 5, 10, and 15 minutes. The ozonation process involved injecting ozone gas directly into the juice. Gaseous ozone was generated from oxygen using a portable ozone sterilization device model: MAS-10G, capacity: 10g/h).

(This ozone was bubbled into the juice at a flow rate of 10g/h with a concentration of 0.1gO_3/L O_2 at an ambient temperature of 18–20°C. The biochemical properties of the juice were analyzed in two stages: first, immediately after ozone exposure (single treatment) and second, after one week where samples were treated with ozone and stored under freezing conditions -18°C (combined treatment) as shown in Figure 1.

Figure 1. The ozone generator device and the different ozonated samples compared to untreated

The following biochemical characters were determined in both freshly treated and the frozen sugar cane juice:

Physiochemical analysis

The total soluble solids (°Brix) was measured using a ATAGO (N-1E) hand refractometer (made in Japan). After calibrating the refractometer with distilled water, a drop of treated juice was placed on the prism surface, and the cover was closed. The °Brix value was recorded at the point where the measurement line intersected on the refractometer scale.

The pH values were measured using a TLEAD CT-986 multifunctional pH/TDS/temperature test pen (Qingdao, China). Prior to testing, the pH meter was calibrated with standard buffer solutions following AOAC guidelines (2012).

Color parameters, including lightness, redness, yellowness, chroma, and hue angle, were measured as follows: 50 ml of juice at a height of 0.3 cm was placed in a Petri plate on a white surface at 28 °C. Color measurements were taken using a CR-410 chromameter (KONICA MINOLTA, Japan), following CIE standards (1976).

Sugar compositions (Total sugars, reducing sugars and sucrose percentage)

Total Sugars was determined by the phenol-sulfuric acid method Dubois et al., (1956) after thermohydrolysis (water bath 100°C/30 min then immediate cooling under water tap) of the aqueous solution. Reducing sugars (RS%) were determined using the picric acid method (Thomas and Dutcher, 1924). Sucrose percentage was determined using a saccharimeter device according to AOAC (2012).

Determination of total phenols and total flavonoid contents

The total phenols content was estimated using the Folin–Ciocalteu (FC) method, while the total flavonoid content was measured using the aluminum chloride colorimetric assay (Kamtekar ***et al.,*** 2014). Gallic acid and quercetin served as calibration standards. Results for phenolic content were expressed as mg of gallic acid equivalent (GAE) per ml of juice, and flavonoid content was expressed as mg of quercetin equivalent (QE) per ml of juice.

Invertase and polyphenol oxidase (PPO) enzyme activities

Invertase activity was determined following the method described by Bergmeyer (1979), while reducing monosaccharide content was measured according to Thomas and Dutcher (1924). Polyphenol oxidase (PPO) enzyme activity was measured based on the method by Saxena et al. (2018).

Enzyme activity was calculated in units per ml (U/ml), where one unit (U) represents the amount of enzyme that causes an absorbance increase of 0.001 Δabs/min. The calculation formula used was:

Activity (U/ml) = (A_b. sample−A_b. blank)/ $0.001X_t$

Where: A_b= sample is the sample absorbance

A_b= blank is the blank absorbance and

t = incubation time (min).

Statistical analysis

The data were analyzed using IBM SPSS Version 20.0 statistical software for Windows (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was performed as described by Snedecor and Cochran (1980), and mean comparisons were made using the least significant difference (LSD) test at a 5% significance level. A correlation matrix was also created following the method of Dewey and Lu (1959).

3. Results and Discussion

Effect of ozone and combined ozone - freezing treatments on the total Sugars, Reducing Sugars, sucrose and Invertase of the sugarcane juice

Total sugars were not significantly affected by either volume or time. In general, the highest volume (1000 ml at 5, 10, and 15 minutes) showed the lowest total sugar content, ranging from 17.5 to 17.7, while other treatments recorded values close to or equal to the untreated juice (27.6). Conversely, reducing sugars (RS) were significantly influenced by all parameters except for the triple interaction (S*V*T). The untreated juice showed the lowest RS% content (1.24%). Results indicated that as exposure time increased, RS% rose for the 250 ml and 500 ml treatments. In contrast, the 1000 ml treatment exhibited a reverse trend, with RS% initially decreasing and then increasing again at a certain point. The highest RS% was observed in 1000 ml/15 min, followed by 250 ml/15 min and 500 ml/10 min (4.74, 4.40, and 4.00%, respectively), representing increases of 282%, 255%, and 223% compared to the untreated juice level. Invertase activity was significantly influenced by both volume (V) and time (T). All three volume treatments (500, 1000, and 250 ml) showed a noticeable response, with activity decreasing as exposure time increased from 5 to 10 minutes for the 250 ml and 1000 ml treatments. A sharp decrease was observed at 15 minutes, correlating with changes in reducing sugars. However, the 500 ml treatment demonstrated an opposite trend, with a positive relationship between invertase activity and ozone exposure time, resulting in the highest invertase activity at 500 ml/15 min (0.228).

Sucrose content was not significantly affected by either time or volume, with values ranging from 12.12 to 25.05 for the 1000 ml/15 min treatment and untreated, respectively. Consistent with these findings, a previous study reported no significant variations during sugarcane ozonation. All measured biochemical components were significantly affected by the combined treatment of ozonation and one-week storage. Total sugars and sucrose content (%) increased significantly after a week across all treatments, with the 1000 ml treatment showing the most notable increases in total soluble sugars and sucrose, by 63, 62, and 64% and by 97, 82, and 104%, respectively as compared to fresh ozonated juice. While reducing sugars decreased overall, the 500 ml/10 min and 500 ml/15 min treatments showed minimal changes, maintaining higher reducing sugar levels of 4.87 and 4.40, respectively, with increases of 21.75 and 23.60%. The untreated sample recorded the highest significant increase in reducing sugars 540%. Invertase activity significantly decreased in all treatments, with the untreated sample being particularly affected. An inverse relationship was observed between pH and invertase activity, with pH increases leading to decreases in invertase activity. This reduction in invertase activity contributed to a corresponding decrease in reducing sugars and an increase in sucrose content, especially in the ozonated frozen juice. Supporting this trend, Bootsarin et al. (2014) found that the activity levels of soluble acid invertase (SAI) were significantly and negatively correlated with sucrose accumulation, suggesting that invertase enzymes, particularly SAI, play a crucial role in sucrose concentration during ripening.

Table 1. Total Sugars, Reducing Sugars, sucrose and Invertase percentages in sugarcane juice subjected to ozone and combined ozone -freezing treatments

Treatments		Traits											
		Total Sugars %			Reducing sugars%			Invertase%			Sucrose %		
Volume (V) ml	Time (T) min	Fresh	frozen	Mean (V*T)	Fresh	frozen	Mean (V*T)	Fresh	frozen	Mean (V*T)	Fresh	frozen	Mean (V*T)
250 ml	Untreated	27.6	28.1	27.85	1.24	7.94	4.59	0.132	0.017	0.07	25.05	19.15	22.10
	5	27.6	27.1	27.35	1.40	1.68	1.54	0.127	0.025	0.08	24.89	24.11	24.50
	10	25.6	28.9	27.25	3.22	1.42	2.32	0.090	0.020	0.06	21.97	26.06	24.02
	15	25.6	28.9	27.25	4.40	3.08	3.74	0.185	0.022	0.10	20.14	24.48	23.31
Mean (V1)		26.60	28.21	27.43	2.56	3.53	3.05	0.133	0.021	0.08	22.84	23.45	23.23
500 ml	Untreated	27.6	28.1	27.85	1.24	7.94	4.49	0.132	0.017	0.07	25.05	19.15	22.10
	5	25.7	28.9	27.30	3.91	2.90	3.41	0.120	0.028	0.07	20.70	24.65	22.68
	10	25.6	28.4	27.00	4.00	4.87	4.44	0.224	0.019	0.12	20.52	22.30	21.41
	15	25.6	28.6	27.10	3.56	4.40	3.98	0.228	0.016	0.12	20.99	22.99	21.99
Mean (V2)		26.14	28.48	27.31	3.18	5.03	4.10	0.176	0.020	0.10	21.81	22.27	22.04
1000 ml	Untreated	27.6	28.1	27.85	1.24	7.94	4.59	0.132	0.017	0.07	25.05	19.15	22.10
	5	17.7	28.8	23.25	4.16	1.70	2.93	0.136	0.015	0.08	12.82	25.21	19.02
	10	17.7	28.7	23.20	3.14	2.24	2.69	0.122	0.017	0.07	13.79	25.09	19.44
	15	17.5	28.7	23.10	4.74	2.69	3.72	0.165	0.010	0.09	12.12	24.73	18.43
Mean (V3)		20.10	28.59	24.36	3.32	2.36	3.48	0.139	0.015	0.08	15.94	24.92	19.75
Mean (T)	Untreated	27.60	28.10	27.85	1.24	7.94	4.59	0.132	0.017	0.07	25.05	19.15	22.10
	5	23.65	28.23	25.94	3.16	1.68	2.42	0.128	0.023	0.08	19.47	25.21	23.34
	10	22.95	28.65	25.80	3.45	1.42	2.44	0.145	0.019	0.08	18.52	25.09	21.81
	15	22.92	28.72	25.82	4.23	3.08	3.66	0.192	0.016	0.10	17.75	24.73	21.24
Mean (S)		24.28	28.43		3.02	3.64		0.149	0.019		20.20	23.55	
LSD at 0.05													
Storage (S)			2.11			0.50			2.20			2.20	
Volume (V)			NS			0.50			NS			NS	
Time (T)			NS			0.58			NS			NS	
S*V			3.66			0.87			3.82			3.82	
S*T			NS			1.00			4.41			4.41	
V*T			NS			1.00			NS			NS	
S*V*T			NS			NS			NS			NS	

NS: Not significant

Effect of ozone and combined ozone - freezing treatments on the physical properties of the sugarcane juice

Brix, an important industrial parameter, serves as an indicator of ripeness. In the sugarcane industry, particularly in São Paulo, juice destined for industrial processing must have a minimum °Brix of 18, representing 18% total soluble solids (Fernandes, 2000). Sugarcane varieties differ significantly in sucrose levels, with °Brix values ranging from 15 to 23%; °Brix closer to 23% is associated with high-quality cane sugar (Elewa et al., 2020). pH is also crucial, as it influences sucrose content; a low pH leads to sucrose hydrolysis, raising juice temperature during processing (Lopes et al., 2012). Exposure of fresh sugarcane juice to ozone did not significantly affect °Brix, though volume had an impact. The 250 ml volume showed higher °Brix values (19.17°, 19.00°, and 18.43°) than the 500 ml and 1000 ml volumes, though it did not exceed the untreated juice (19.43°). This finding aligns with Davis (2001), who noted that total soluble solids (TSS) are less impacted by ozone in low-acid foods, as the ozone reacts through oxygen radicals without degrading sugar molecules. pH showed an acidifying trend after ozone treatment, decreasing significantly with longer ozonation times. This pH drop was attributed to the formation of organic acids (e.g., acetic, formic, oxalic) during ozone decomposition (Matsumoto et al., 2021). Chen and Chou (1993) observed that sugarcane juice from fully mature plants has a pH of 5.2–5.4. In their study, ozonation reduced the pH of variety RB 855453 to 4 after 16 minutes and to 2.9 after 100 minutes. Chou et al. (2006) noted that a pH below 4.2 indicates juice deterioration. Silva et al. (2015), however, found pH levels remained relatively constant during ozonation under both heated and unheated conditions. The °Brix was not significantly affected by the combined treatment, but pH values increased significantly, by 1.120 and 0.27 in the 500 ml and 1000 ml samples, respectively as shown in Figure (2A-B). In contrast, the untreated juice showed decreases in both °Brix and pH. Among the volumes, the 1000 ml sample recorded the lowest ° Brix values (1.50 and 2.88 lower than those of the 250 ml and 500 ml samples). Acidity increased in the 500 ml sample subjected to ozone. However, the ozonated frozen juice showed increased pH, while the untreated juice pH dramatically decreased, indicating deterioration. Significant interactions between variables were observed for °Brix, except in the storage treatment. The highest °Brix value (26.00) was recorded after one week for the 500 ml/15 min treatment. For pH, the highest recorded value was 6.17 in the 500 ml sample with the longest exposure time (15 min). This result can be considered as a positive factor because the raise in pH decrease the rate of sucrose inversion to reducing sugars (glucose and fructose) and consequently formation of undesired colored compounds (Riffer, 1988).

Regarding the color parameters, fresh sugarcane juice typically ranges in color from yellow to dark green. This characteristic coloration is mainly due to the presence of plant pigments such as chlorophylls, anthocyanins, and others (Singh et al., 2015). Additionally, other coloring agents in sugarcane juice include polyphenolic compounds, caramels, and sugar degradation products like those formed through the Maillard reaction (Smith and Paton, 1985). Figure (2C-G) clearly demonstrates the significant impact of the ozonation process on the color parameters of the juice. The analysis included five key color parameters: “L” (lightness), “C” (chroma), “H” (hue angle), “a+/a-” (indicating red or green values), and “b” (yellow value).

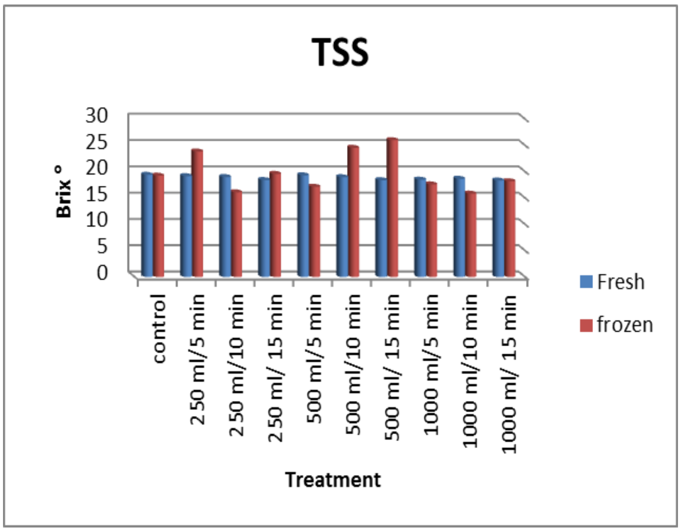
In our study, the untreated juice exhibited the highest values for “L,” “C,” and “H.” The effect of ozone on lightness varied depending on the juice volume and exposure time. For the 250 ml sample, lightness increased with longer exposure time, whereas for the 500 ml and 1000 ml samples, there was an inverse relationship between lightness and exposure time. Chroma (“C”) showed a direct relationship with exposure time, increasing from 5 to 15 minutes, with the highest chroma (5.13) observed in the 1000 ml sample after 15 minutes of treatment.

A similar trend was observed for hue angle (“H”) and the red/green value (“a+/a-”), both of which increased as the exposure time increased from 5 to 15 minutes for the 250 ml sample. However, for the 500 ml sample, increasing the exposure time from 5 to 10 minutes reduced these parameters, whereas a 15-minute exposure significantly increased “H” and “a+/a-.” The ozonated juice also showed a notable increase in the yellow value (“b”) compared to the untreated sample, which had the lowest “b” value.

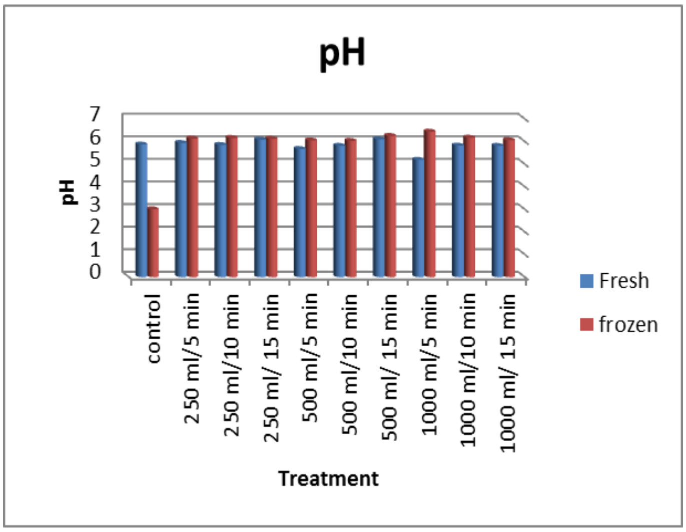
Hamerski (2009) highlighted that the final color of sugar is a crucial parameter for the sugarcane industry as it determines the quality and market value of the product. Silva et al. (2015) reported a marked reduction in color indexes at the initial stage of their study, indicating a significant decrease in the ICUMSA color of the sugarcane juice after ozone treatment compared to the untreated juice.

Furthermore, Davis et al. (1998) suggested that the high oxidative potential of ozone (E^o = 2.42 V) allows it to attack various functional groups, such as phenolic groups, leading to the breakdown of aromatic structures and the oxidation of amino groups (precursors of Maillard reactions) to nitrates.

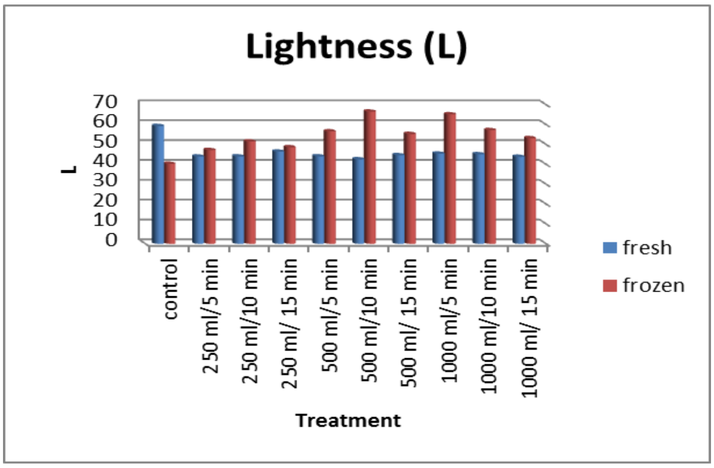
They noted that ozone's mechanisms for destroying pigmented compounds are both direct and varied.
Similarly, Chakraborty et al. (2014), Rosenfeld et al. (2015), and Panigrahi et al. (2022) proposed that ozone inactivates enzymes by modifying functional groups and active sites through ozone decomposition free radicals, resulting in structural changes and a loss of polyphenol oxidase (PPO) enzyme activity. The "L" parameter (lightness) of 500 and 1000L treatments increased by 15% and 17% following the freezing process, compared to the freshly ozonated juice. In contrast, the untreated sample showed an opposite trend, with a 32.1% decrease in lightness. The highest significant "L" values were observed in samples treated with ozone for 10 minutes/500 ml and 5 minutes/1000 ml, with recorded values of 66.53 and 65.16, respectively.
For the "C" parameter (chroma), the untreated sample had the highest values across all treatments. The general trend indicated a reduction in chroma, with the lowest significant value (0.21) observed in the 5 -minute ozone treatment of the 1000 ml in the frozen treatment. The "H" (hue angle), "a" (red/green value), and "b" (yellow value) parameters exhibited a similar trend. The untreated sample showed a decrease in all these parameters after freezing, with reduction rates of 20.3% and 12% for H and b, respectively. In contrast, the combined ozone treatment had a positive effect on these parameters, significantly increasing their values across all studied treatments. The lowest significant hue angle ("H") was 21.39, recorded after freezing with 5-minute ozone treatment of the 250 ml sample.
For the "a" parameter (red value), all treatments showed an increase after freezing. The 5-minute/250 ml and 10-minute/500 ml treatments had the lowest red values after freezing, with recorded values of 21.65 and 21.91, respectively. A similar trend was observed for the "b" parameter (yellow value), where the 5-minute/250 ml treatment after freezing recorded the lowest value of 76.14. The significant increases in these parameters compared to the untreated juice were accompanied by a decrease in reducing sugars (Table 1).



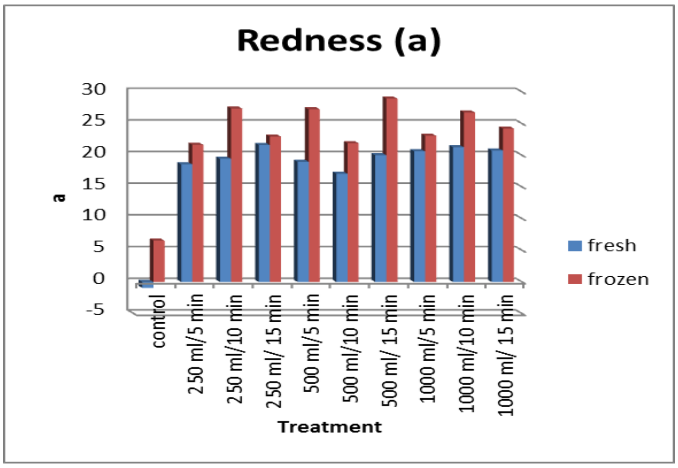
A: The effect of sugar cane juice ozonation and combined ozone -freezing treatments on ^{0}Brix (TSS)



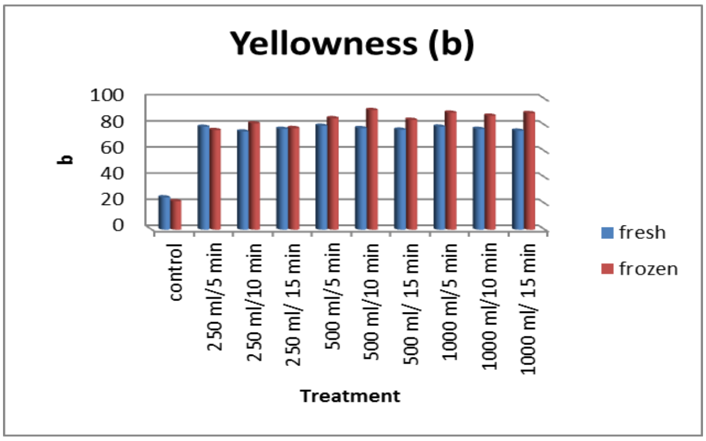
B: The effect of sugar cane juice ozonation and combined ozone -freezing treatments on pH



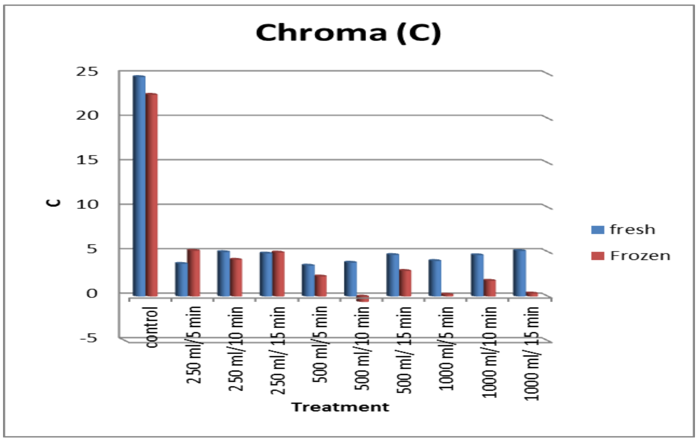
C: The effect of sugar cane juice ozonation and combined ozone -freezing treatments on Lightness (L) color coordinate



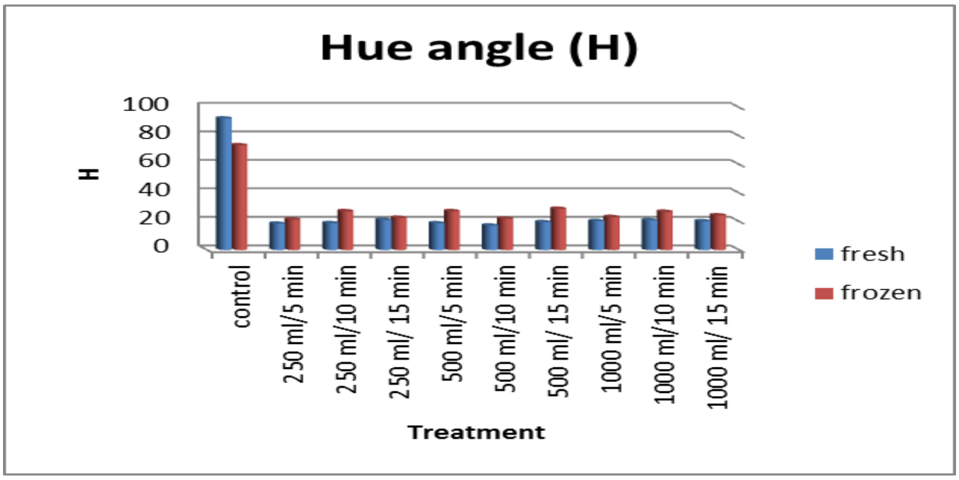
D: The effect of sugar cane juice ozonation and combined ozone -freezing treatments on Redness (a) color coordinate



E: The effect of sugar cane juice ozonation and combined ozone -freezing treatments on yellowness (b) color coordinate



F: The effect of sugar cane juice ozonation and combined ozone -freezing treatments on Chroma (C) color coordinate



G: The effect of sugar cane juice ozonation and combined ozone -freezing treatments on hue angle (H) color coordinate

Figure 2(A-G). The effect of sugar cane juice ozonation and combined ozone -freezing treatments on ^{0}Brix , pH and color parameter

Effect of ozone and combined ozone - freezing treatments on the on Total Phenol (TP), Total Flavonoid (TF) and Poly Phenol Oxidase (PPO) enzyme of the sugarcane juice

Sugarcane is a rich source of polyphenols, which have been linked to various health benefits (Carvalho et al., 2021; Rana et al., 2022). The levels of these bioactive compounds can be influenced by various factors, including genetic factors, environmental conditions, and processing techniques. Table 2 presents the impact of ozone treatment on total phenols (TP), total flavonoids (TF), and polyphenol oxidase (PPO) in fresh and stored sugarcane juice.

Total Phenols (TP)

- Time: A significant decrease in TP was observed with increasing exposure time. This could be attributed to the slower oxidation rate of phenols by ozone compared to hydroxyl radicals, allowing some compounds to remain relatively unaffected (Onopiuk et al., 2017; Zeng et al., 2013).
- Volume: The lowest decrease in TP was observed for 500 ml/5 min and 1000 ml/10 min treatments.

Total Flavonoids (TF):

- Volume: A significant increase in TF was observed with increasing volume.
- Time and Volume Interaction: Longer exposure times, especially for 500 ml and 1000 ml treatments, led to increased TF levels. This could be due to the complex interactions between ozone and flavonoids, which can involve both oxidation and structural modifications (Cullen et al., 2010).

Polyphenol oxidase (PPO)

- Time, Volume, and Interaction: PPO activity was significantly affected by all these factors. Longer exposure times and larger volumes generally led to lower PPO activity.
- Mechanism of Inactivation: Ozone inactivates PPO by oxidizing the enzyme's active site and altering its structure, leading to loss of activity (Tentscher et al., 2019; Chakraborty et al., 2014; Rosenfeld et al., 2015).

In conclusion, ozone treatment can significantly impact the levels of TP, TF, and PPO in sugarcane juice. The optimal treatment conditions need to be determined to balance the desired effects on these compounds while minimizing negative impacts on other quality attributes.

Correlation between physiochemical properties for Sugarcane Juice Subjected to Ozonation and Combined Ozone – Freezing Treatments

1. Fresh Sugarcane Juice

The correlation matrix for the technological properties of fresh sugarcane juice is presented in Table 3. Among the physicochemical parameters, a strong and significant positive correlation was observed between total sugars and sucrose (0.979) as well as between TSS (Total Soluble Solids) and sucrose (0.701). Positive but non-significant correlations were found between total sugars and TSS (0.628), total sugars and pH (0.546), and sucrose and pH (0.521). These relationships can be explained by the scientific observation that sugarcane juice consists of approximately 75–82% water and 18–25% soluble solids. These soluble solids include sugars (sucrose, glucose, and fructose in varying ratios), as well as organic, inorganic, and non-sugar substances (Jeronimo, 2018). The enzymatic activity within the juice alters the sugar composition, where reducing sugars are inversely proportional to sucrose content. Additionally, the effect of pH on total sugar and sucrose content is indirect, primarily influencing the activity of the invertase enzyme.

Regarding color parameters, a strong positive correlation was found between Total Phenolic Content (TPC) and “L” (lightness), “C” (chroma), and “H” (hue angle), with correlation coefficients of 0.801, 0.818, and 0.837, respectively. However, there was a strong negative correlation between TPC and the “b” (yellow) value (-0.821). Total Flavonoid Content (TFC) showed a weak positive correlation with “L,” “C,” “H,” and “a” (red/green value), with coefficients of 0.389, 0.356, 0.387, and 0.270, respectively. Reducing sugars were positively correlated with the “b” coordinate (0.614), while a significant negative correlation was observed between TSS and the “a” coordinate (-0.714).

Table 2. The effect of sugar cane juice ozonation and combined ozone -freezing treatments on Total phenols, Flavonoids and Poly Phenol Oxidase

Treatments		Traits								
		Total phenols (mg equivalent GAE/ml juice)			Total flavonoids(mg QE/ml juice)			Poly Phenol Oxidase PPO (U/ml)		
Volume (V) ml	Time (T) min	Fresh	frozen	Mean (V*T)	Fresh	frozen	Mean (V*T)	Fresh	frozen	Mean (V*T)
250 ml	Untreated	80.14	37.19	58.67	3.17	0.77	1.97	1.04	2.52	1.78
	5	50.74	6.48	28.60	2.69	0.69	1.69	1.67	2.18	1.93
	10	49.42	12.14	30.78	2.15	1.14	1.64	1.36	1.01	1.19
	15	40.51	10.76	25.63	2.58	0.93	1.75	1.65	1.99	1.82
Mean (V1)		55.20	16.64	35.92	2.65	0.93	1.76	1.43	1.93	1.68
500 ml	Untreated	80.14	37.19	58.67	3.17	0.77	1.97	1.04	2.52	1.78
	5	60.21	8.97	34.58	2.79	0.69	1.73	1.73	1.35	1.54
	10	48.51	6.16	27.33	2.81	0.99	1.90	1.71	1.47	1.59
	15	54.55	3.86	29.20	2.89	0.66	1.77	1.54	1.00	1.27
Mean (V2)		60.85	14.05	74.89	2.91	0.78	1.84	1.50	1.59	1.55
1000 ml	Untreated	80.14	37.19	58.67	3.17	0.77	1.97	1.04	2.52	1.78
	5	52.97	10.71	31.83	3.00	1.49	2.24	2.02	0.20	1.11
	10	60.07	13.61	36.84	3.31	1.25	2.28	1.74	0.09	0.92
	15	48.76	3.96	26.36	2.91	0.75	1.82	1.06	1.68	1.37
Mean (V3)		60.48	16.37	38.43	3.10	1.07	2.08	1.46	1.12	1.30
Mean (T)	Untreated	80.14	37.19	58.67	3.17	0.77	1.97	1.04	2.52	1.78
	5	54.64	8.72	31.68	2.83	0.69	1.76	1.81	1.24	1.53
	10	52.67	10.64	31.66	2.76	1.14	1.95	1.60	0.86	1.23
	15	47.94	6.19	27.07	2.91	0.93	1.92	1.42	1.56	1.49
Mean (S)		58.85	15.69		2.89	0.88		1.47	1.55	
LSD at 0.05										
Storage (S)			4.93			0.15			NS	
Volume (V)			NS			0.15			0.13	
Time (T)			5.70			NS			1.50	
S*V			NS			NS			2.24	
S*T			NS			0.31			2.58	
V*T			NS			0.31			2.58	
S*V*T			NS			NS			0.45	

2. Frozen Sugarcane Juice

The correlation matrix for the technological properties of frozen sugarcane juice is shown in Table 4. The results revealed a strong, significant negative correlation between reducing sugars and sucrose (-0.965), reducing sugars and pH (-0.838), sucrose and TPC (-0.657), and pH and TPC (-0.921). Conversely, a strong positive correlation was found between reducing sugars and TPC (0.659) and between pH and sucrose (0.850). Strong significant correlations between physicochemical properties and color coordinates were observed as follows:

- Positive correlations between reducing sugars and "C" (chroma) and "H" (hue angle), with coefficients of 0.859 and 0.816, respectively.
- Positive correlations between sucrose and the "a" and "b" coordinates (0.817 and 0.785, respectively).
- Positive correlations between pH and the "L," "a," and "b" coordinates (0.635, 0.913, and 0.965, respectively). On the other hand, there were strong negative correlations between:
- Reducing sugars and the "a" and "b" coordinates (-0.41 and -0.746, respectively).
- Sucrose and the "C" and "H" coordinates (-0.897 and -0.785, respectively).

pH and the "C" and "H" coordinates (-0.969 and -0.978, respectively). In addition, TPC was positively correlated with the "C" and "H" coordinates (0.916 and 0.931, respectively), but negatively correlated with the "a" and "b" coordinates (-0.842 and -0.920, respectively). For the frozen juice, a strong significant correlation was observed between PPO

(Polyphenol Oxidase) activity and TFC (Total Flavonoid Content), as well as between PPO activity and the "L" coordinate, with coefficients of -0.725 and -0.706, respectively.

The relation between sucrose, reducing sugars, pH and color coordinated might be attributed to the activity of enzymes which is strongly affected by pH through which the rate of sucrose inversion to reducing sugars (glucose and fructose) is determined in addition to the other decomposition products especially colored and/or acidic compounds (Chou et al. 2006) . Also, the relation between TPC and PPO activity was align with Manohar, et al. (2013) who reported that as high phenolic content increase enzymic browning increased as well due to polyphenol oxidase (PPO) activity. In our study the application of ozone and ozone-freeze treatments led to inhibition of PPO activity of cane juice which can be considered as a positive impact.

Table 3. The correlation between different properties of sugarcane Juice Subjected to Ozonation

Trait	Total sugars	RS	invertase	Sucrose	TSS	pH	TPC	TFC	PPO	L	C	H	a	b
Total sugars	1	-												
RS	-.522-	1												
invertase	.104	.382	1											
Sucrose	.979**	-.681*	-.023-	1										
TSS	.628	-.688*	-.465-	.701*	1									
pH	.546	-.229-	.359	.521	-.013-	1								
TPC	.150	-.627-	-.313-	.268	.593	-.111-	1							
TFC	-.442-	-.143-	.141	-.375-	-.068-	-.231-	.583	1						
PPO	-.167-	.249	.059	-.213-	-.140-	-.438-	-.358-	.039	1					
L	.275	-.587-	-.179-	.366	.413	.073	.801**	.389	-.509-	1				
C	.302	-.603-	-.153-	.396	.466	.111	.818**	.356	-.624-	.978**	1			
H	.303	-.616-	-.165-	.398	.490	.076	.837**	.387	-.576-	.985**	.997**	1		
a	-.575-	.336	-.152-	-.581-	-.714*	-.002-	-.078-	.270	-.091-	.859**	.642	.994**	1	
b	-.321-	.614	.151	-.415-	-.487-	-.115-	-.821**	-.344-	.627	-.972**	-.999**	-.996**	-.282-	1

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Table 4. The correlation between different properties of combined ozone treated and frozen sugarcane juice

Trait	Total sugars	RS	invertase	Sucrose	TSS	pH	TPC	TFC	PPO	L	C	H	a	B
Total sugars	1													
RS	-.145-	1												
invertase	-.235-	-.156-	1											
Sucrose	.390	-.965**	.098	1										
TSS	-.527-	.333	.057	-.439-	1									
pH	.271	-.838**	.090	.850**	.042	1								
TPC	-.121-	.659*	-.036-	-.657*	-.304-	-.921**	1							
TFC	.391	-.394-	-.269-	.419	-.498-	.277	.048	1						
PPO	-.522-	.515	.253	-.586-	.349	-.577-	.331	-.725*	1					
L	.390	-.334-	-.150-	.379	.057	.635*	-.570-	.522	-.706*	1				
C	-.368-	.859**	.045	-.897**	.097	-.969**	.916**	-.286-	.640	-.799**	1			
H	-.176-	.816**	-.137-	-.807**	-.091-	-.978**	.931**	-.225-	.442	-.611-	.947**	1		
A	.429	-.741*	.104	.817**	-.044-	.913**	-.842**	.137	-.626-	.531	-.911**	-.837**	1	
B	.350	-.746*	-.014-	.785**	.021	.965**	-.920**	.288	-.612-	.763*	-.998**	-.953**	.891**	1

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

4. Conclusion

Using ozone gas had positive effects on sugar cane juice which can be directed to two sides. The first side is the farmers who can get benefit of ozonation process as a side small business in addition of increasing the added value for this crop and the local shops that sell the fresh juice. Ozonation of 250ml/5 min increased the sucrose percentage and this result was enhanced by cooling. There is a portable ozone machine generator which can be easily used. On the industrial levels, the previous treatment demonstrated favorable industrial characteristics, including high levels of total soluble sugars and sucrose, and lower levels of reducing sugars. Overall, these findings agree with producer and consumer needs who want natural, safe food preservatives and healthy food product.

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