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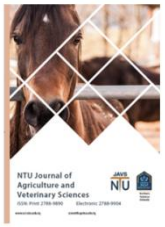

Influence of ozone treatment on quality of stored orange juice

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A B S T R A C T

This study aimed to treat Orange Juice with Ozone Gas and store the juice for 30 days. The characteristics and Laboratory tests were evaluated every 7 days to determine the effect of Ozone treatment on the juice properties during different storage periods.

At the end of the storage period, an increase was observed in the Total Phenolic Compound values and Total Acidity values, while the Total Soluble Solids, pH, and Browning Index values decreased. Additionally, the concentration of Ascorbic Acid decreased after 30 days of storage

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Introduction

Orange juice contains other phytochemicals such as flavonoids, which may have beneficial effects on human health, including anti-inflammatory, anti-allergic, cardioprotective, anti-hyperglycemic, neuroprotective, and antioxidant effects. Consumption of foods rich in flavonoids is associated with a lower incidence of chronic diseases (such as type 2 diabetes, cardiovascular diseases, and dyslipidemia) [1].

There are more than 170 chemical compounds in orange juice, including 60 flavonoid compounds. Most of these have been found to have antioxidant, anti-inflammatory, and anti-tumor benefits [2]. The orange color of orange juice is attributed to various carotenoids, including alpha-carotene, zeaxanthin (yellow), violaxanthin (yellow), beta-sitosterol (reddish-orange), and beta-cryptoxanthin (orange) [3].

Thermal treatment techniques, such as pasteurization, can be used to extend the shelf life of extracted fruit juice by reducing microbial contamination and preventing enzymatic and non-enzymatic processes that contribute to juice spoilage. Since pasteurization does not eliminate all microbes and enzymes, the quality of the juice may vary during storage (Ampofo-Asiama and Quaye, 2018). Some of these treatments may affect the juice's flavor and color, potentially reducing its sensory acceptance during storage. Additionally, many bioactive compounds, such as ascorbic acid, degrade during treatment, which decreases the nutritional value of the juice. It is also possible for bacteria to regain their activity and growth during storage if the juice is not kept at the appropriate storage temperature [4].

Ozone is produced from three oxygen atoms through electrical corona discharge or ultraviolet light (Figure 2 shows the method of ozone gas production). It is colorless and tasteless. Due to its strong oxidizing power and bactericidal properties, it has the ability to eliminate microorganisms such as bacteria, protozoan parasites, and viruses from food products, thus extending their shelf life compared to other thermal treatments [5]. Ozone is a fast, effective, cost-efficient, accessible technology that can be used as an alternative to other treatments. It has antibacterial properties, such as releasing free oxygen atoms that can kill many microbes and prolong the shelf life of food. Ozone has been used in some food industries, such as for orange, tomato, and apple juices, because it does not negatively affect the acidity or pH of fruit juices or cause non-enzymatic browning (NEB) [6]; [7].

Ozone is widely used for disinfecting fruits, meats, and poultry, as well as for washing and sanitizing plant-based foods and dairy products. It is also used in silos, storage rooms, and tanks to protect and preserve grains, fruits, and vegetables. The direct use of ozone during storage helps maintain a clean and sanitized environment, ensuring cleanliness and improving the color, aroma, and visual appearance of these products, which generally allows for increased food storage capacity [8]; [9].

Mechanism of Action of Ozone

Ozone gas oxidizes organic materials and then transforms into oxygen and hydrogen peroxide, which is a byproduct of this process, without forming any carcinogenic or toxic secondary substances, thus it is classified as environmentally friendly. The first oxygen atom is the main factor responsible for the distinctive action in the oxidation and reduction of various complex substances such as ketones, aldehydes, alcohols, and acids. It also reacts with unsaturated hydrocarbons, thiol groups (SH), amines, and aromatic compounds, breaking them down and removing them from water or air by decomposing them into their basic components, which include water, carbon dioxide, carbon, nitrogen, sulfur, oxygen, hydrogen, chlorine, and other elements. Additionally, it oxidizes some inorganic materials such as iron, manganese, cyanide, sulfates, phenol, lead, and nitrates in water, as well as certain heavy metals and some natural organic compounds, particularly oxalic acid and acetic acid, along with synthetic compounds like nitro and chloro-benzenes [10]; [11]; [12].

MATERIAL AND METHODS

Estimation of Total Dissolved Solids (%)

The estimation of total dissolved solids (%) was conducted using a handheld refractometer after stabilizing the temperature of the treated juice samples at 20°C, following the method described by [13].

Estimation of Total Titratable Acidity (%)

The percentage of total titratable acidity in orange juice was estimated based on citric acid. The titration process was performed using sodium hydroxide solution until reaching the endpoint, indicated by the formation of pink color in the presence of phenolphthalein indicator, according to the method of [14].

The percentage of total titratable acidity was calculated using the following equation:

A= B*C*D \ E *100

Where:

A = Total titratable acidity (%)

B = Volume of NaOH consumed in the titration (mL)

C = Molarity of NaOH used in the titration

D = 0.064, which is the equivalent weight of citric acid

E = Volume of the sample taken for estimation

Estimation of pH*

The pH of the juice undergoing the study treatments was measured using a pH meter at a temperature of 20°C, following the method described by [15].

Estimation of Ascorbic Acid Content

The ascorbic acid content in the orange juice samples was estimated using the titrimetric method described by [16], which concludes when a light blue color appears after titrating the samples with a 0.01 molar iodine solution. By determining the volume of iodine solution consumed, the following equation can be applied to calculate the ascorbic acid content in mg/100 mL:

A = B * C * D * E

Where:

A = Amount of ascorbic acid

B = Concentration of the prepared iodine solution, which equals 0.01 molar

C = Volume of iodine solution consumed in the titration (mL)

D = A constant value of 2

E = Molecular weight of ascorbic acid, which equals 176.12 g/mol

Total Phenols Estimation

The concentration of total phenols was estimated according to the method of [17], with some modifications.

Estimation Solutions

A. Folin Reagent Solution

This solution was prepared by diluting 0.1 mL of Folin reagent with 0.9 mL of distilled water in a volumetric flask.

B. Sodium Carbonate (Na2CO3) Solution at 7.5% Concentration

This was prepared by dissolving 7.5 grams of sodium carbonate powder in distilled water, then completing the volume to 100 mL in a volumetric flask.

Estimation Steps

1. The orange juice samples were diluted with distilled water before adding the Folin reagent solution. The mixture was left at room temperature for 8 minutes before adding the 7.5% sodium carbonate solution. The volume was then completed with distilled water to 5 mL and left at 40°C for 30 minutes before measuring the absorbance at 760 nanometers.
2. Comparison samples were prepared by replacing the juice samples with distilled water.
3. Different concentrations of kalic acid dissolved in methanol were used to prepare the standard curve, as shown in Appendix (2-3).

Estimation of Browning Index

The values of the browning index were estimated at a wavelength of 420 nanometers after mixing the orange juice samples with ethanol, according to the method described by [18].

Total Bacterial Count

The number of viable bacterial cells in the prepared dilutions was calculated using the drop method, according to [19].

Estimation Steps

1. A series of tenfold dilutions was prepared by adding 1 mL of orange juice sample to the first dilution tube containing 9 mL of sterile distilled water, and the tube was shaken well to obtain a homogeneous mixture.
2. After withdrawing 1 mL of the dilution mixture from the first tube using a micropipette under sterile conditions inside a hood, it was added to the second dilution tube containing 9 mL of sterile distilled water, and the tube was shaken well.
3. The remaining dilutions were completed following the same two steps until reaching the appropriate dilution (the sixth dilution).
4. A ready-made nutrient medium solution (Nutrient Agar, NA) was prepared according to the manufacturer's instructions and sterilized in an autoclave at 121°C for 15 minutes under 1 atmosphere of pressure.
5. The nutrient medium (NA) was poured into Petri dishes and left until it solidified.
6. Sixty microliters of the required dilutions were added using a micropipette in a dropwise manner onto the plates containing the nutrient medium (NA).
7. The plates were incubated upside down at 37°C for 24 hours.
8. The number of growing colonies was calculated using the following equation:

A= B*C*D

Where:

A = Number of viable bacterial cells in 1 mL

B = Number of growing bacterial colonies

C = A constant value of 50

D = The reciprocal of the dilution

Total Yeast and Mold Count

The indirect method was used to calculate the total number of yeasts and molds in the juice sample, specifically the Standard Plate Count (SPC), following the method of [20].

Estimation Steps

1. A series of tenfold dilutions was prepared by adding 1 mL of orange juice sample to the first dilution tube containing 9 mL of sterile distilled water, and the tube was shaken well to obtain a homogeneous mixture.
2. After withdrawing 1 mL of the dilution mixture from the first tube using a micropipette under sterile conditions inside a hood, it was added to the second dilution tube containing 9 mL of sterile distilled water, and the tube was shaken well.
3. The remaining dilutions were completed as before until reaching the appropriate dilution (the sixth dilution).
4. A ready-made nutrient medium solution (Potato Dextrose Agar, PDA) was prepared according to the manufacturer's instructions and sterilized in an

autoclave at 121°C for 15 minutes under 1 atmosphere of pressure.
5. One milliliter of the required dilutions was added using a micropipette to sterile Petri dishes before adding the nutrient medium (PDA) to ensure the sample spread evenly across the dish.
6. The plates were incubated upside down at 28°C for 4 days.
7. The number of growing mold and yeast colonies was counted at the end of the incubation period, and the obtained numbers were multiplied by the reciprocal of the dilution, using the following equation:
A= B*C
Where:
A = Number of colonies in 1 mL
B = Number of growing colonies
C = Reciprocal of the dilution

Statistical Analysis
Statistical analysis of the study data was conducted using a complete random design in a factorial experiment (FCRD) within the Statistical Analysis System (SAS, 2017). Different letters indicate significant differences among treatment means according to Duncan's multiple range test at a significance level of \(P < 0.05 \).

RESULTS AND DISCUSSION

Effect of Ozone Treatment on Total Soluble Solids (Brix Degree) of Orange Juice During Cold Storage for 30 Days

The results of the statistical analysis shown in Figure (4-1) indicate that treating orange juice with ozone did not lead to significant changes in the total soluble solids values at the level of \(P > 0.05 \) during the storage periods (0, 7, 14, and 21 days). The recorded values were (12.05, 12, 11.95, and 11.92 Brix) respectively. A gradual decrease in the total solids values was observed throughout the storage period, with the total soluble solids value reaching (11.86 Brix) at the end of the 30-day storage period. This decline may be attributed to the breakdown of some solids during storage, such as the fermentation of sugars into alcohols, carbon dioxide, and water. Our results are consistent with those found by [21] when treating kino juice with ozone, where they indicated that ozone treatment reduced the total soluble solids values during storage.Additionally, [22] noted that the total solids values decreased when strawberries were exposed to ozone at various concentrations.

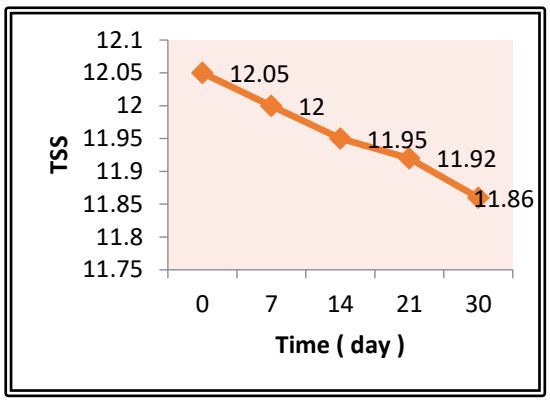

Figure 1. The effect of treating orange juice with ozone on the values of dissolved solids (Brix), stored at (4)°C for (30) days.

Effect of Ozone Treatment on the Corrected Total Acidity (%) Values of Orange Juice During Refrigerated Storage for 30 Days.

The results of the statistical analysis shown in Figure (4-2) indicate that the treatment of orange juice with ozone did not result in significant changes in the corrected total acidity (%) values at the level (P>0.05) during the storage periods (0, 7, 14, and 21 days), where the values were (0.47, 0.49, 0.49, and 0.52) %, respectively. A slight significant difference was observed at the level (P<0.05) during the 30-day storage period, where the value was (0.55) %. Our results are consistent with those reported [23].

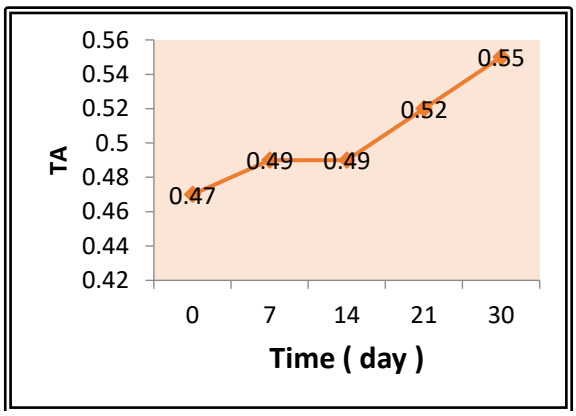

Figure 2. The effect of treating orange juice with ozone on the total acidity values (%) of orange juice during refrigerated storage for a period of (30) days.

Effect of Ozone Treatment on pH Values of Orange Juice During Refrigerated Storage for 30 Days.

The results of the statistical analysis shown in Figure (4-3) indicate that the treatment of orange juice with ozone did not result in significant changes in the pH values at the level (P>0.05) during the storage periods (0, 7, 14, 21, and 30 days), with the mentioned values being (3.41, 3.39, 3.37, 3.36, and 3.35) respectively. Our results align with those reported by Sroy et al. (2019) when they

treated watermelon juice with ozone and noted no significant differences in pH values.

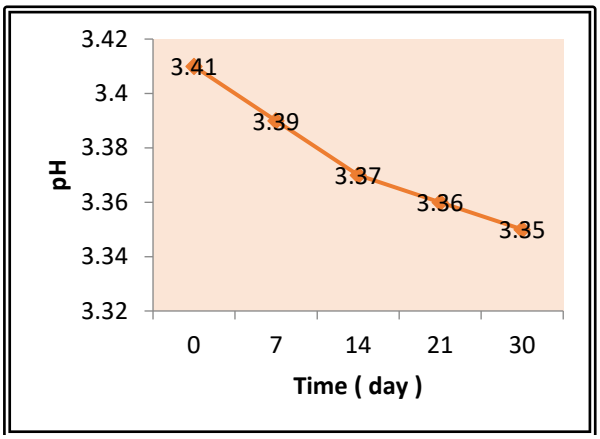

Figure 3. The effect of treating orange juice with ozone on the pH of orange juice during refrigerated storage for a period of (30) days.

Effect of Ozone Treatment on Ascorbic Acid Values (mg/100 ml) in Orange Juice During Refrigerated Storage for 30 Days.

The results of the statistical analysis shown in Figure (4-4) indicate that the treatment of orange juice with ozone led to significant changes in ascorbic acid values at the level (P>0.05), resulting in a decrease in ascorbic acid content. This decline persisted throughout the storage period (0, 7, 14, 21, and 30 days), with the values being (16.71, 12.4, 8.45, 5.11, and 2.23) respectively. This decrease is attributed to the high oxidative activity of ozone, which is a strong oxidizer. [24], supported the decline in ascorbic acid values in his study when he treated orange juice with ozone. [25] also noted a decrease in ascorbic acid values in ozone-treated apple juice. Our results are consistent with those of [26], who observed a significant drop in ascorbic acid values when treating orange juice with ozone.

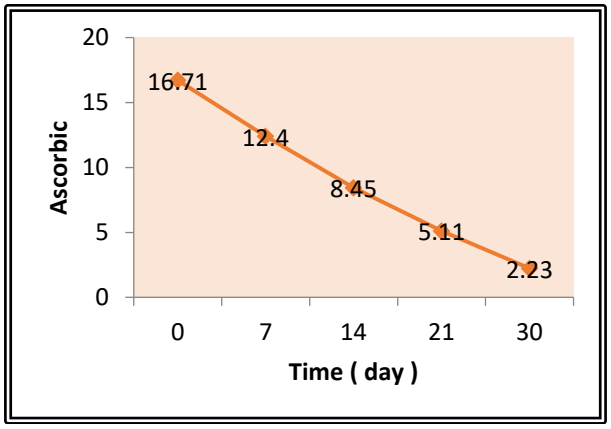

Figure 4. The effect of treating orange juice with ozone on the values of ascorbic acid (mg/100 ml) for orange juice during cold storage for a period of (30) days.

Effect of Ozone Treatment on Total Phenolic Content Values (mg/100 ml) in Orange Juice During Refrigerated Storage for 30 Days.

The results of the statistical analysis shown in Figure (4-5) indicate that the treatment of orange juice with ozone did not lead to significant changes in total phenolic content values (mg/100 ml) at the level (P>0.05) during the storage periods (0, 7, 14, and 21 days), with the values being 75, 72, 75, and 84 mg/100 ml, respectively. A gradual increase in solid content was observed throughout the storage period, with the total soluble solids reaching 88 mg/100 ml by the end of the 30-day storage period. Our results are consistent with those reported by [27], who demonstrated that there were no significant differences in ozone-treated orange juice. However, these results did not align with those found by [28], who noted a significant decrease in total phenolic values when treating apple juice with ozone.

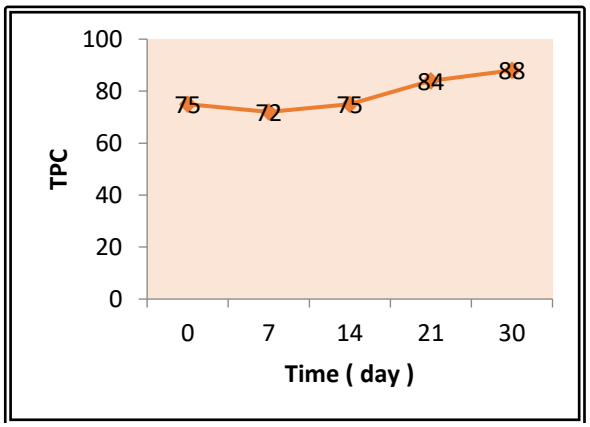

Figure 5. The effect of treating orange juice with ozone on the values of total phenols content (mg/100 ml) for orange juice during refrigerated storage for a period of (30) days.

Effect of Ozone Treatment on Browning Index Values (Abs. at 420 nm) in Orange Juice During Refrigerated Storage for 30 Days.

The results of the statistical analysis shown in Figure (4-6) indicate that the treatment of orange juice with ozone did not result in significant changes in browning index values (Abs. at 420 nm) at the level (P>0.05) during the storage periods (0, 7, 14, 21, and 30 days), with the values being (0.137, 0.135, 0.135, 0.134, and 0.131) Abs. at 420 nm, respectively. The results also indicated a non-significant decrease in the browning index values of ozone-treated orange juice. This finding is consistent with [29] and [30], who reported a decrease in the browning index values of ozone-treated sugarcane juice under different periods and ozone concentrations.

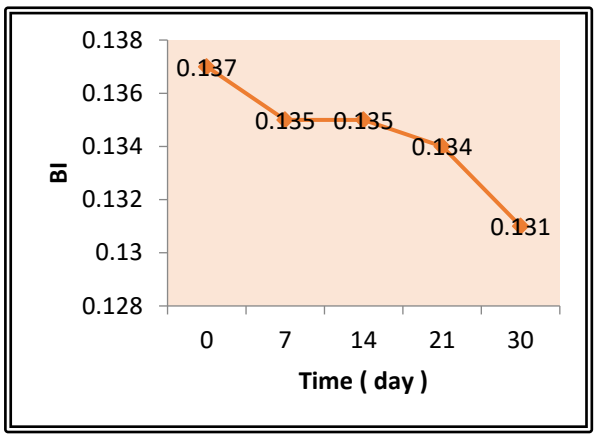

Figure 6. The effect of treating orange juice with ozone on the browning index values (Abs. at 420 nm) for orange juice during refrigerated storage for a period of (30) days.

Effect of Ozone Treatment on Total Bacterial Count (Log cfu/ml) in Orange Juice During Refrigerated Storage for 30 Days.

The results of the statistical analysis shown in Figure (4-7) indicate that the treatment of orange juice with ozone led to a reduction in total bacterial count to 1.47 Log cfu/ml. There were no significant changes in total bacterial count at the level (P>0.05) during the storage periods (0 and 7 days), with the values being 1.47 and 1.6 Log cfu/ml, respectively. A gradual increase in total bacterial count was observed throughout the storage period, reaching 1.63, 1.69, and 1.75 Log cfu/ml at 14, 21, and 30 days, respectively. Our results are consistent with those of [31], who observed a decrease in total bacterial count in grapefruit juice after ozone treatment, as well as an increase in total bacterial count during the storage period until the end of the storage duration.

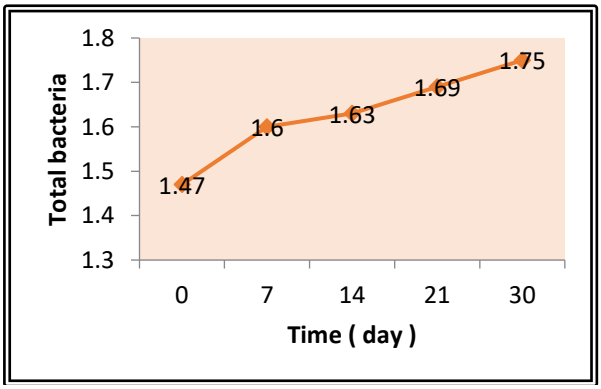

Figure 7. The effect of treating orange juice with ozone on the total bacterial count (Log cfu/ml) of orange juice during refrigerated storage for a period of (30) days.

Effect of Ozone Treatment on the Number of Molds and Yeasts (Log cfu/ml) in Orange Juice During Refrigerated Storage for 30 Days

The results of the statistical analysis shown in Figure (4-8) indicate that the treatment of orange juice with ozone did not lead to significant changes in the numbers of yeasts and molds at the level of ($P > 0.05$) during the storage periods of 0, 7, and 14 days, with counts of 0.89, 0.92, and 0.94 Log cfu/ml, respectively. A slight significant difference was observed at the level of ($P < 0.05$) during the storage periods of 21 and 30 days, with counts of 0.98 Log cfu/ml. Our results are consistent with those observed a decrease in the total bacterial count in grapefruit juice after ozone treatment, as well as an increase in the total bacterial count during the storage period until the end of the storage duration.

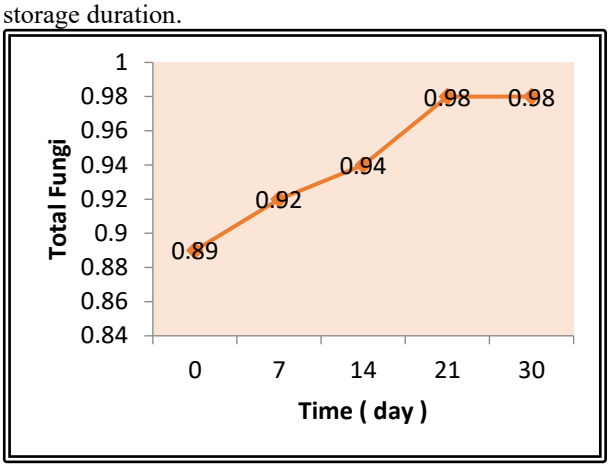

Figure 8. The effect of treating orange juice with ozone on the number of molds and yeasts (Log cfu/ml) of orange juice during cold storage for a period of (30) days.

Conclusions

1. It was observed that the use of ozone resulted in a slight decrease in the total soluble solids (TSS) values, which reached 11.86 at the end of the storage period, compared to 12.05 before storage.
2. 2. The treatment with ozone led to an increase in the total acidity values, rising from 0.46 to 0.55.
3. The use of ozone resulted in a decrease in pH values during the different storage periods.
4. A significant loss in ascorbic acid concentration occurred in the ozone-treated orange juice at the end of the storage period.
5. The concentration of total phenolic compounds increased after treatment with ozone, rising from 75 mg/100 ml in the juice before storage to 88 mg/100 ml after 30 days.
6. Ozone gas played a role in reducing the browning index values during the different storage periods.
7. The treatment of orange juice with ozone led to a slight increase in the total count of bacteria and yeasts.

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Competing Interests
There are no competing interests.

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