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Research Article

Effect of Preservatives and Storage Conditions on Physicochemical and Sensory Quality of Sweet Orange Juice

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Abstract

Fruit juices are susceptible to spoilage in the absence of preservatives. The aim of this study is to evaluate the effects of preservatives and storage conditions on physicochemical and sensory quality of sweet orange juice. The experiment focused on using treatments of different preservatives *viz*: P_0: No preservative (Control), P_1: Honey @ 50 ml/L, P_2: Ginger extract @ 3 ml/L, P_3: Cardamom extract @ 5 ml/L, and two storage conditions *viz*: T_0: Ambient condition (25 ± 2°C) and T_1: Refrigerator condition (4 ± 1 ℃). The results revealed that the highest pH (4.05, 4.00, 3.90 and 3.75), total soluble solid (11.64, 10.64, 10.15 and 8.64 °Brix), and ascorbic acid content (48.42, 44.49, 41.67 and 37.67 mg/100g) and lowest microbial growth (2.20, 3.13, 4.33 and 5.80 $\times 10^3$ cfu/ml) was recorded in honey treated sweet orange juice stored in the refrigerator at 5, 10, 15 and 20 days after storage respectively. The honey-treated juice was the most organoleptically accepted of the treatments. It can be inferred that the quality attributes of honey-treated juice were minimized while maintaining physicochemical and sensory quality when stored in refrigerated conditions. The current work is basically a very useful tool for the industry because it allows the correct adjustment of both sensory quality and microbiological parameters, leading to less returned and spoiled products.

Keywords

Sweet Orange, Preservative, Juice, Microbial Growth, Sensory Quality

1. Introduction

Sweet orange (*Citrus sinensis* Osbeck), belonging to the family Rutaceae, originated from Southeast Asia and is the most widely cultivated species among citrus fruits worldwide [1]. Globally, oranges represent an economically important fruit crop, with an annual production of approximately 75.57 million metric tonnes from 9.93 million hectares as reported in 2023 [2]. Sweet orange is rich in essential nutrients such as folacin, calcium, potassium, thiamine, niacin, and magnesium, as well as a wide range of phytochemicals including limonoids, synephrine, hesperidin flavonoids, polyphenols, and pectin [3]. It is well-known as an excellent natural source of vitamin C, a powerful antioxidant that supports the body's immune system [4].

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Sweet orange juice is widely consumed for its refreshing taste and health-promoting properties. The juice is enjoyed in various forms, including fresh, canned, and processed beverages, making it a versatile choice for nutrient intake [5]. Sweet orange juice, with its high moisture and sugar content, is highly perishable and prone to rapid microbial spoilage and enzymatic deterioration. These factors lead to degradation of nutrients, undesirable changes in flavor and color, and reduced shelf life, posing challenges for storage, distribution, and consumer safety [6].

To mitigate these issues, preservatives are added to inhibit microbial growth and enzymatic activity, thereby maintaining the juice's freshness, safety, and nutritional value over time. Historically, chemical preservatives such as sodium benzoate, potassium sorbate, and sulfur dioxide have been commonly used due to their effective antimicrobial and antioxidant capabilities [7]. Long-term or excessive consumption of synthetic preservatives has been linked to allergic reactions, respiratory issues such as asthma, digestive discomfort, and in some studies, possible carcinogenic effects [8]. Additionally, chemical preservatives can sometimes impart off-flavors or alter the natural taste of the juice, diminishing consumer acceptance. Environmental concerns regarding the accumulation and disposal of synthetic chemicals further limit their desirability in food products [9].

Bio-preservatives possess inherent antimicrobial and antioxidant properties that help inhibit microbial growth, reduce oxidative damage, and preserve the nutritional value of sweet orange juice [10]. Honey is widely recognized for its potent antimicrobial effects, which are attributed to its high sugar concentration creating osmotic pressure unfavorable to microbes, its acidic pH, and the enzymatic generation of hydrogen peroxide, together effectively suppressing spoilage microorganisms [11]. Lemon juice extract, rich in citric acid and vitamin C, lowers the pH of the juice and offers antioxidant protection, thereby enhancing microbial safety and delaying nutrient degradation [12]. Similarly, ginger extract contains bioactive compounds such as phenolic acids, flavonoids, and essential oils with strong antioxidant and antimicrobial activities, contributing to improved juice quality and sensory attributes [13]. Cardamom extract, rich in terpenes, flavonoids, and phenolic compounds such as cineole and α-terpinyl acetate, exhibits broad-spectrum antimicrobial properties and effectively inhibits lipid oxidation, thereby enhancing shelf life and maintaining flavor stability [14]. The synergistic application of these natural bio-preservatives offers a promising, clean-label alternative for maintaining the freshness, safety, and nutritional profile of sweet orange juice, while minimizing the risks associated with synthetic chemicals [15]."

Furthermore, storage conditions significantly influence the shelf life and quality of sweet orange juice. Storage at ambient temperatures accelerates microbial proliferation and enzymatic reactions, leading to rapid nutrient loss, flavor deterioration, and discoloration, which shorten shelf life [16]. A thorough understanding of how bio-preservative treatments interact with different storage conditions is essential for developing optimized preservation strategies that deliver natural, safe, and high-quality juice products to consumers [17]. Given the increasing consumer demand for sweet orange juice and the importance of maintaining its quality during storage, this study aims to evaluate the effects of natural bio-preservatives on the quality and shelf life of sweet orange juice under varying storage conditions.

2. Materials and Methods

2.1. Materials

2.1.1. Experimental Site

The experiment was conducted at the Postharvest Laboratory o of the Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh during the period of May to June, 2025.

2.1.2. Experimental Materials

The sweet oranges (*Citrus sinensis*) used in this study were purchased from a local market (Kawran Bazaar), Dhaka, Bangladesh. To ensure consistency throughout the experiment, only firm, mature, and uniformly ripened fruits free from visible defects were carefully selected for juice extraction. Then the fruits were washed, surface sanitized with ozonized water for 20 minutes. The fruits were subjected to different bio preservatives as treatment. During the experiment, the average temperature and humidity were 25 ± 2°C and 65±2% respectively.

2.1.3. Treatments of the Experiment

The experiment was designed to study the quality assessment of sweet orange juice preserved with natural preservatives under different storage conditions. The experiment had two factors, which are as follows:

Factor A: Four different types of bio-preservatives

P_0: No preservative (Control)

P_1: Honey (50 ml/L)

P_2: Zinger extract (3 ml/L)

P_3: Cardamon extract (5 ml/L)

Factor B: Different storage conditions

T_0: Ambient condition (25 ± 2 ℃)

T_1: Refrigerator condition (4 ± 1 ℃)

2.2. Methods

2.2.1. Extraction and Sealing of Juice

The sweet orange fruits were thoroughly washed with clean tap water, followed by two rinses in chlorinated water and a final rinse with distilled water. The fruits were weighed using a digital balance, peeled, and reweighed to determine peel weight. Sweet orange juice was extracted using an electronic juice extractor and filtered through a tea sieve to remove pulp and coarse particles. To the filtered juice, 100 g of sugar, 2 g of citric acid, and 500 mg of ascorbic acid were added, then blended with potable water at a 7:3 (v/v) juice-to-water ratio using a Panasonic MX800S multifunction blender (Malaysia) until a uniform consistency was achieved. The juice was further strained through muslin cloth before the addition of bio-preservatives, namely honey, lemon juice extract, ginger extract, and cardamom extract, according to treatment specifications. The prepared sweet orange juice was pasteurized at 80 °C for 20 seconds, then, allowed to cool to room temperature. After cooling, the juice was carefully poured into 250 mL glass jars and sealed tightly with airtight lids to ensure optimal preservation. The sealed samples were properly labeled and stored in two storage conditions.

Table 1. Bio-preservatives added according with par treatment requirement.

Treatments (Bio-preservatives)	Fruit Juice (mL)	Water (mL)	Ascorbic Acid (mg)	Citric Acid (g)	Sugar (g)
No preservative (Control)	700	300	500	2	100
Honey (50 ml/L)	700	300	500	2	100
Ginger extract (3 ml/L)	700	300	500	2	100
Cardamom extract (ml/L)	700	300	500	2	100

2.2.2. Storage Studies

Quality parameters were monitored throughout a month-long storage of processed dragon fruit jellies at room temperature (25 ±1 ℃) and in a refrigerator (10 ±1 ℃). At 0, 5, 10, 15 and 20 days after storage (DAS), the preserved orange juice was subjected to chemical analysis, organoleptic evaluation, and microbial examination.

2.2.3. Total Soluble Solids (TSS) Content and pH Determination

A digital refractometer (MA871; Romania) was used to measure the TSS content of sweet orange juice. Using a dropper, a drop of dragon fruit juice was extracted and put on the refractometer prism. The total soluble solid reading was displayed by the refractometer.

A digital pH meter (HI 2211; Romania) was used to measure the pH after the fruit juices of each treated sweet orange were filtered independently.

2.2.4. Titratable Acidity (TA%)

In order to determine the titratable acidity, the 5 ml of fruit juice samples were filtered and 100 milliliters of water were added. Two drops of phenolphthalein were then added to 10 milliliters of stock solution in a conical flask. 1N NaOH was used to titrate the solution three times. Until the pink hue emerged, the titration was stopped.

2.2.5. Ascorbic Acid Determination

5 ml of sweet orange fruit was filtered (Whatman No. 1) to sieve the liquid. A solution of 5% oxalic acid was added to get the volume up to 100 milliliters. The dye solution 2, 6-dichlorophenol indophenol, was used for the titration. Using the L-ascorbic acid standard, the mean observations yielded the quantity of dye needed to oxidize a specific amount of L-ascorbic acid solution at an unknown concentration. Each time titration was performed using a 5 ml solution, and the pink color marked final point of titration, which persisted for 10 seconds.

2.2.6. Reducing Sugar Content

Three milliliters of the extract and three milliliters of DNS reagent were pipetted into each test tube and thoroughly combined. For five minutes, the test tube was heated in a bath of boiling water. After the color had developed, 1 milliliter of 40% Rochelle salt was added to the tubes while the contents were still warm. The test tubes were then cooled using running water from the faucet. A reagent blank was made by mixing 3 ml of DNS reagent with 3 ml of distilled water in a tube, and it was then treated in the same way. A colorimeter was used to measure the solution's absorbance at 575 nm.

$$\text{Reducing sugar (\%)} = \text{Amount of reducing sugar obtained/ Weight of sample} \times 100 \qquad (1)$$

2.2.7. Sensory Analysis of Sweet Orange Juice

The sensory qualities of dragon fruit jellies were examined. A panel of ten semi-trained individuals used a nine-point Hedonic scale to assess sensory quality attributes. The Dragon fruit jellies were assessed for color, flavor, taste, appearance, and overall acceptability. The evaluation was conducted at four intervals: 0, 10, 20, and 30 days after storage.

Table 2. Sensory Evaluation using 9-Point Hedonic Scale.

Score / Rating	Hedonic Scale
9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much

2.2.8. Microbiological Count of Sweet Orange Juice

The microbial population in sweet orange juice stored under different conditions was analyzed using the spread plate method [18]. Nutrient agar was used as the selective medium for microbial enumeration. One milliliter of fruit juice was pipetted into a sterile test tube containing 9 ml of distilled water, resulting in a 10^{-1} dilution. To ensure consistent dispersion of microbial cells, the test tube was shaken thoroughly. From this 10^{-1} dilution, 1 ml was transferred to another test tube containing 9 ml of distilled water, creating a 10^{-2} dilution. For enumeration, 0.1 ml of the 10^{-2} dilutions of both fresh and treated juice samples were pipetted onto nutrient agar plates. The samples were then spread uniformly using a sterilized glass L-rod. These dilutions were selected based on preliminary studies and a thorough review of literature. The plates were kept in upright position for few minutes. The plates were inverted and incubated at 35 ℃ for 24 to 48 hours. The colonies were counted after incubation. The average number of bacteria per ml of juice was estimated by using the following formula:

$$\text{Total plate count (cfu/ml) of sample} = \frac{\text{Average number of colonies from duplicate plates}}{\text{Dilution factor} \times \text{Volume plated}} \quad (2)$$

2.2.9. Statistical Analysis

The experiments used a complete randomized design (CRD) with two factors. The Statistical Analysis System (SAS), version 9.4 (SAS Institute, Cary, NC, USA), was used for statistical analyses. The mean value among the treatments were considered to be statistically significant when $P<0.05$. All results were presented with mean ± SE from the replicates.

3. Results

3.1. Proximate Composition of Sweet Orange Fruit and Its Juice

Table 3. Physico-chemical properties of sweet orange (Citrus sinensis) fruit and juice.

Sl. No	Parameters	Mean values
a	Physical parameters of fruit	
1	Fruit colour	Yellow-orange
2	Fruit segment colour	Yellowish orange
3	Fruit shape	Roundish oval
4	Average weight of sweet orange (g)	188.9
5	Horizontal diameter (cm)	6.37
6	Vertical diameter (cm)	6.67
7	Thickness of the rinds (cm)	0.18
8	Number of segments per fruit	10.33
9	Number of seeds per fruit	13.67
b	Chemical parameters of juice	
1	Moisture (%)	87.60
2	pH	3.90
3	TSS, ^{0}Brix	10.67
4	Titrable acidity,%	0.41
5	Reducing sugars,%	1.83
6	Ascorbic acid, mg/100 g	45.67

*Each value is an average of three determinations

The physico-chemical properties of sweet orange (*Citrus sinensis*) fruit and its juice were thoroughly evaluated to provide a comprehensive understanding of its quality attributes, as presented in Table 3. The fruit displayed a characteristic yellow-orange peel color and yellowish-orange segments, with a roundish oval shape. The average fruit weight was (188.9 g), with horizontal and vertical diameters of 6.37 cm and 6.67 cm, respectively. The rind thickness was 0.18 cm and each fruit contained about 10.33 segments and 13.67 seeds. Chemically, the juice contained 87.60% moisture, a pH value of 3.90, total soluble solids (TSS) of 10.67 °Brix, acidity of 0.41%, reducing sugars at 1.83% and ascorbic acid content was 45.67 mg/100 g.

3.2. pH and Titratable Acidity

Effect of bio-preservatives and temperature significantly influenced the pH levels of preserved sweet orange juice at different storage periods (Table 4). The maximum pH (4.05, 4.00, 3.90, and 3.75 at 5, 10, 15, and 20 DAS respectively) was recorded in P_1T_1 treatment and the minimum (3.65, 3.15, 3.05, and 2.90 at 5, 10, 15, and 20 DAS respectively) was recorded in P_0T_0 treatment.

There was a significant variation in titratable acidity (TA) of sweet orange juice during storage due to various bio-preservatives and storage conditions (Table 4). The highest value of TA was found (0.53, 0.74, 0.86, and 0.98% at 5, 10, 15, and 20 DAS respectively) was recorded in P_0T_0 treatment, and the lowest (0.42, 0.49, 0.52, and 0.59% at 5, 10, 15, and 20 DAS respectively) was found in P_1T_1 treatment.

Table 4. *Effect of preservatives and different storage conditions on the pH level of sweet orange juice at different days after storage (DAS).*

Treatments	pH levels				TA (%)			
	5 DAS	10 DAS	15 DAS	20 DAS	5 DAS	10 DAS	15DAS	20DAS
P_0T_0	3.65 e	3.15 f	3.05 f	2.90 f	0.53 a	0.74 a	0.86a	0.98 a
P_0T_1	3.67 de	3.45 e	3.22 e	3.00 ef	0.47cd	0.63 b	0.74b	0.82 b
P_1T_0	4.00 ab	3.85 bc	3.60 c	3.35 c	0.45 e	0.54 e	0.59e	0.65 d
P_1T_1	4.05 a	4.00 a	3.90 a	3.75 a	0.42 f	0.49 f	0.52 f	0.59 e
P_2T_0	3.75 d	3.65 d	3.30 e	3.10 de	0.50 b	0.59 c	0.68c	0.82 b
P_2T_1	3.95 bc	3.80 c	3.55 cd	3.35 c	0.46de	0.57 cd	0.62d	0.70 c
P_3T_0	3.90 c	3.70 d	3.45 d	3.20 d	0.48 c	0.56 de	0.64d	0.72 c
P_3T_1	4.00 ab	3.90 b	3.75 b	3.50 b	0.46de	0.51 f	0.58e	0.66 d
$LSD_{(0.05)}$	0.08	0.07	0.10	0.10	0.01	0.02	0.02	0.03
CV (%)	1.28	1.20	1.771	1.82	1.93	2.15	2.58	3.01

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. LSD (0.05) = Least significant difference at 5% level and CV (%) = coefficient of variation in percent. Here, P_0: No preservative (control), P_1: Honey, P_2: Zinger, P_3: Cardamom, T_0: Ambient temperature (25±2 ℃) and T_1: Refrigerator temperature (4±1 ℃).

3.3. Total Soluble Solids and Vitamin C Content

Bio-preservatives and storage conditions had the significant impacts on the total soluble solids (TSS) content of sweet orange juice during different DAS (Table 5). P_1T_1 treatment had the highest TSS values i.e. 11.64, 10.64, 10.15, and 8.64 °Brix at 5, 10, 15, and 20 DAS respectively. However, P_0T_0 treatment had the lowest TSS values i.e. 45.33, 45.90, and 46.67 °Brix at 10, 20, and 30 DAS respectively.

Table 5. *Effect of -preservatives and different storage conditions on the total soluble solids (TSS) and ascorbic acid content of sweet orange juice at different days after storage (DAS).*

Treatments	TSS (°Brix)				Ascorbic acid (mg/100g)			
	5 DAS	10 DAS	15 DAS	20 DAS	5 DAS	10 DAS	15 DAS	20 DAS
P_0T_0	8.55 f	7.10 g	5.90 e	4.85 f	37.60 g	29.62 g	21.33 f	12.49 g
P_0T_1	8.70 f	7.35 f	6.05 e	5.00 f	40.90 f	34.81 f	28.98 e	20.67 f
P_1T_0	11.22 b	9.80 b	8.37 b	7.29 bc	45.86 bc	39.89 c	36.00 c	30.24 c

Treatments	TSS (°Brix)				Ascorbic acid (mg/100g)			
	5 DAS	10 DAS	15 DAS	20 DAS	5 DAS	10 DAS	15 DAS	20 DAS
P_1T_1	11.64 a	10.64 a	10.15 a	8.64 a	48.42 a	44.49 a	41.67 a	37.67 a
P_2T_0	9.60 e	7.94 e	7.09 d	6.05 e	44.36 de	38.81 d	31.24 d	26.83 d
P_2T_1	9.66 e	8.64 d	7.60 c	7.05 c	46.20 b	43.56 b	37.33 b	32.32 b
P_3T_0	10.00 d	8.75 d	7.54 c	6.49 d	43.56 e	37.68 e	30.42 d	24.39 e
P_3T_1	10.24 c	9.00 c	8.10 b	7.55 b	45.14 cd	40.73 c	35.60 c	30.67 c
$LSD_{(0.05)}$	0.18	0.17	0.30	0.32	0.82	0.87	0.86	0.93
CV(%)	1.05	1.18	2.26	2.80	1.10	1.29	1.51	1.97

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. LSD (0.05) = Least significant difference at 5% level and CV (%) = coefficient of variation in percent. Here, P_0: No preservative (control), P_1: Honey, P_2: Zinger, P_3: Cardamom, T_0: Ambient temperature (25±2 °C) and T_1: Refrigerator temperature (4±1 °C).

Experimental result reveals that at 5, 10, 15, and 20 days of storage, the highest value of vitamin C content 48.42, 44.49, 41.67, and 37.67 mg/100g at 5, 10, 15, and 20 DAS respectively were observed in the P_1T_1 treatment. The lowest vitamin C content 37.60, 29.62, 21.33, and 12.49 mg/100g was recorded in the P_0T_0 treatment at 5, 10, 15, and 20 days of storage respectively (Table 5).

3.4. Reducing Sugars (%) and Microbial Count (cfux10^3)

The maximum reducing sugar 3.67, 4.10, 4.72, and 5.86% was found in P_0T_0 treatment at 5, 10, 15, and 20 DAS and the lowest reducing sugar 2.87, 2.90, 2.98, and 3.07% were recorded in the P_0T_1 treatment at 5, 10, 15, and 20 DAS respectively (Table 6).

The study demonstrated the antibacterial qualities of honey by successfully preventing the growth of bacteria in the sweet orange juice. This impact may help to ensure the product's safety for consumption and prolong its shelf life. The highest microbial counts (5.00, 11.60, 15.60, and 22.27 × 10^3 cfu x10^3 at 10, 20, and 30 DAS, respectively) was recorded in samples treated with no preservatives and stored at ambient condition (P_0T_0). However, the lowest microbial count (2.20, 3.13, 4.33, and 5.80 × 10^3 cfu/ml at 5, 10, 15 and 20 DAS, respectively) was found in honey-treated juice stored at refrigerator (Table 6).

Table 6. *Effect of preservatives and different storage conditions on the reducing sugar content and microbial count of sweet orange juice at different days after storage (DAS).*

Treatments	Reducing sugar (%)				Microbial count (10^3cfu/ml)			
	5 days	10 days	15 days	20 days	5 days	10 days	15 days	20 days
P_0T_0	3.67 a	4.10 a	4.72 a	5.86 a	5.00 a	11.60 a	15.60 a	22.27 a
P_0T_1	3.15 b	3.55 b	4.17b	4.90 b	3.33 c	8.07 b	11.67 b	14.33 b
P_1T_0	2.92 de	3.00 e	3.10 e	3.33 e	3.07 d	4.67 e	6.00 e	7.67 e
P_1T_1	2.87 e	2.90 e	2.98 e	3.07 f	2.20 f	3.13 g	4.33 f	5.80 f
P_2T_0	3.17 b	3.48 bc	3.90 c	4.83 b	3.13 d	5.00 d	8.00 d	9.67 d
P_2T_1	3.05 bc	3.30 d	3.44 d	4.25 d	2.67 e	4.33 f	6.33 e	8.00 e
P_3T_0	3.10 bc	3.35 cd	3.78 c	4.56 c	3.67 b	5.40 c	8.66 c	11.00 c
P_3T_1	3.00 cd	3.22 d	3.45 d	4.05 d	3.27 c	4.80 de	7.67 d	9.27 d
$LSD_{(0.05)}$	0.12	0.15	0.18	0.23	0.13	0.30	0.48	0.70

Treatments	Reducing sugar (%)				Microbial count (10^3cfu/ml)			
	5 days	10 days	15 days	20 days	5 days	10 days	15 days	20 days
CV (%)	2.25	2.57	2.90	3.13	2.41	2.96	3.26	3.66

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. LSD (0.05) = Least significant difference at 5% level and CV (%) = coefficient of variation in percent. Here, P_0: No preservative (control), P_1: Honey, P_2: Zinger, P_3: Cardamom, T_0: Ambient temperature (25±2 ℃) and T_1: Refrigerator temperature (4±1 ℃).

Sensory quality of juice

The organoleptic evaluation such as color, flavor, taste, appearance and overall acceptability of sweet orange juice was significantly affected by bio-preservative and storage conditions (Tables 7, 8 & 9). At 20 days of storage, the highest score of color, flavor, taste, appearance and overall acceptability 6.67, 6.67, 6.00, 6.33 and 6.42 respectively, was recorded for juice preserved with honey and stored at refrigerator temperature (P_1T_1). However, the lowest score of color, flavor, taste, appearance and overall acceptability 3.00, 2.67, 3.00, 2.33 and 2.75 respectively, was recorded for juice preserved with honey and stored at ambient temperature with using no preservatives (P_0T_0).

Table 7. *Organoleptic score of color of preserved sweet orange juice at different days after storage (DAS).*

Treatments	Color				Flavor			
	5 DAS	10DAS	15 DAS	20 DAS	5 DAS	10DAS	15 DAS	20 DAS
P_0T_0	5.75 e	5.29 f	4.00 f	3.00 g	5.32 f	4.35 d	3.39 e	2.67 g
P_0T_1	6.00 d	5.67 e	4.33 e	3.92 f	5.98 e	5.97 c	5.95 cd	4.33 e
P_1T_0	7.00 c	6.96 c	6.92 b	6.00 c	7.04 c	7.00 b	6.93 b	5.00 d
P_1T_1	8.67 a	7.92 a	7.33 a	6.67 a	8.33 a	8.00 a	7.94 a	6.67 a
P_2T_0	6.92 c	5.96 d	5.92 cd	5.00 e	6.04 e	6.02 c	5.97 cd	4.33 e
P_2T_1	7.96 b	7.67 b	7.00 b	6.33 b	7.33 b	7.00 b	6.95 b	5.67 b
P_3T_0	6.00 d	5.83 de	5.67 d	4.04 f	6.00 e	5.95 c	5.67 d	4.00 f
P_3T_1	7.04 c	6.96 c	6.00 c	5.33 d	6.67 d	6.94 b	6.00 c	5.33 c
$LSD_{(0.05)}$	0.20	0.23	0.25	0.25	0.24	0.29	0.31	0..22
CV (%)	1.73	2.05	2.49	2.85	2.15	2.59	2.93	2.73

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. LSD (0.05) = Least significant difference at 5% level and CV (%) = coefficient of variation in percent. Here, P_0: No preservative (control), P_1: Honey, P_2: Zinger, P_3: Cardamom, T_0: Ambient temperature (25±2 ℃) and T_1: Refrigerator temperature (4±1 ℃).

Table 8. *Organoleptic score of color of preserved sweet orange juice at different days after storage (DAS).*

Treatments	Taste				Appearance			
	5 DAS	10DAS	15 DAS	20 DAS	5 DAS	10DAS	15 DAS	20 DAS
P_0T_0	5.33 g	4.96 g	3.67 f	3.00 g	4.96 f	4.57 f	3.96 f	2.33 g
P_0T_1	5.67 f	5.33 f	4.92 e	3.58 f	5.67 e	4.96 e	4.67 e	3.67 e
P_1T_0	7.00 c	6.33 c	6.00 b	5.67 b	7.02 b	5.95 c	5.93 c	5.00 c

Treatments	Taste				Appearance			
	5 DAS	10DAS	15 DAS	20 DAS	5 DAS	10DAS	15 DAS	20 DAS
P_1T_1	7.67 a	7.00 a	6.33 a	6.00 a	8.00 a	6.94 a	6.90 a	6.33 a
P_2T_0	7.00 c	6.33 c	5.67 c	5.00 d	6.02 d	5.33 d	5.00 d	4.67 d
P_2T_1	7.33 b	6.67 b	6.00 b	5.33 c	7.96 a	6.67 b	6.33 b	5.33 b
P_3T_0	5.95 e	5.67 e	5.00 e	4.33 e	5.88 de	5.00 e	4.83 de	3.33 f
P_3T_1	6.33 d	6.00 d	5.33 d	5.00 d	6.33 c	6.00 c	5.04 d	4.67 d
$LSD_{(0.05)}$	0.24	0.24	0.28	0.27	0.25	0.26	0.29	0.27
CV (%)	2.16	2.36	3.02	3.25	2.27	2.70	3.14	3.53

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. LSD (0.05) = Least significant difference at 5% level and CV (%) = coefficient of variation in percent. Here, P_0: No preservative (control), P_1: Honey, P_2: Zinger, P_3: Cardamom, T_0: Ambient temperature (25±2 ℃) and T_1: Refrigerator temperature (4±1 ℃).

Table 9. *Organoleptic score of color of preserved sweet orange juice at different days after storage (DAS).*

Treatments	Overall acceptability			
	5 DAS	10 DAS	15 DAS	20 DAS
P_0T_0	5.34 f	4.79 f	3.76 f	2.75 f
P_0T_1	5.83 e	5.48 e	4.97 e	3.88 e
P_1T_0	7.02 c	6.56 c	6.45 b	5.42 b
P_1T_1	8.17 a	7.47 a	7.13 a	6.42 a
P_2T_0	6.50 d	5.91 d	5.64 c	4.75 d
P_2T_1	7.65 b	7.00 b	6.57 b	5.50 b
P_3T_0	5.96 e	5.61 de	5.29 de	3.93 e
P_3T_1	6.59 d	6.48 c	5.59 cd	5.08 c
$LSD_{(0.05)}$	0.28	0.30	0.32	0.28
CV(%)	2.48	2.80	3.25	3.49

Means in the column followed by the same letter(s) are not significantly different at 5% level of significance. LSD (0.05) = Least significant difference at 5% level and CV (%) = coefficient of variation in percent. Here, P_0: No preservative (control), P_1: Honey, P_2: Zinger, P_3: Cardamom, T_0: Ambient temperature (25±2 ℃) and T_1: Refrigerator temperature (4±1 ℃).

4. Discussion

The use of bio-preservatives in sweet orange (*Citrus sinensis*) juice is gaining attention as a safer and more sustainable alternative to synthetic preservatives, aiming to extend shelf life while maintaining physicochemical and sensory qualities. Preservatives aid to minimize microbial count in all juice sample treatments held at 4±1 ℃ and ambient temperature 25±2 ℃. Honey showed the highest reduction in sweet orange juice. Different types of honey, when used as natural preservatives, significantly reduced microbial proliferation in unpasteurized avocado juice via a variety of antimicrobial mechanisms, including high sugar concentration, low pH, hydrogen peroxide production, and the presence of organic acids [19]. Fruit juice kept in refrigeration showed a noticeably lower rise in microbial count over longer storage times than fruit juice kept at room temperature [20]. Similarly, a previous study reported that storage at ambient temperature (25 ±2 ℃) accelerated microbial proliferation in fruit beverages by promoting enzymatic reactions that created favorable conditions for spoilage and pathogenic microorganisms [21].

It is probable that honey was responsible for the synergistic antimicrobial and buffering properties, which collectively reduced microbial growth and subsequent acid production during storage. This effectively maintained higher pH levels and preserved the purity of the juice. Honey's enzymatic activity and natural antimicrobial compounds are responsible for the slowing of pH reduction in citrus-based products [22]. Refrigerated conditions were able to maintain higher pH stability by delaying the biochemical processes that are responsible for acid formation in fruit juice systems [23].

Honey-based preservatives effectively maintained TSS levels in citrus juices by inhibiting enzymatic activities responsible for sugar degradation [24]. In line with this, refrigerated storage at 4 ℃ greatly reduced TSS loss in fruit juices by limiting invertase enzyme activity and microbial fermentation [25]. Likewise previous study reported that the degradation of soluble carbohydrates in fruit beverages was accelerated by ambient temperature storage due to increased microbial metabolic activities and enhanced enzymatic reactions [26].

Honey-based preservation systems significantly reduced titratable acidity in fruit juices by inhibiting lactic acid bacteria and other acid-producing microorganisms [27]. This study found that refrigerated storage at 4 ± 1 ℃ considerably lowered titratable acidity [28]. Ambient temperature storage accelerated organic acid formation in fruit beverages through enhanced microbial metabolism and increased enzymatic conversion of carbohydrates into acidic byproducts [29].

The effectiveness of honey was attributed to the antimicrobial and enzymatic inhibition properties of honey and lemon juice extract, which effectively prevented the breakdown of complex carbohydrates into reducing sugars by suppressing invertase and other sugar-hydrolyzing enzymes, while simultaneously reducing microbial activities responsible for polysaccharide degradation during extended storage. These findings were consistent with previous study, which found that honey-based preservation systems dramatically reduced reducing sugar production in fruit juices by blocking the enzymatic breakdown of sucrose and other complex sugars [30]. Refrigerating fruit juices at 4 ± 1 ℃ suppressed invertase and other sugar-hydrolyzing enzymes, leading to a reduction in the formation of reducing sugars [31]. Carbohydrate deterioration in fruit blended juices was increased by ambient temperature storage, which promoted enzyme activities that transformed complex sugars into simpler reducing compounds [32].

Our findings revealed that sweet orange juice treated with honey contained more ascorbic acid than the other treatments. This may be due to the fact that honey successfully shielded ascorbic acid from oxidative deterioration by chelating metal ions that catalyze the breakdown of vitamin C and scavenging free radicals. It also created a protective matrix that reduced exposure to light and oxygen during prolonged storage. These findings aligned with previous studies who found that the presence of antioxidant compounds, such as flavonoids and phenolic acids, in bio-preservatives like honey greatly improved the retention of ascorbic acid in fruit juices by preventing oxidative losses [33]. Lower temperatures significantly reduced oxidative reactions that cause ascorbic acid degradation and reduced enzymatic activity involving ascorbate oxidase and peroxidase enzymes that typically catalyze vitamin C breakdown through oxidation processes, effectively slowing the conversion of L-ascorbic acid into dehydroascorbic acid and other degradation products during extended storage. This finding was consistent with that of previous study that discovered that lower storage temperatures considerably reduced ascorbic acid breakdown in fruit juices by reducing enzymatic and oxidative activity [34]. Similarly, chilling slowed the enzymatic breakdown of ascorbic acid, allowing it to be stored for a longer time [23].

Natural preservatives significantly preserved the color and appearance of fruit juices [35]. Adding honey to a mixed apple and passion fruit beverage improved its chemical composition and sensory properties [36]. This finding was consistent with that previous study reported that refrigeration delayed the degradation of sensory qualities in carbonated pomegranate juice, helping to maintain product stability throughout the storage periods [37]. Similarly, [38] who demonstrated that refrigeration combined with natural additives effectively maintained the sensory quality of citrus-based beverages. Furthermore, refrigeration combined with natural bio-preservatives effectively sustained consumer preference by protecting all organoleptic qualities, thereby ensuring better marketability and customer loyalty for premium juice products [39].

5. Conclusions

Natural bio-preservatives significantly enhance the quality and shelf life of sweet orange juice, while at ambient temperature; the control sample saw more compositional changes and lost the most quality. Natural preservation, combined with refrigerator, can readily be used to extend the shelf life of juices. Honey-treated juice showed a higher pH, TSS, ascorbic acid, and reduced microbiological development. According to the study's findings, honey-based juice stored in the refrigerator offered the best results, effectively increasing shelf life while maintaining physicochemical properties and consumer appeal.

Abbreviations

TSS	Total Soluble Solid
TA	Titratable Acidity
SAS	Statistical Analysis System
LSD	Least Significant Difference
CV	Coefficient of Variation
SE	Standard Error

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Conflicts of Interest

The authors declare no conflicts of interest.

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