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Effect of Thermal and Non-Thermal Treatments on Bell Pepper–Pineapple Juice during Cold Storage

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This study evaluated the effects of five preservation methods control, UV-C (254 nm, 40 min), mild thermal pasteurization (65 °C, 15 min), ultrasound (65 °C, 15 min), and chitosan addition ($0.2\ g \cdot 200\ mL^{-1}$) on a 1:1 ($v \cdot v^1$) bell pepper-pineapple juice blend stored at 4 ± 2 °C for 13 days. The untreated juice was rich in vitamin C, polyphenols, and flavonoids but prone to spoilage and oxidation. All treatments led to gradual declines in quality, though the chitosan group best preserved pH, total phenolic content (TPC), flavonoids (TFC), and DPPH antioxidant activity, and showed the lowest microbial growth. Ultrasound and UV-C provided moderate protection, whereas thermal treatment caused the most significant degradation of nutrients. The results highlight chitosan as the most effective strategy, supporting the use of non-thermal and bioactive-integrated technologies for extending the shelf life of antioxidant-rich juice blends in a sustainable manner.
Keywords: Antioxidants, Bell pepper, Chitosan, Cold storage, Non-thermal preservation, Pineapple juice.

1. Introduction

The rising challenges of climate change, resource depletion, and non-communicable diseases have increased demand for food products that are nutritious, safe, and environmentally sustainable. Fruit and vegetable juices are valued for their high content of polyphenols and flavonoids, which provide antioxidant and anti-inflammatory benefits (Thuphairo et al., 2019). However, these compounds are highly unstable during processing and storage, and many commercial juices still depend on synthetic preservatives. Such additives may raise health concerns and contribute to environmental burdens (Singh and Sharma, 2017). Chitosan is a natural cationic polysaccharide that is widely studied as a bio-preservative. Its antimicrobial effect comes from protonated amino groups ($–NH_3^+$) that bind to microbial cell membranes, increase permeability, and cause cell leakage, and to its ability to chelate metal ions, thereby limiting microbial growth and protecting antioxidant compounds (Liu et al., 2023; Zheng and Zhu, 2003). It is important to distinguish between its applications: as an edible coating, chitosan forms a physical barrier against microbial penetration (Xing et al., 2016), whereas in juice formulations it remains dissolved in the aqueous matrix and improves microbiological and physicochemical stability without film formation (Belgheisi and EsmaeilZadeh Kenari, 2019). In addition to chitosan, non-thermal technologies such as UV-C irradiation and ultrasound are gaining attention. These methods extend shelf life by suppressing microbial growth and delaying oxidation while minimizing nutrient loss compared with thermal pasteurization (Khursheed et al., 2024; Shamsudin et al., 2020). Bell pepper (*Capsicum annuum*) and pineapple (*Ananas comosus*) are nutrient-rich fruits cultivated in Southeast Asia, containing abundant vitamin C, phenolics, and flavonoids (Anaya-Esparza et al., 2021). When blended, their juice is functional but highly perishable due to its relatively low pH (~4.7) and high sugar content (~10 °Brix), conditions that favor microbial spoilage (Kaddumukasa et al., 2017). Despite this, few studies have systematically compared thermal and non-thermal preservation approaches in such juice systems. Therefore, this study evaluates the effects of UV-C, ultrasound, mild thermal processing, and chitosan addition on the physicochemical and microbiological stability of bell pepper-pineapple juice during cold storage.

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2. Materials and methods

2.1 Materials

Fresh yellow bell peppers (*Capsicum annuum*) and pineapples (*Ananas comosus*) were procured from Hoc Mon commune, Ho Chi Minh City, Vietnam (10.9174° N, 106.5897° E). Chitosan powder (LanShan-Shaanxi,China), Folin–Ciocalteu reagent, gallic acid, sodium carbonate (Na_2CO_3), Aluminum chloride ($AlCl_3$), quercetin, ethanol (96%), sodium acetate (CH_3COONa), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox, 3,6-dichlorophenol-indophenol (DCPIP), Sodium hydroxide (NaOH, 0.1 N) all chemical reagents used in this study were of analytical grade.

2.2 Preparation and treatment protocols

Fresh yellow bell peppers (90 days after flowering) and pineapples (105 days) were harvested, inspected, washed, and prepared (peppers: deseeded and trimmed; pineapples: peeled, cored, and de-eyed). Juices were extracted mechanically, blended at a 1:1 ($v\cdot v^{-1}$) ratio, homogenized, and filled into sterilized 200 mL glass bottles. Five treatments were applied: Control (C), no further processing; Thermal treatment (TT), heating at 65 ± 2 °C for 15 min; UV-C treatment (UV-C), exposure to 254 nm light (40 min, 10 cm distance, 1.5 $mW\cdot cm^{-2}$); Ultrasound treatment (SA), sonication at 37 kHz, 80 W, 65 °C for 15 min; and Chitosan treatment (CS), addition of 0.2 g chitosan to 200 mL juice, followed by homogenization at 100 rpm for 10 min (Abd and Niamah, 2012). All samples were stored at 4 ± 2 °C and analyzed on days 0, 1, 3, 5, 7, 9, 11, and 13. At each time point, three bottles per treatment were tested, and the entire experiment was performed in triplicate.

2.3 Analysis of physicochemical, antioxidant, and microbiological indicators

The pH of the juice was measured using a calibrated pH meter (HM105, Vietnam), and total soluble solids (TSS) were determined with a handheld refractometer (RHB0-90) and expressed in °Brix. Titratable acidity (TA) was quantified by titration with 0.1 N NaOH and expressed as $g_{citric\ acid}\cdot L^{-1}$ using Eq(1):

$$X\ (g_{acid\ citric}\cdot L^{-1}) = \frac{V_1\ \cdot C \cdot 1000}{V_o} \tag{1}$$

where X is the total acid content ($g_{acid\ citric}\cdot L^{-1}$), V_1 is the volume of 0.1N NaOH solution used (mL), C is the concentration of NaOH (0.1N), and V_0 is the volume of the sample (mL).
Ascorbic acid was determined by iodine titration (Nweze et al., 2015) and expressed as $mg\cdot 100\ mL^{-1}$ Eq(2):

$$X = \frac{D \cdot C \cdot V \cdot 100}{V_m} \tag{2}$$

where X is the ascorbic acid content ($mg\cdot 100\ mL^{-1}$), D is the equivalent weight of ascorbic acid (88 mg), C is the concentration of iodine solution (0.01 N), V is the volume of iodine used (mL), and V_m is the volume of the juice sample titrated (mL).Total phenolic content (TPC) was determined using a colorimetric method (Singleton and Rossi, 1965) with slight modifications. Briefly, 0.5 mL of diluted juice was mixed with 2 mL Folin-Ciocalteu reagent and, after 6 - 8 min, 2.5 mL of 7.5 % Na_2CO_3 was added. The mixture stood for 60 min at room temperature before measuring absorbance at 765 nm. A blank was prepared using distilled water. TPC was calculated from a gallic acid standard curve ($y = 0.0076x + 0.0429$, $R^2 = 0.9995$) and expressed as $mg_{GAE}\cdot mL^{-1}$ juice. All analyses were performed in triplicate.

Total flavonoid content (TFC) was determined using a colorimetric method (Chang et al., 2002) with slight modifications. In brief, 0.5 mL juice was mixed with 0.1 mL $AlCl_3$, 1.5 mL of 96 % ethanol, and 0.1 mL of 1N sodium acetate. After standing at room temperature for 40 min, absorbance was measured at 415 nm. A blank was prepared with distilled water. TFC was calculated using a quercetin standard curve ($y = 0.0032x + 0.0505$, $R^2 = 0.9946$) and expressed as $mg_{QE}\cdot mL^{-1}$ juice. All measurements were done in triplicate.

Antioxidant activity (DPPH•) was evaluated using the DPPH radical scavenging assay (Brand-Williams et al., 1995) with modifications. A 0.1 mM DPPH solution (4 mg in 100 mL of 99.5 % ethanol) was freshly prepared. For analysis, 0.5 mL of diluted juice was mixed with 4 mL DPPH solution and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm. Radical scavenging (%) was calculated from the absorbance of the control (ACT) and the sample (ASP). Antioxidant activity was expressed as mM Trolox equivalents using the standard curve ($y = 0.9589x – 6.9575$, $R^2 = 0.9975$).

Microbiological stability was assessed by pour plate method on Plate Count Agar (PCA). Serial dilutions were plated and incubated at 30 °C for 72 h. Colony counts were expressed as $CFU\cdot mL^{-1}$ and calculated using Eq(3):

$$N = \frac{\sum C}{V \cdot (n_1 + 0.1 \cdot n_2)\cdot d \cdot 1000} \tag{3}$$

where C is total colonies, V plated volume (L), n_1 and n_2 plate numbers at first and second dilution, and d the dilution factor. All analyses were performed in triplicate.

2.4 Statistical analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). One-way ANOVA ($P < 0.05$) was conducted using Statgraphics Centurion XV.I, and graphs were created with Microsoft Excel 2019.

3. Results and discussions

3.1 Initial physicochemical characteristics of bell pepper–pineapple juice blend

The initial analysis revealed that yellow bell pepper had a higher flesh yield (77.17 %) and moisture content (90.76 %) than pineapple, contributing to better juice extraction and lighter texture. It also exhibited higher titratable acidity (3.33 $g_{acid\ citric}\cdot L^{-1}$) and lower pH (4.49), supporting its role in enhancing microbial stability. In terms of bioactive compounds, bell pepper contained significantly more vitamin C (139.04 $mg\cdot 100\ mL^{-1}$) and phenolics (476.38 $mg_{GAE}\cdot mL^{-1}$), while pineapple was richer in flavonoids (41.33 $mg_{QE}\cdot mL^{-1}$) (Table 1). These complementary profiles justify the 1:1 blending ratio, offering a balanced phytochemical composition. Overall, the compositional data are consistent with previous studies (Anaya-Esparza et al., 2021; Thuphairo et al., 2019) and establish a strong basis for evaluating preservation strategies.

Table 1: Chemical composition of yellow bell pepper and pineapple

Composition	Yellow bell pepper	Pineapple
Fruit peel percentage (%)	22.83 ± 3.84	27.68 ± 2.03
Fruit flesh percentage (%)	77.17 ± 3.84	72.32 ± 2.03
Moisture (%)	90.76 ± 0.75	86.51 ± 0.33
pH	4.49 ± 0.01	5.22 ± 0.01
TSS (°Brix)	10.01 ± 0.01	9.70 ± 0.17
TA - Titrable Acidity ($g_{acid\ citric}\cdot L^{-1}$)	3.33 ± 0.29	2.17 ± 0.06
Vitamin C ($mg\cdot 100\ mL^{-1}$)	139.04 ± 1.34	102.96 ± 0.51
TPC ($mg_{GAE}\cdot mL^{-1}$)	476.38 ± 0.38	236.25 ± 0.66
TFC ($mg_{QE}\cdot mL^{-1}$)	12.27 ± 0.24	41.33 ± 0.54

3.2 Changes in physicochemical parameters during storage

Table 2 presents juice pH trends during storage, indicating microbial activity under different treatments.

Table 2: pH variation of treated juice samples over 13-day cold storage

Sample	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
Control	4.72 ± 0.01^{h}	4.71 ± 0.01^{g}	4.66 ± 0.01^{f}	4.57 ± 0.01^{e}	4.34 ± 0.01^{d}	4.32 ± 0.01^{c}	4.27 ± 0.01^{b}	4.23 ± 0.01^{a}
UV-C	4.64 ± 0.01^{h}	4.62 ± 0.00^{g}	4.51 ± 0.00^{f}	4.45 ± 0.01^{e}	4.39 ± 0.00^{d}	4.37 ± 0.00^{c}	4.28 ± 0.01^{b}	4.26 ± 0.00^{a}
TT	4.67 ± 0.00^{f}	4.66 ± 0.02^{f}	4.54 ± 0.01^{e}	4.49 ± 0.01^{d}	4.45 ± 0.00^{c}	4.41 ± 0.01^{b}	4.30 ± 0.01^{a}	4.29 ± 0.00^{a}
SA	4.61 ± 0.00^{f}	4.60 ± 0.01^{f}	4.54 ± 0.01^{e}	4.52 ± 0.02^{e}	4.44 ± 0.00^{d}	4.42 ± 0.02^{c}	4.38 ± 0.02^{b}	4.31 ± 0.01^{a}
CS	4.7 ± 0.01^{g}	4.63 ± 0.01^{f}	4.54 ± 0.01^{e}	4.48 ± 0.01^{d}	4.46 ± 0.01c	4.35 ± 0.01^{b}	4.34 ± 0.01^{ab}	4.33 ± 0.01^{a}

Different superscript letters within the same row indicate significant differences ($p < 0.05$).

During storage, the pH of all juice samples decreased due to the accumulation of organic acids. The decline was most pronounced in the control group, where pH dropped sharply from 4.72 to 4.34 by day 7. This rapid acidification is attributed to unchecked microbial activity, as evidenced by the elevated total viable counts (TVC) reaching 5.26 $log_{CFU}\cdot mL^{-1}$. In contrast, samples treated with chitosan (CS), ultrasound (SA), and thermal treatment (TT) showed improved pH stability, maintaining final pH values between 4.29 and 4.33 by day 13. This suggests a stronger inhibitory effect on microbial proliferation. The inverse relationship between pH decline and microbial growth was clearly observed in the experimental data, where lower pH values consistently corresponded with higher microbial loads (Table 2). These findings are consistent with previous studies on microbial suppression through sub-lethal treatments (Khursheed et al., 2024). In particular, the antimicrobial activity of chitosan contributed to pH stabilization by disrupting microbial membranes and inhibiting cellular functions (Liu et al., 2023). As shown in Table 3, the titratable acidity (TA) of the bell pepper-pineapple juice changed slightly over 13 days of cold storage at 4 ± 2 °C. Initial TA values ranged from 3.22 to 3.91 $g_{acid\ citric}\cdot L^{-1}$. The control and UV-C groups showed minor increases (up to 3.50 $g_{acid\ citric}\cdot L^{-1}$), while TT and SA treatments led to a gradual rise, reaching 4.73 and 5.43 $g_{acid\ citric}\cdot L^{-1}$, respectively, likely due to thermal or mechanical release of organic acids. The CS group exhibited moderate changes (3.91 - 4.43 $g_{acid\ citric}\cdot L^{-1}$).

Overall, all treatments helped stabilize acidity during storage. Similar trends were reported by (Shamsudin et al., 2020), whereas (Kaddumukasa et al., 2017) found acidity declined in untreated juices. The TA stability in our study indicates that combining preservation methods with cold storage effectively limits microbial activity and maintains juice quality.

Table 3: Changes in titratable acidity (TA, $g_{citric\ acid} \cdot L^{-1}$) of juice samples during 13 days of cold storage.

Sample	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
Control	3.27 ± 0.12^{a}	3.37 ± 0.15^{ab}	3.40 ± 0.00^{ab}	3.43 ± 0.06^{b}	3.50 ± 0.10^{b}	3.70 ± 0.00^{c}	4.07 ± 0.06^{d}	4.33 ± 0.06^{e}
UV-C	3.23 ± 0.25^{a}	3.27 ± 0.12^{ab}	3.27 ± 0.12^{ab}	3.4 ± 0.00^{abc}	3.47 ± 0.12^{bc}	3.57 ± 0.06^{c}	3.87 ± 0.06^{d}	4.47 ± 0.06^{e}
TT	3.87 ± 0.06^{a}	4.00 ± 0.00^{b}	4.17 ± 0.06^{c}	4.20 ± 0.00^{c}	4.47 ± 0.06^{d}	4.53 ± 0.06^{d}	4.63 ± 0.06^{e}	4.73 ± 0.06^{f}
SA	3.83 ± 0.06^{a}	3.87 ± 0.06^{a}	4.13 ± 0.06^{b}	4.17 ± 0.06^{bc}	4.23 ± 0.06^{c}	4.87 ± 0.06^{d}	5 ± 0.00^{e}	5.43 ± 0.06^{f}
CS	3.33 ± 0.06^{a}	3.43 ± 0.06^{ab}	3.47 ± 0.06^{bc}	3.50 ± 0.00^{bc}	3.57 ± 0.06^{cd}	3.67 ± 0.06^{d}	3.93 ± 0.12^{e}	4.43 ± 0.12^{f}

Different superscript letters within the same row indicate significant differences ($p < 0.05$).

As shown in Table 4, total soluble solids (TSS) declined across all treatments due to microbial and enzymatic sugar degradation, with the sharpest drop in the control (to 8.70 °Brix). CS and SA retained the highest TSS values (> 9.1 °Brix), reflecting better control over degradation processes, also supported by a strong negative correlation between TSS and TVC. These results confirm that non-thermal and bioactive treatments, particularly chitosan, effectively delayed physicochemical deterioration during refrigerated storage (Xing et al., 2016).

Table 4: Changes in total soluble solids (TSS, °Brix) of juice samples during 13 days of cold storage

Sample	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
Control	10.07 ± 0.06^{h}	9.80 ± 0.00^{g}	9.50 ± 0.00^{f}	9.00 ± 0.00^{e}	8.70 ± 0.00^{d}	8.5 ± 0.06^{c}	8.0 ± 0.06^{b}	7.9 ± 0.00^{a}
UV-C	10.40 ± 0.00^{h}	10.20 ± 0.00^{g}	9.87 ± 0.06^{f}	9.67 ± 0.12^{e}	9.00 ± 0.00^{d}	8.80 ± 0.00^{c}	8.3 ± 0.00^{b}	8 ± 0.00^{a}
TT	9.83 ± 0.06^{f}	9.80 ± 0.00^{f}	9.67 ± 0.12^{e}	9.57 ± 0.06^{e}	9.40 ± 0.00^{d}	9.13 ± 0.12^{c}	9.00 ± 0.00^{b}	8.87 ± 0.06^{a}
SA	10.30 ± 0.00^{f}	10.17 ± 0.12^{e}	9.80 ± 0.0^{d}	9.77 ± 0.06^{d}	9.57 ± 0.06^{c}	9.40 ± 0.00^{b}	9.23 ± 0.06^{a}	9.27 ± 0.06^{a}
CS	10.43 ± 0.06^{f}	10.37 ± 0.06^{ef}	10.27 ± 0.12^{de}	10.20 ± 0.00^{d}	9.80 ± 0.00^{c}	9.77 ± 0.06^{c}	9.63 ± 0.12^{b}	9.13 ± 0.06^{a}

Different superscript letters within the same row indicate significant differences ($p < 0.05$).

3.3 Changes in antioxidants during storage

The antioxidant potential of the bell pepper-pineapple juice blend declined during 13 days of cold storage, as evidenced by reductions in total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging activity (Tables 5-7). As shown in Table 5, the control and UV-C groups experienced sharp TPC losses, dropping to 161.69 and 139.32 $mg_{GAE} \cdot mL^{-1}$, respectively, by day 7.

Table 5: Changes in total phenolic content (TPC, mgGAE·mL-1) of juice samples during 13 days of cold storage.

Sample	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
Control	470.68 ± 0.38^{h}	416.73 ± 0.38^{g}	439.98 ± 0.38^{f}	345.24 ± 0.38^{e}	161.69 ± 0.38^{d}	104.23 ± 0.38^{c}	44.15 ± 1.74^{b}	31.86 ± 0.38^{a}
UV-C	485.59 ± 0.00^{h}	433.62 ± 0.66^{g}	545.45 ± 0.38^{f}	293.27 ± 0.38^{e}	139.32 ± 0.38^{d}	138.44 ± 1.00^{c}	117.61 ± 0.38^{b}	103.36 ± 0.00^{a}
TT	350.94 ± 0.38^{g}	322.21 ± 0.76^{f}	259.28 ± 0.00^{e}	236.03 ± 0.38^{d}	258.52 ± 0.00^{e}	183.84 ± 1.00^{c}	104.45 ± 0.38^{b}	96.12 ± 0.00^{a}
SA	454.45 ± 0.38^{g}	430.77 ± 1.00^{f}	350.94 ± 0.38^{d}	329.67 ± 0.00^{c}	403.36 ± 0.66^{e}	212.79 ± 1.37^{b}	107.52 ± 0.38^{a}	107.30 ± 0.66^{a}
CS	475.94 ± 0.38^{h}	446.78 ± 0.66^{f}	461.91 ± 0.00^{g}	383.62 ± 0.66^{e}	293.05 ± 0.38^{d}	286.25 ± 0.00^{c}	129.45 ± 1.00^{b}	127.48 ± 0.38^{a}

Different superscript letters within the same row indicate significant differences ($p < 0.05$).

Table 6 illustrates a parallel trend in flavonoid degradation, where all samples showed a marked decline, particularly in UV-C and SA treatments.

Table 6: Changes in total flavonoid content (TFC, $mg_{QE} \cdot mL^{-1}$) of juice samples during 13 days of cold storage

Sample	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
Control	41.33 ± 0.00^{g}	27.47 ± 0.09^{f}	26.80 ± 0.54^{e}	8.26 ± 0.09^{d}	7.94 ± 0.24^{d}	4.35 ± 0.09^{c}	1.85 ± 0.09^{b}	1.38 ± 0.09^{a}
UV-C	37.79 ± 0.09^{h}	36.74 ± 1.02^{g}	19.82 ± 0.09^{f}	14.77 ± 0.00^{e}	13.36 ± 1.34^{d}	7.06 ± 0.09^{c}	5.81 ± 0.09^{b}	3.88 ± 0.09^{a}
TT	29.92 ± 0.00^{h}	28.83 ± 0.00^{g}	21.74 ± 0.09^{f}	20.96 ± 0.18^{e}	11.33 ± 0.00^{d}	6.59 ± 09^{c}	5.70 ± 0.00^{b}	3.93 ± 0.09^{a}
SA	32.32 ± 0.09^{g}	31.02 ± 0.16^{f}	24.51 ± 0.24^{e}	24.35 ± 0.59^{e}	10.29 ± 0.09^{d}	8.52 ± 0.16^{c}	5.49 ± 0.86^{b}	2.47 ± 0.09^{a}
CS	39.24 ± 0.24^{g}	38.93 ± 0.24^{g}	37.79 ± 0.39^{f}	28.72 ± 0.09^{e}	12.16 ± 0.09^{d}	7.32 ± 0.09^{c}	4.24 ± 0.24^{b}	3.36 ± 0.00^{a}

Different superscript letters within the same row indicate significant differences ($p < 0.05$).

Corresponding reductions in antioxidant activity are also reflected in Table 7**,** with DPPH values falling sharply across all treatments, reaching as low as 14.66 mg$_{TE}$·mL^{-1} in the control by day 7. Thermal treatment (TT) caused the most severe degradation across all indicators, consistent with the breakdown of polyphenols under heat. In contrast, chitosan (CS) preserved antioxidant capacity most effectively (e.g., DPPH: 9.34 mg$_{TE}$·mL^{-1}, highest on day 13), attributed to its chelating and barrier-forming properties (Xing et al., 2016). Ultrasound (SA) offered moderate protection, likely due to enhanced dispersion and localized cell disruption, while UV-C benefits diminished after day 7. These patterns are consistent with prior findings on bioactive retention in treated fruit matrices (Khursheed et al., 2024).The data further revealed that samples with higher TPC also retained more TFC and exhibited stronger DPPH activity, underscoring the interdependence of phenolic compounds in maintaining antioxidant capacity. Additionally, the inverse correlation between antioxidant levels and total viable counts (TVC) suggests microbial proliferation may accelerate antioxidant degradation, particularly in untreated controls. Collectively, the results confirm that combining non-thermal processing with chitosan offers superior preservation of antioxidant and physicochemical stability in sensitive juice systems, aligning with sustainable food processing strategies (Singh and Sharma, 2017).

Table 7: DPPH radical scavenging activity (DPPH, mg$_{TE}$·mL^{-1}) of juice samples treated by different methods during 13 days of cold storage

Sample	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
Control	91.03 ± 0.11^{e}	76.37 ± 0.06^{c}	65.23 ± 0.15^{d}	43.90 ± 0.06^{b}	14.66 ± 0.00^{a}			
UV-C	89.58 ± 0.06^{f}	64.83 ± 0.16^{e}	54.21 ± 0.06^{d}	34.28±0.19c	32.81 ± 0.00^{b}	10.22 ± 1.07^{a}		
TT	56.78 ± 0.00^{g}	36.30 ± 0.16^{f}	28.03 ± 0.06^{e}	18.61 ± 0.17^{d}	14.13 ± 0.06^{e}	10.22 ± 0.07^{c}	9.24 ± 0.15^{b}	7.78 ± 0.00^{a}
SA	87.72 ± 0.13^{g}	70.72 ± 0.21^{f}	54.17 ± 0.12^{e}	53.16 ± 0.00^{d}	32.45 ± 0.06^{c}	11.46 ± 0.17^{b}	7.56 ± 0.00^{a}	7.54 ± 0.06^{a}
CS	91.69 ± 0.00^{h}	87.74 ± 0.06^{g}	65.99 ± 0.12^{f}	52.65 ± 0.06^{e}	33.93 ± 0.06^{d}	21.65 ± 0.07^{c}	10.29 ± 0.10^{b}	9.34 ± 0.16^{a}

Different superscript letters within the same row indicate significant differences ($p < 0.05$).

3.4 Microbiological stability during cold storage (total viable count)

Aerobic mesophilic bacteria are major contributors to fruit juice spoilage. In this study, microbial counts increased across all treatments during 13 days of cold storage, with the untreated control exceeding acceptable limits by day 5 (Table 8). The decline in pH due to organic acid accumulation likely inhibited many spoilage organisms, yet acid-tolerant bacteria and yeasts still proliferated, explaining the continued microbial rise despite increasing acidity (Fleet, 1992). In general, a lower pH slows down most spoilage bacteria. However, acid-tolerant species such as lactic acid bacteria and yeasts can adapt and continue to grow, which explains the higher microbial counts even when the juice became more acidic. UV-C can inactivate microorganisms while preserving sensory attributes in the short term, but nutrient degradation and off-flavor development remain dose- and product-dependent (Koutchma, 2009). Ultrasound moderately suppressed microbial growth and enhanced clarity, though excessive sonication may cause nutrient degradation, especially vitamin C (Tiwari et al., 2009). However, chitosan can darken juice slightly and may not be suitable for shellfish-sensitive consumers. Taken together, these results highlight chitosan's superior antimicrobial performance and suggest that combining it with non-thermal methods provides a sustainable approach to extend shelf life while preserving nutrition and sensory quality.

Table 8: Changes in total viable counts (TVC, log$_{CFU}$·mL^{-1}) of juice samples during 13 days of cold storage.

Sample	Day 0	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
Control	5.09 ± 0.09	5.15 ± 0.04	5.24 ± 0.21	5.24 ± 0.11	5.26 ± 0.07	5.31 ± 0.02	5.40 ± 0.00	5.42 ± 0.02
UV-C	4.95 ± 0.05	5.02 ± 0.11	5.04 ± 0.03	5.04 ± 0.12	5.09 ± 0.06	5.13 ± 0.16	5.27 ± 0.04	5.30 ± 0.05
TT	4.53 ± 0.05	4.70 ± 0.16	4.75 ± 0.30	4.77 ± 0.16	4.85 ± 0.10	4.89 ± 0.08	5.09 ± 0.08	5.14 ± 0.15
SA	4.56 ± 0.03	4.71 ± 0.01	4.72 ± 0.04	4.88 ± 0.02	4.89 ± 0.05	4.98 ± 0.00	5.02 ± 0.00	5.13 ± 0.02
CS	4.28 ± 0.00	4.36 ± 0.00	4.58 ± 0.01	4.70 ± 0.01	4.88 ± 0.01	4.95 ± 0.00	5.01 ± 0.01	5.10 ± 0.00

Different superscript letters within the same row indicate significant differences ($p < 0.05$).

Each preservation method also showed effects on sensory quality. UV-C kept the juice with fresh color and aroma, but long exposure sometimes caused browning. Ultrasound helped keep the natural flavor and improved clarity, but it could lower vitamin C. Chitosan made the juice clearer but slightly darker, and it may not be suitable for people who are allergic to shellfish. These points show both the benefits and the limits of each method when used in practic.

4. Conclusions

This study demonstrated that the bell pepper–pineapple juice blend possesses a high nutritional profile but is vulnerable to quality degradation during cold storage. Among the preservation methods tested, chitosan proved most effective in maintaining physicochemical and microbiological stability, as its antimicrobial and chelating properties contributed to delayed microbial growth and better preservation of antioxidants when incorporated into the juice matrix. Ultrasound and UV-C treatments offered moderate benefits, while thermal treatment resulted in the greatest nutrient losses. Overall, the findings highlight that combining chitosan with non-thermal preservation methods provides a sustainable and health-conscious strategy for extending the shelf life of antioxidant-rich juice blends.

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