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Research Article

Impact of Non-Thermal Technologies on Wood Apple Juice Quality

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ABSTRACT

Wood apple (Feronia limonia or Limoniaacidissima) is an underutilized dry-land fruit with significant nutritional and medicinal potential, commonly consumed fresh or processed into juice at household and cottage-industry levels in India; however, limited scientific information is available on its preservation using non-thermal techniques. In this study, an electronic sensing–assisted approach, integrated with conventional analytical methods, was employed to evaluate the impact of emerging non-thermal processing technologies—ultrasound, ozonation, and high-pressure processing (HPP)—on the quality and storage stability of wood apple juice under refrigerated conditions (4 °C). Juice samples were treated using ultrasonication at 60 kHz for 10 min (with and without ice bath cooling), ozonation at 60 g Nm^{-3} for 5 and 10 min, and HPP at 350 and 650 MPa for 10 min. Physicochemical and bioactive parameters, including pH, titratable acidity, total soluble solids (°Brix), viscosity, ascorbic acid content, total phenolic content, and antioxidant activity (IC_{50}), were monitored at 7-day intervals over a 28-day storage period, supported by sensor-based observations for rapid quality assessment. The results indicated that pH and viscosity remained statistically unchanged ($p > 0.05$) across all treatments, while total soluble solids and titratable acidity showed minimal variation, particularly in ozonation- and HPP-treated samples. In contrast, ascorbic acid, phenolic content, and antioxidant activity exhibited significant changes during storage. Among the evaluated techniques, HPP at 350 MPa for 10 min demonstrated superior retention of bioactive compounds and overall quality stability. The integration of electronic sensing techniques enabled efficient, real-time monitoring of quality attributes, validating their potential for rapid assessment in food processing applications. Overall, the findings confirm that

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non-thermal processing effectively preserves the nutritional and functional quality of wood apple juice, with HPP emerging as the most promising technique for extending shelf life under refrigerated storage.a

Keywords: Juice, non-thermal technique, ultrasonication, ozonisation, high pressure processing, storage stability

Introduction

Wood apple (*Feronia limonia*Swingle), also botanically identified as *Limoniaacidissima L.* or *Feronia elephantum* Correa, is a hardy perennial fruit tree of the Rutaceae family that is native to the Indian subcontinent and Sri Lanka [1], [2]. The species is well known for its exceptional adaptability to adverse agro-climatic environments, including prolonged drought, high ambient temperatures, and poor soil fertility. These physiological traits allow wood apple to thrive in arid and semi-arid regions where conventional horticultural crops often fail, positioning it as a valuable component of climate-resilient and sustainable agricultural systems [3]. A mature tree typically produces 200–300 fruits annually, with individual fruits weighing between 150 and 500 g, offering considerable yield potential even under low-input cultivation practices [4]. Despite these agronomic advantages, wood apple remains an underutilized fruit crop, with its consumption largely restricted to traditional household preparations and limited local markets, and minimal penetration into organized food processing or commercial supply chains [5].

From a nutritional perspective, wood apple pulp is recognized for its high energy density and functional composition. It contains approximately 18–25% carbohydrates and 5–10% dietary fiber, along with 2–4% organic acids that contribute to its characteristic flavor profile [6]. The fruit is also a notable source of essential minerals such as calcium, phosphorus, potassium, and iron, which are critical for metabolic and physiological functions [7]. In addition, wood apple provides moderate quantities of ascorbic acid (5–15 mg/100 g) and is particularly rich in bioactive phytochemicals, including phenolic acids, flavonoids, tannins, and coumarins [8]. Reported total phenolic contents range from 200 to 450 mg gallic acid equivalents per 100 g of pulp, underscoring its potential as a functional food ingredient with antioxidant capacity [9].

The health-promoting properties of wood apple have been extensively described in traditional medicinal systems such as Ayurveda and Unani, where it has been used for centuries to manage gastrointestinal disorders, diabetes, liver dysfunction, inflammatory conditions, and microbial infections [10], [11]. Modern scientific investigations have increasingly validated these ethnomedicinal claims through in vitro and in vivo studies, reporting strong antioxidant, antimicrobial, anti-inflammatory, antipyretic, and hepatoprotective activities [12]–[14]. These biological effects are primarily attributed to the fruit's high phenolic content and its ability to scavenge reactive oxygen species. Dietary intake of antioxidant-rich fruits and fruit-based beverages has been widely associated with reduced oxidative stress, inhibition of lipid peroxidation, and a lower incidence of chronic non-communicable diseases such as cardiovascular disorders, neurodegenerative diseases, and certain forms of cancer [15], [16].

In recent years, increasing consumer awareness regarding health, nutrition, and wellness has driven a significant shift toward functional beverages, natural fruit juices, and minimally processed foods with clean-label attributes [17]. Fruit juices are particularly appealing due to their sensory acceptance, convenience, and enhanced bioavailability of nutrients and phytochemicals. However, the commercialization of wood apple juice is constrained by several postharvest challenges, including pronounced seasonal availability, enzymatic browning, rapid microbial spoilage, and susceptibility to fungal contamination—particularly by *Aspergillus* species [18]. These factors severely limit shelf life and hinder large-scale industrial processing and distribution.

Processing wood apple into juice represents an effective strategy for value addition, reduction of postharvest losses, and year-round utilization. Nevertheless, preserving juice quality during storage remains a major technological challenge. Conventional juice preservation relies primarily on thermal pasteurization, typically conducted at temperatures ranging from 72 to 95 °C for short holding times, often combined with chemical preservatives [19]. While thermal treatments are effective in ensuring microbial safety, they frequently result in undesirable quality degradation, including losses of heat-sensitive nutrients such as ascorbic acid, reductions in total phenolic content, destruction of volatile aroma compounds, and deterioration of color and sensory attributes [20]. Previous studies have reported ascorbic acid losses of 20–60% and phenolic degradation of up to 40% following conventional thermal processing of fruit juices [21]. In parallel, growing consumer resistance to synthetic additives and increasingly stringent regulatory frameworks have intensified the demand for alternative preservation strategies that ensure microbial safety while retaining fresh-like quality.

In this context, emerging non-thermal technologies such as ultrasound (US), ozonation (O_3), and high-pressure processing (HPP) have gained considerable attention as promising alternatives for fruit juice preservation [22]. Ultrasound processing, typically operating at frequencies between 20 and 100 kHz, induces acoustic cavitation that generates localized high temperatures and pressures, leading to microbial cell disruption, enzyme inactivation, and enhanced mass transfer while largely preserving nutritional quality [23]. Ozonation, classified as a Generally Recognized as Safe (GRAS) technology, employs ozone concentrations ranging from 1 to 80 g/Nm³ to achieve rapid microbial inactivation through oxidative damage to cellular membranes and nucleic acids, without leaving harmful chemical residues [24]. High-pressure processing, commonly applied in the range of 300–600 MPa for 5–15 min, is widely regarded as one of the most effective non-thermal pasteurization methods, capable of achieving 4–6 log reductions in vegetative microorganisms while maintaining color, flavor, vitamins, and bioactive compounds [25].

Extensive research has demonstrated the successful application of these non-thermal technologies in preserving the quality of widely consumed fruit juices such as apple, orange, mango, grape, pomegranate, strawberry, and various berry juices [26]–[28]. HPP-treated juices consistently exhibit superior retention of phenolic compounds (up to 90%), ascorbic acid (80–95%), and antioxidant activity compared with thermally processed counterparts, along with extended refrigerated shelf life and improved sensory acceptance [29]. Ultrasound processing has been reported to enhance antioxidant activity by 10–30% due to improved extractability of phenolic compounds, while ozone treatment has effectively reduced microbial loads and extended shelf life with minimal impact on physicochemical and sensory properties [30].

Despite this substantial body of literature, studies specifically addressing the application of non-thermal technologies to wood apple juice are extremely scarce. Existing research on wood apple is largely limited to compositional analysis, traditional processing methods, and a few investigations employing conventional thermal preservation techniques. Comprehensive, comparative evaluations of ultrasound, ozonation, and high-pressure processing on the physicochemical properties, bioactive compound retention, antioxidant activity, and refrigerated storage stability of wood apple juice have not yet been systematically reported. Furthermore, standardized processing parameters and technology-specific recommendations for non-thermal preservation of wood apple juice remain undeveloped, significantly constraining its commercial exploitation.

The present study addresses a critical research gap arising from the absence of systematic and comparative investigations on the influence of multiple non-thermal preservation technologies on the quality and storage stability of wood apple juice. Although non-thermal methods such as ultrasound, ozonation, and high-pressure processing have been widely studied for conventional fruit juices, their application to wood apple juice—particularly with respect to storage-dependent changes in physicochemical properties, bioactive compound retention, and antioxidant activity—remains largely unexplored. The novelty of this work lies in its integrated and comparative evaluation of these three non-thermal technologies under refrigerated storage conditions, providing one of the first comprehensive assessments for an underutilized yet nutritionally and functionally rich fruit. Accordingly, this study aims to evaluate the effects of ultrasound, ozonation, and high-pressure processing on the physicochemical and functional quality attributes of wood apple juice; to monitor variations in ascorbic acid content, total phenolic content, and antioxidant activity during storage; to compare the storage stability of juices subjected to different non-thermal treatments; and to identify the most effective preservation technique for maximizing nutritional and functional quality. The outcomes of this research are expected to contribute to the development of functional beverages, clean-label fruit juice products, and sustainable agro-processing solutions, thereby facilitating the commercial utilization of wood apple as a value-added, climate-resilient fruit crop.

2. Materials and Methods

2.1 Preparation of Wood Apple Juice

Fully ripened wood apple fruits (*Feronia limonia*Swingle), characterized by a hard shell, globular shape, and uniform maturity, were selected for the study. The fruits were procured from the Department of Horticulture, University of Mysore, Mysuru, India. Prior to juice extraction, fruits were washed thoroughly under running water to remove surface contaminants. The shells were manually cracked using a hammer, and the pulp was carefully scooped out using a stainless-steel spoon. For juice extraction, potable water was added to the pulp in a ratio of 2.5:1 (water:pulp, w/w), followed by manual squeezing through a double-layered cheesecloth to separate the juice. The filtered juice was immediately collected in sterilized containers and stored under refrigerated conditions (4 °C) until further processing to minimize enzymatic and microbial degradation [31], [32].

2.2 Non-Thermal Processing Treatments

2.2.1 Ultrasound-Assisted Treatment

Ultrasound treatment was carried out using a probe-type ultrasonic processor (DES500, 500 W, QSONICA Sonicators, USA) equipped with a titanium probe of 1.3 cm diameter. Wood apple juice samples (300 mL) were transferred into 500 mL glass beakers and sonicated at a constant frequency of 60 kHz for 10 min. The probe was immersed to a depth of 20 mm in the juice sample. To evaluate the influence of temperature rise during sonication, treatments were conducted both with and without an external ice bath. In the absence of cooling, the final juice temperature reached 49.5 ± 1.5 °C, whereas with ice bath cooling, the temperature was maintained at 16.5 ± 1.5 °C, as measured using a digital thermometer. Following sonication, the treated juice samples were filled into sterilized 100 mL polyethylene terephthalate (PET) bottles and stored at refrigerated temperature (5 ± 2 °C). Physicochemical, nutritional, and microbial analyses were conducted at 7-day intervals for a storage period of 28 days [33], [34].

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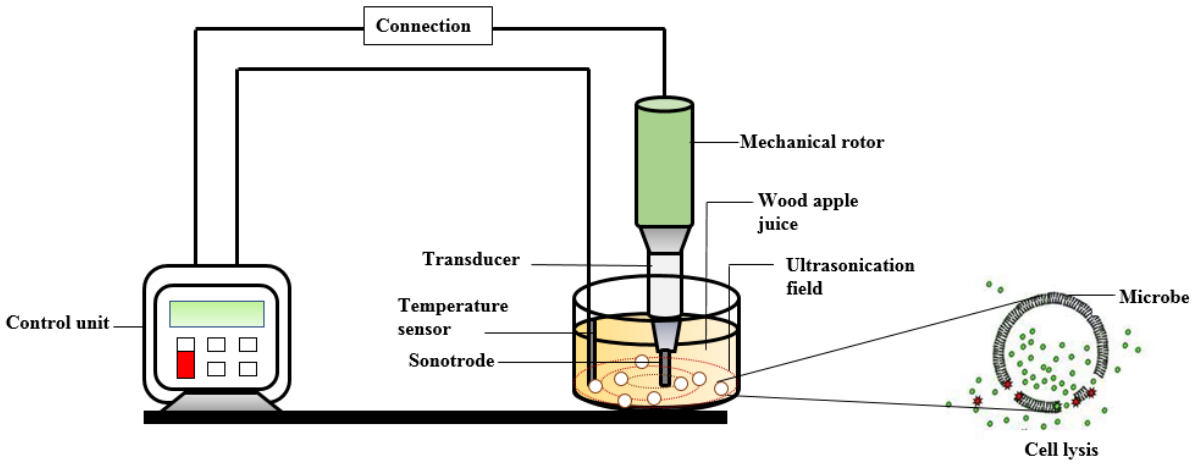

Figure 1:Ultrasonication set up and its effect on microorganisms in wood apple juice

2.2.2 Ozone Treatment

Ozone treatment was performed by bubbling ozone gas directly into 300 mL of wood apple juice placed in a glass beaker using a stainless-steel spurger connected to an ozone generator (OZ-AIR, India). Ozone was applied at a fixed concentration of 60 g/Nm³ for exposure durations of 5 and 10 min. The ozone concentration was continuously monitored using an ozone gas analyzer (OZ-AIR ISM-5). After treatment, residual ozone was allowed to dissipate naturally before packaging. The ozonated juice samples were transferred into 100 mL PET bottles and stored under refrigerated conditions (5 ± 2 °C). Analyses were performed at weekly intervals over a 28-day storage period [35], [36].

2.2.3 High-Pressure Processing

For high-pressure processing, freshly extracted wood apple juice was immediately filled into 100 mL PET bottles with polypropylene screw-cap closures to minimize heat exchange. Pressure treatments were carried out using a high-pressure processing unit with a vessel capacity of 5 L and an operating pressure range of 300–650 MPa. Water was used as the pressure-transmitting medium. The juice samples were subjected to pressures of 350 and 650 MPa for 10 min at ambient temperature (18–20 °C). After processing, samples were stored at refrigerated temperature (5 ± 2 °C) and analyzed at 7-day intervals for up to 28 days [37], [38].

2.3 Experimental Design

The experimental design was developed based on preliminary trials and followed a completely randomized design (CRD). Wood apple juice samples were subjected to different non-thermal treatments, including ultrasound, ozonation, and high-pressure processing, with two processing intensities for each technique. The detailed treatment combinations are summarized in Table 1. Each treatment was performed in triplicate to ensure statistical reliability.

Table 1: Experimental design of wood apple juice

S. No.	Non-thermal treatments					
	Ultrasonication		Ozonization		High pressure processing	
	Frequency (kHz)	Time (min)	Concentration (g/Nm³)	Time (min)	Pressure (MPa)	Time (min)
1	60	10	60	5	350	10
2	60	10	60	10	650	10

2.4 pH and Titratable Acidity

The pH of wood apple pulp and juice samples was determined using a digital pH meter (Systronics India Ltd., Ahmedabad, India) after calibration with standard buffer solutions of pH 4.0, 7.0, and 9.1. Titratable acidity was estimated following the standard method described by Ranganna [39]. For this purpose, a known volume of juice sample was diluted with distilled water and titrated against 0.1 N sodium hydroxide solution using 1% phenolphthalein as the indicator until a persistent light pink endpoint was obtained. Titratable acidity was calculated and expressed as:

$$\text{Acidity (\% citric acid)} = \frac{T \times N \times V_m \times E \times 100}{V_a \times V_s \times 1000}$$

Where, T represents the titre value, N denotes the normality of the alkali, V_m is the volume made up, E is the equivalent weight of the acid, V_a is the volume of aliquot taken for estimation, and V_s is the volume of sample taken for estimation.

2.5 Viscosity

The apparent viscosity of wood apple juice samples was measured using a Brookfield Digital Viscometer (Model DV-II+ Pro, Brookfield Engineering Laboratories, USA) fitted with spindle S-2. Measurements were carried out at a constant rotational speed of 100 rpm and a controlled temperature of 28.2 ± 0.5 °C to ensure consistency. Shear stress (τ) and shear rate ($\dot{\gamma}$) data were recorded using Rheocalc software (version 3.2). Apparent viscosity (η) was calculated using the fundamental rheological relationship:

$$\eta = \frac{\tau}{\dot{\gamma}}$$

where η is the apparent viscosity (Pa·s), τ is the shear stress (Pa), and $\dot{\gamma}$ is the shear rate (s^{-1}). The procedure followed standard rheological measurement protocols for fruit juices as reported in previous studies [40].

2.6 Total Soluble Solids (TSS)

Total soluble solids (TSS) content of the juice samples was determined using an Abbe hand refractometer in accordance with AOAC standard methods [41]. Prior to measurement, the refractometer was calibrated using distilled water (0 °Brix). A few drops of juice were placed on the prism surface, and readings were recorded at room temperature. The results were expressed as °Brix, representing the percentage of soluble solids present in the juice.

2.7 Ascorbic Acid Content

Ascorbic acid content was estimated using the 2,6-dichlorophenolindophenol (DCPIP) titrimetric method as described by AOAC and Ranganna [39], [41]. Juice samples (5 g) were homogenized with 3% metaphosphoric acid to stabilize ascorbic acid and prevent oxidation. The extract was filtered and titrated against standardized DCPIP dye until a light pink endpoint persisted for 15 s. Ascorbic acid content was calculated using the following equation:

$$\text{Ascorbic acid (mg/100g)} = \frac{V \times D \times 100}{W}$$

where$V$ is the volume of dye consumed (mL), D is the dye factor, and W is the weight of the sample (g).

2.8 Extraction of Phenolic Compounds and Antioxidant Activity

Phenolic compounds were extracted following the method described by previous researchers [42]. Briefly, 2 mL of juice sample was mixed with 10 mL of 80% ethanol (v/v) and homogenized

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thoroughly. The mixture was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was filtered and stored at −18 °C until further analysis.

2.8.1 Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method with minor modifications [43]. An aliquot of the ethanolic extract was mixed with Folin–Ciocalteu reagent and sodium carbonate solution, followed by incubation in the dark. Absorbance was measured at 765 nm using an ELISA plate reader. Total phenolic content was quantified using a gallic acid calibration curve and expressed as mg gallic acid equivalents (GAE) per mL of extract.

2.8.2 DPPH Radical Scavenging Activity

The procedure was performed following the reported method, using Trolox as the standard [43].The scavenging activity of the extracts were expressed as IC_{50} values which is the inhibition concentration to inhibit 50% of the radicals. DPPH was prepared at the concentration 0.1mM in methanol in dark and kept away from light. DPPH scavenging was tested with various gradients of concentration of the extract and standard. Methanol was kept as blank. The inhibition was visualized by bleaching of deep violet colour of DPPH. The absorbance was taken at 517nm using ELISA plate reader. The IC_{50} value (the concentration required to scavenge 50% DPPH free radicals) was calculated from the graph ofconcentration vs % inhibition, and it was determined in triplicate.

$$\% \text{ inhibition} = \frac{A\ blank - A\ sample}{A\ blank} \times 100$$

Where, $A_{control}$-Absorbance of control; $A_{extract}$-Absorbance of extract

2.9 Statistical Analysis

All experimental analyses were conducted in triplicate to ensure reliability and reproducibility of the data, and the results were expressed as mean ± standard deviation (SD). The statistical evaluation of the data was performed using analysis of variance (ANOVA) under a completely randomized design (CRD) to examine the effects of different treatments and storage intervals. The analysis was carried out using OPSTAT statistical software, which is widely used for agricultural and experimental data analysis. The significance of differences among treatment means was assessed at a 95% confidence level ($p < 0.05$). Where applicable, appropriate post-hoc comparisons were considered to identify significant variations between treatments and storage durations. This statistical approach ensured accurate interpretation of the experimental results and validation of observed trends.

3. Experimental Procedure

Fully ripened wood apple fruits (Feronia limonia Swingle) of uniform maturity were procured and thoroughly washed to remove surface impurities. The fruits were manually cracked to extract the pulp, which was then diluted with potable water in a 2.5:1 (water:pulp, w/w) ratio. The mixture was filtered through a double-layered cheesecloth to obtain clear juice, which was immediately stored under refrigerated conditions (4 ± 1 °C) prior to processing. The extracted juice was subjected to three non-thermal preservation techniques, namely ultrasound, ozonation, and high-pressure processing (HPP). For ultrasound treatment, 300 mL juice samples were processed using a probe-type ultrasonic processor at 60 kHz for 10 min, both with and without an external ice-bath cooling system to control temperature rise, after which the samples were aseptically filled into sterilized PET bottles. In ozonation treatment, ozone gas at a concentration of 60 g/Nm^3 was bubbled into the juice samples for 5 and 10 min, followed by natural dissipation of residual ozone before packaging. High-pressure processing was carried out by sealing juice samples in PET bottles and subjecting them to pressures of 350 and 650 MPa for 10 min using water as the pressure-transmitting medium at ambient temperature. All treated samples, along with untreated control samples, were stored under refrigerated conditions (5 ± 2 °C) and analyzed at 7-day intervals over a 28-day storage period. The quality evaluation included physicochemical parameters such as pH, titratable acidity, total soluble solids, and viscosity, along with nutritional attributes including ascorbic acid and total phenolic content, and antioxidant activity using the DPPH radical scavenging assay. All experiments were conducted in triplicate under a completely randomized design (CRD), and the data obtained were statistically analyzed using analysis of variance (ANOVA), with significance considered at $p < 0.05$. Figure 2 illustrates the experimental flow diagram for non-thermal processing of wood apple juice.

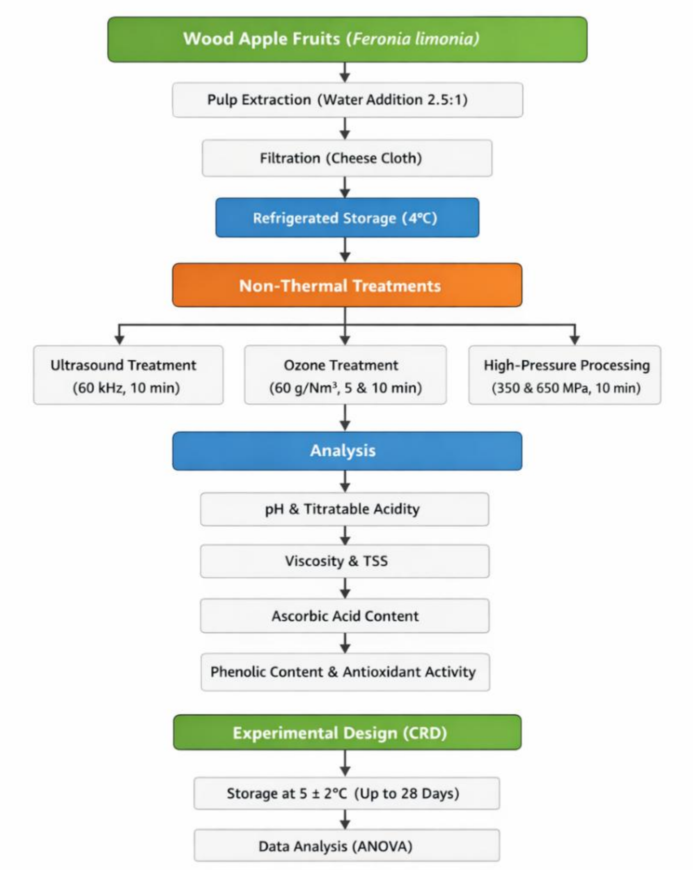

Figure 2: Experimental Flow Diagram for Non-Thermal Processing of Wood Apple Juice

3. Results and discussion

3.1 Ultrasound- assisted treatment

Ultrasound-assisted processing is a promising non-thermal technique that helps preserve the quality of fruit juices by reducing thermal damage. In this study, wood apple juice was treated using ultrasonication at 60 kHz for 10 minutes, both with and without an ice bath cooling system, and its quality attributes were evaluated over 28 days of refrigerated storage. The results presented in Table 2 highlight the effect of ultrasound treatment and storage duration on important physicochemical and bioactive parameters such as acidity, pH, total soluble solids (TSS), viscosity, ascorbic acid, phenolic content, and antioxidant activity (IC_{50}).

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Table 2: Effect of ultrasound treatment and storage period on nutritional quality of wood apple juice

Sample	Storage Period (Days)	Acidity (%)	pH	TSS (°Bx)	Viscosity (cps)	Ascorbic acid (mg/100 mL)	Phenolic content (mg GAE/mL)	IC 50 (mg/mL)
A60T1	0	0.74±0.01	3.15±0.01	3.20±0.17	40.67±0.23	2.20±0.13	38.98±0.98	0.74±0.06
	7	0.72±0.01	3.17±0.01	3.23±0.06	40.80±0.00	1.91±0.20	38.06±0.48	0.94±0.04
	14	0.70±0.01	3.17±0.01	3.42±0.21	40.93±0.23	1.97±0.09	36.57±0.34	1.01±0.05
	21	0.68±0.01	3.18±0.01	3.47±0.06	40.90±0.23	1.65±0.19	36.04±0.30	1.04±0.04
	28	0.66±0.02	3.18±0.01	3.56±0.12	41.33±0.23	1.44±0.07	34.00±0.56	1.11±0.03
C.D.		0.03*	NS	0.15*	NS	0.11*	1.91*	0.04*
SE(m)		0.01	0.05	0.05	0.64	0.03	0.60	0.01
C.V.		2.33	2.61	2.44	2.71	3.28	2.83	2.54
A60T1	0	0.73±0.06	3.17±0.06	3.20±0.00	40.53±0.23	2.58±0.19	40.04±0.44	0.63±0.01
(C)	7	0.70±0.01	3.18±0.01	3.27±0.06	41.53±0.23	2.46±0.19	38.02±0.39	0.72±0.03
	14	0.68±0.01	3.17±0.01	3.33±0.06	41.27±0.12	2.29±0.33	37.10±0.13	0.74±0.02
	21	0.67±0.02	3.18±0.01	3.40±0.06	41.33±0.12	1.95±0.13	35.41±0.56	0.85±0.01
	28	0.61±0.01	3.18±0.01	3.46±0.12	41.53±0.58	1.84±0.28	34.83±0.38	0.90±0.03
C.D.		0.04*	NS	0.12*	NS	0.10*	2.24*	0.03*
SE(m)		0.01	0.03	0.04	0.58	0.03	0.70	0.01
C.V.		3.49	1.75	1.95	2.44	2.48	3.29	1.91

* Significant at 5% level with respect to storage days; **NS**= Non-significant;**IC50**= Half maximal inhibitory concentration; **A60T10**= Treated with amplitude 60 kHz for 10 min without ice bath cooling system;**A60T10 (C)** = Treated with amplitude 60 kHz for 10 min with ice bath cooling system.

The findings show that acidity decreased gradually during storage in both treatments, from 0.74% to 0.66% (without cooling) and from 0.73% to 0.61% (with cooling), indicating a significant effect at the 5% level. This decline may be due to biochemical changes occurring during storage. However, pH values remained stable (3.15–3.18), suggesting that ultrasonication did not significantly affect the overall acidity balance of the juice. Similarly, viscosity showed minimal variation (~40–41 cps), indicating that the consistency of the juice remained unchanged.

A slight increase in TSS was observed during storage, rising from 3.20 to 3.56 °Brix (without cooling) and from 3.20 to 3.46 °Brix (with cooling), possibly due to the breakdown of complex compounds into simpler sugars. Ascorbic acid content decreased significantly over time, with a higher reduction in samples without cooling (about 34.5%) compared to those with cooling (about 28.7%). This loss is

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mainly due to oxidation reactions, which are more pronounced at higher temperatures, demonstrating the protective effect of the ice bath.

Phenolic content also declined during storage, likely due to enzymatic oxidation by polyphenol oxidase (PPO). This reduction led to a decrease in antioxidant activity, as reflected by the increase in IC_{50} values. Since lower IC_{50} values indicate higher antioxidant activity, the results confirm a gradual decline in antioxidant capacity, although samples treated with cooling showed better retention.

Statistical analysis, including critical difference (C.D.), standard error (SE), and coefficient of variation (C.V.), confirms the accuracy and reliability of the data. Overall, Table 1 demonstrates that ultrasound treatment effectively maintains the physicochemical properties of wood apple juice, while the use of an ice bath cooling system significantly improves the retention of nutritional and antioxidant qualities during storage.

3.2 Ozone treatment

Total soluble solids (TSS) and acidity are critical quality parameters that govern the flavor balance of fruit juices, representing the sugar–acid ratio of the system. The effect of ozone treatment on the physicochemical and bioactive properties of wood apple juice during 28 days of refrigerated storage is presented in Table 3. Statistical analysis revealed that pH, TSS, and viscosity did not show significant variation ($p > 0.05$) for both ozone-treated samples (O60T5 and O60T10), indicating that ozonation had minimal impact on these parameters during storage. The TSS values remained nearly constant throughout storage, with retention levels of approximately 97.83% (O60T5) and 97.81% (O60T10), confirming that ozone treatment effectively preserves soluble solids without causing major compositional changes. Similarly, pH values remained stable (3.17–3.19), and viscosity showed negligible variation (~40–41 cps), indicating preservation of juice consistency and structural stability.

Acidity exhibited a significant decreasing trend ($p < 0.05$) during storage, declining from 0.75% to 0.66% (O60T5) and 0.74% to 0.64% (O60T10) over 28 days. This reduction may be attributed to metabolic and biochemical changes occurring during storage. A notable decline was also observed in ascorbic acid content, which decreased significantly due to the oxidative nature of ozone. In O60T5 samples, ascorbic acid reduced from 1.96 to 1.42 mg/100 mL, whereas in O60T10 samples, it declined from 1.97 to 1.67 mg/100 mL. This degradation is mainly influenced by oxidation reactions facilitated by dissolved oxygen and ozone exposure, consistent with earlier findings in ozonized fruit juices.

The total phenolic content also decreased significantly during storage. For O60T5 samples, it reduced from 35.00 to 28.75 mg GAE/mL, while for O60T10 samples, it declined from 34.26 to 28.12 mg GAE/mL. This reduction can be attributed to oxidative degradation of phenolic compounds due to ozone's strong oxidizing potential. Consequently, antioxidant activity decreased, as indicated by the increase in IC_{50} values. In O60T5 samples, IC_{50} increased from 0.90 to 1.25 mg/mL, and in O60T10 samples, from 1.09 to 1.44 mg/mL, indicating a decline in antioxidant capacity over storage time.

The statistical parameters (C.D., SE(m), and C.V.) confirm the reliability and precision of the experimental results. Overall, Table 2 demonstrates that ozone treatment effectively maintains physicochemical stability (pH, TSS, viscosity), while significantly affecting bioactive compounds such as ascorbic acid, phenolics, and antioxidant activity due to oxidative reactions during storage.

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Table 3: Effect of Ozone treatment and storage period on nutritional quality of wood apple juice

Sample	Storage Period (Days)	Acidity (%)	pH	TSS (°Bx)	Viscosity (cps)	Ascorbic acid (mg/100 mL)	Phenolic content (mg GAE/mL)	IC 50 (mg/mL)
O60T5	0	0.75±0.06	3.17±0.01	3.23±0.06	40.67±0.12	1.96±0.05	35.00±0.41	0.90±0.01
	7	0.73±0.01	3.18±0.00	3.27±0.06	40.33±0.12	1.88±0.09	34.43±0.51	1.10±0.02
	14	0.71±0.06	3.18±0.00	3.23±0.15	40.80±0.35	1.75±0.20	32.19±0.42	1.11±0.03
	21	0.69±0.02	3.19±0.02	3.30±0.00	40.73±0.23	1.70±0.14	31.00±0.52	1.19±0.01
	28	0.66±0.01	3.19±0.00	3.30±0.10	41.00±0.35	1.42±0.10	28.75±0.40	1.25±0.04
C.D.		0.03*	NS	NS	NS	0.07*	1.50*	0.02*
SE(m)		0.01	0.05	0.06	0.54	0.02	0.47	0.01
C.V.		2.49	2.66	3.23	2.29	2.29	2.52	1.05
O60T10	0	0.74±0.02	3.18±0.01	3.20±0.10	40.4±0.35	1.97±0.09	34.26±1.09	1.09±0.01
	7	0.73±0.02	3.18±0.00	3.27±0.6	40.67±0.42	1.86±0.10	32.65±0.47	1.25±0.03
	14	0.70±0.02	3.19±0.01	3.27±0.06	40.80±0.00	1.83±0.06	32.09±0.52	1.31±0.08
	21	0.66±0.01	3.19±0.01	3.27±0.06	41.13±0.31	1.61±0.23	30.82±0.29	1.34±0.08
	28	0.64±0.01	3.19±0.01	3.27±0.06	41.20±0.00	1.67±0.12	28.12±0.66	1.44±0.08
C.D.		0.04*	NS	NS	NS	0.07*	1.27*	0.08*
SE(m)		0.01	0.05	0.06	0.49	0.02	0.39	0.03
C.V.		3.27	2.69	3.31	2.09	2.27	2.18	3.50

* Significant at 5% level; **NS**= Non-significant; **IC50**= Half maximal inhibitory concentration; **O60T5**= Treated with 60 g/Nm^3 ozone dose for 5 min;**O60T10**= Treated with 60 g/Nm^3 ozone dose for 10 min.

3.3 High pressure processing

The experimental results presented in Table 3 demonstrate the effect of high-pressure processing (HPP) treatments (350 MPa and 650 MPa for 10 min) on the physicochemical and bioactive properties of wood apple juice during 28 days of refrigerated storage.The parameters such as titratable acidity, pH, total soluble solids (TSS), and viscosity showed non-significant (NS) changes at the 5% level of significance throughout the storage period for both treatments (P350T10 and P650T10). For instance, acidity decreased slightly from 0.72% to 0.55% (P350T10) and 0.71% to 0.58% (P650T10) over 28 days, but these changes were statistically insignificant. Similarly, pH values remained almost constant (~3.18–3.21), indicating that HPP did not alter the acidity balance of the juice. The TSS values (3.21–3.30 °Brix) and viscosity (approximately 37.66 to 41.00 cps) also

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exhibited minimal variation, confirming that the physical stability and consistency of the juice were well preserved during storage.

Table 4: Effect of Ozone treatment and storage period on nutritional quality of wood apple juice

* Significant at 5% level; **NS**= Non-significant; **IC50**= Half maximal inhibitory concentration; **P350T10**= Treated at350 MPa pressure for 10 min; **P650T10**= Treated at650 MPa pressure for 10 min.

Sample	Storage Period (Days)	Acidity (%)	pH	TSS (°Bx)	Viscosity (cps)	Ascorbic acid (mg/100 mL)	Phenolic content (mg GAE/mL)	IC 50 (mg/mL)
P350T1	0	0.72±0.02	3.19±0.05	3.21±0.06	37.66±0.31	2.69±0.07	39.74±0.42	0.44±0.01
	7	0.69±0.01	3.19±0.06	3.21±0.06	38.73±0.48	2.53±0.04	37.94±0.28	0.57±0.01
	14	0.67±0.01	3.2±0.09	3.23±0.11	40.07±0.23	2.28±0.04	36.79±0.33	0.59±0.01
	21	0.61±0.01	3.20±0.09	3.23±0.03	40.13±0.44	1.98±0.05	36.59±0.87	0.68±0.04
	28	0.55±0.01	3.20±0.03	3.27±0.07	40.33±1.04	1.98±0.01	34.86±0.46	0.78±0.02
C.D.		NS	NS	NS	NS	0.09*	2.11*	0.03*
SE(m)		0.01	0.05	0.05	0.67	0.03	0.66	0.01
C.V.		3.13	2.71	2.71	2.93	2.07	3.08	2.83
P650T1	0	0.71±0.00	3.18±0.08	3.22±0.06	38.14±0.33	2.54±0.06	38.72±1.30	0.51±0.01
	7	0.68±0.01	3.20±0.07	3.24±0.10	38.92±1.35	2.37±0.08	36.75±0.46	0.64±0.02
	14	0.67±0.00	3.20±0.11	3.27±0.08	39.47±0.93	2.16±0.02	35.36±0.99	0.67±0.01
	21	0.62±0.01	3.21±0.05	3.28±0.03	40.07±1.09	1.95±0.03	34.42±0.61	0.70±0.01
	28	0.58±0.01	3.21±0.03	3.30±0.11	41.00±0.88	1.83±0.01	33.46±0.10	0.81±0.01
C.D.		NS	NS	NS	NS*	0.14*	1.74*	0.03*
SE(m)		0.01	0.04	0.07	0.62	0.04	9,54	0.01
C.V.		3.04	2.01	3.61	2.74	3.39	2.64	2.33

These findings indicate that non-thermal HPP treatment effectively maintains the structural and physicochemical integrity of juice, which aligns with earlier studies reporting negligible changes in pH, TSS, and viscosity in HPP-treated fruit juices during storage.In contrast, bioactive compounds showed statistically significant ($p < 0.05$) changes over time. The ascorbic acid (AA) content decreased progressively during storage in both treatments. In P350T10 samples, AA reduced from 2.69 to 1.98 mg/100 mL, while in P650T10, it decreased from 2.54 to 1.83 mg/100 mL over 28 days. This indicates that vitamin C degradation occurred during storage, although better retention was observed at 350

MPa (73.6%) compared to 650 MPa (72.04%), suggesting that lower pressure conditions are more favorable for nutrient preservation.

Similarly, the total phenolic content showed significant reduction ($p < 0.05$). In P350T10, phenolic content decreased from 39.74 to 34.86 mg GAE/mL (12.28% loss), whereas in P650T10 it declined from 38.72 to 33.46 mg GAE/mL (13.58% loss). This reduction may be attributed to oxidative degradation of phenolic compounds during storage, even under refrigeration.The antioxidant activity, expressed as IC_{50} values, increased significantly during storage (from 0.44 to 0.78 mg/mL in P350T10 and 0.51 to 0.81 mg/mL in P650T10), indicating a decline in antioxidant capacity over time. Since higher IC_{50} values correspond to lower antioxidant activity, this trend confirms the gradual degradation of functional compounds.

The statistical parameters (C.D., SE(m), and C.V.) further validate the reliability of the results. Non-significant C.D. values for acidity, pH, TSS, and viscosity confirm their stability, while significant C.D. values for ascorbic acid, phenolics, and IC_{50} highlight meaningful changes in bioactive components during storage.

4. Conclusion

The study clearly demonstrates that ultrasonication (60 kHz, 10 min), ozone treatment (60 g/Nm^3), and high-pressure processing (350 and 650 MPa) are effective non-thermal methods for preserving wood apple juice during 28 days of refrigerated storage, minimizing thermal degradation and maintaining overall juice quality.

1. Across all treatments, key physicochemical parameters such as pH (≈3.15–3.21), total soluble solids (≈3.20–3.56 °Brix), and viscosity (≈40–41 cps) remained non-significantly affected, confirming that these techniques preserve the structural integrity, consistency, and sensory acceptability of the juice throughout storage.
2. A gradual and statistically significant decline in titratable acidity was observed in all treatments (e.g., ultrasound: 0.74% → 0.66%; ozone: 0.75% → 0.64%; HPP: ~0.72% → 0.55%), likely due to biochemical reactions during storage, while a slight increase in TSS in ultrasound-treated samples suggests hydrolysis of complex compounds into simpler sugars.
3. Significant reductions in ascorbic acid, phenolic content, and antioxidant activity (increase in IC_{50} values) were observed across all treatments during storage, indicating oxidative and enzymatic degradation. For instance, ascorbic acid decreased notably in ultrasound (up to ~34.5% loss without cooling) and ozone-treated samples due to oxidation.
4. Ultrasonication with an ice bath cooling system showed better retention of nutrients (ascorbic acid loss ~28.7%) and antioxidant activity compared to without cooling, highlighting the importance of temperature control, whereas ozone treatment resulted in comparatively higher degradation due to its strong oxidative nature.
5. Among all methods, HPP—especially at 350 MPa for 10 minutes—exhibited the best retention of nutritional quality, with higher ascorbic acid retention (≈73.6%), lower phenolic loss (≈12.28%), and relatively stable antioxidant activity compared to higher pressure (650 MPa) and other treatments.

While all non-thermal techniques effectively maintain physicochemical stability, HPP at moderate pressure (350 MPa) is the most suitable method for preserving both nutritional and functional quality of wood apple juice, making it a promising technology for extending shelf life and enhancing the commercial value of fruit juices under refrigerated storage conditions.

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Conflict of Interest

The authors hereby declare that there are no conflicts of interest, financial or otherwise, that could have influenced the outcomes, interpretation, or presentation of the research findings reported in this study.

Data Availability

All relevant data generated and analyzed during the course of this study are included within the article. Any additional details, supporting datasets, or supplementary information required for further clarification can be made available by the corresponding author upon reasonable request

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