

"THE IMPACT OF DIFFERENT PROCESSING METHODS ON APPLE JUICE QUALITY: A COMPARATIVE ANALYSIS OF ULTRASONIC, HPP, AND PASTEURIZATION"**Jakhongir Bakhtiyorovich Pernapasov,**

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Introduction

Apple juice is a herbal product this is very sensitive to the increase of microorganisms. Ensuring its high-satisfactory and protection is an crucial project for cutting-edge meals technology. "The purpose of this study is to apply specific technologies, such as High-Pressure Processing (HPP), ultrasonic waves, and pasteurization, to eliminate microorganisms and assess the quality of the juice.". "Along with killing dangerous microbes, such as Escherichia coli, Salmonella spp., and Listeria monocytogenes, this study also examines the physical and chemical properties of the juice, including pH, color, and sugar content Today, numerous technology are normally utilized in meals processing, which includes High-Pressure Processing (HPP), ultrasonic waves, and pasteurization. High-Pressure Processing (HPP) reduces the amount of microorganisms by breaking their cellular structures under very high pressure (Prakash Kumar Nayak, et al., 2017). Ultrasonic waves additionally play an important role by affecting the cellular membranes of microbes and making them inactive (Muhammad Abid et al., 2013). Pasteurization, which has been used for a long time, relies on heat to kill harmful microorganisms. It is one of the most widely used methods for ensuring the safety of juice (AV Charles-Rodríguez et al., 2007).

Approach: Evaluating the juice's fine after processing is likewise very crucial. For example, modifications withinside the pH stage can display how acidic the juice is and whether or not any chemical reactions or microbial interest came about at some point of the process. The colour of the juice is some other key aspect as it influences the juice's look and the way clients be given it. Additionally, the sugar content material is checked as it affects the flavor and shelf existence of the juice (Zahra Moaddabdoost Baboli et al., 2020).

Studying these technologies and evaluating their effectiveness helps improve the safety and quality of apple juice. The findings from this study could play an important role in developing better juice processing technologies in the future.

Materials and methods

Apple juice was prepared by grinding whole apples in a blender. The ground apples were then placed in a specialized pressing machine to extract the juice efficiently. For this study, 'Red Delicious' apples were specifically used. The juice was utilized immediately after extraction to ensure its freshness during the experiments.

1. High-Pressure Processing (HPP)

In this approach, apple juice was treated using specialized high-pressure equipment, DRG High-pressure equipment (STANSTED FLUID POWER LTD), capable of applying 600 MPa (approximately 6000 atmospheres) for a duration of 15 minutes. The system consisted of a high-pressure chamber and a hydraulic device that uniformly distributed pressure throughout the liquid. This high-pressure regime effectively broke the cellular walls of microorganisms and bacteria, rendering them inactive.

**Fig 1 HPP model**

High-Pressure Processing is a non-thermal technique, that means it does not now no longer depend on heat, which facilitates hold the herbal taste, color, and dietary great of the juice. The system guarantees uniform strain distribution, so the juice's great stays consistent. This approach is especially useful for keeping the freshness of apple juice even as extending its shelf existence with the aid of using lowering microbial pastime to a secure level.

2. Ultrasonic treatment (Citizen Scale YJ 5120-1 Digital Ultrasonic Cleaner Bath)

For this technique, an ultrasonic generator was used, which emitted sound waves at a frequency of 40 kHz. The ultrasonic waves were transmitted into the juice via a sonicator probe or an ultrasonic bath (Citizen Scale YJ 5120-1 Digital Ultrasonic Cleaner Bath). These sound waves created microbubbles in the liquid through a process known as cavitation. When these bubbles collapsed, they released energy that disrupted the structure of microbial cells, effectively reducing their activity and preventing further growth. The treatment was performed for 30 minutes to ensure sufficient microbial reduction.

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and nutrients. Additionally, ultrasonic system is efficient, eco-friendly, and does not now no longer require the addition of chemicals, making it a sustainable desire for meals processing..

3. Pasteurization (95°C for 15 mins)

Pasteurization worried heating the apple juice to a temperature of 95°C for 15 mins the use of a pasteurization unit ready with temperature controls. The device blanketed a heating chamber and temperature sensors to keep constant warmness degrees at some stage in the system. The warmness successfully destroyed dangerous microorganisms, inclusive of bacteria, yeasts, and molds, making sure the juice's safety.

This approach is extensively used withinside the meals and beverage enterprise because of its reliability and simplicity. However, it's miles a thermal system, which means it is able to reason

n Scale YJ 5120-1 Digital Ultrasonic Cleaner Bath)

achieved for half-hour to make certain enough microbial
is it's far mild at the juice, keeping its herbal flavor, color,

moderate adjustments within the juice's colour and taste. Despite this, pasteurization stays one of the simplest strategies for microbial discount and lengthening the shelf existence of apple juice.

By combining unique heating and managed timing, the pasteurization system ensured the destruction of microbes with out appreciably affecting the juice's quality. The device used for this step additionally blanketed a cooling device to fast convey the juice returned to a secure garage temperature, retaining its sensory and dietary characteristics.

Evaluation of Chemical and Physical Properties

1. Using a pH meter (SevenCompact pH meter S210), the apple juice's pH level is determined both before and after the treatment. This made it easier to identify any changes made to the juice's acidity stage. These modifications should indicate microbial activity or chemical interactions that occurred during or following the processing methods.

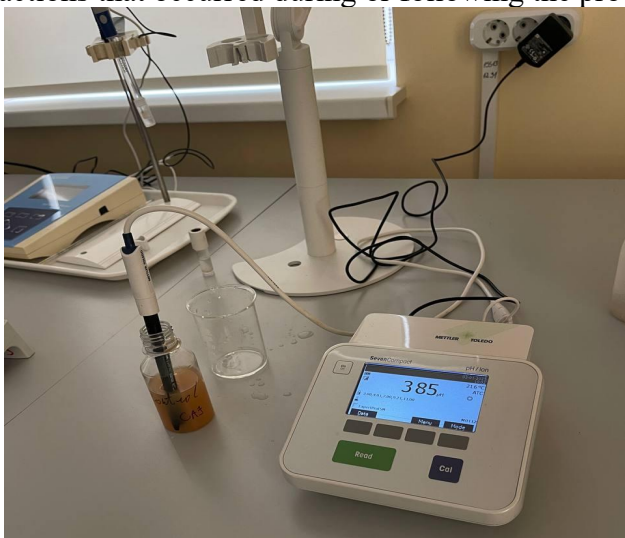


Fig.2 pHCounter (SevenCompact pH meter S210),

2. Color Analysis

1. Control: No treatment was applied. 2. Ultrasonic treatment (Usonic): Treated with ultrasonic waves. 3. HPP: Treated with high-pressure processing (HPP). 4. PAJ: Pasteurized juice. The color of the juice samples was measured using the COLORFLEX EZ STANDARDS BOX (HunterLab, USA) color measurement device. This device measures color in the CIELAB color system where: L: Represents lightness (0 = black, 100 = white). a: Represents the green-red axis (negative = green, positive = red). b: Represents the blue-yellow axis (negative = blue, positive = yellow). The samples were prepared by ensuring they were homogeneous and free of bubbles. Measurements were taken by placing the juice in a transparent container, calibrating the device with a white tile, and recording the L, a, and b values. The average values of L, a, and b for each group were calculated. To compare the color changes, the total color difference (ΔE) was used. Delta E was calculated with this formula

$$\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$



Fig.3 . COLORFLEX EZ STANDARDS BOX

3. Soluble Sugar Content:

The quantity of dissolved sugar within the apple juice is measured using a refractometer. This check is carried out to reveal the various stages of the juice and to make sure that the sugar content material remained solid after the treatments, because it performs a critical function in flavor and average quality.



Fig 4. Digital Brix Refractometer

Microbiological analysis

Each shake sample underwent serial dilution in 0.1% (w/v) peptone water to achieve appropriate concentrations for plating.

1) 1 mL of each sample was transferred into a test tube containing 9 mL peptone water, homogenized using a vortex mixer (IKA Vortex mixers), and serially diluted.

2) A sample from each dilution was then plated onto the designated agar media using a clean sterile spreader or L-shaped spreader (spread plate technique) or pour plate method.



Fig.5.Plated petri dishes.

Media and incubation conditions

a) Plate Count Agar (PCA): To determine total viable microorganisms counts, samples were plated twice at both 10^{-1} and 10^{-2} dilutions. Incubation was conducted at 30 °C for 72 hours. (pour plate method).

b) ENDO Agar: For the detection of *E. coli*, 1 plate per dilution was prepared and incubated at 37 °C for 24 hours. (pour plate method).

c) Violet Red Bile Dextrose (VRBD) Agar: For Enterobacteriaceae detection, 0,1 mL of the 10 dilution was plated and incubated at 37 °C for 24 hours. (spread plate technique).

d) Malt Extract Agar (MEA): For mold and yeast detection, two plates per 10 dilution were prepared and incubated at 27 °C for 3 days. (pour plate method).

Colony counting

Following incubation, colonies were counted using the Interscience Scan 500 yeast and mold counter. Colony counts were recorded and expressed as CFU/mL based on dilution factors



Fig 6.MODEL;GUARDIAN MSC T1200. Fig 7 MEMMETER MODEL UNB200

Results and discussion

Table 1.

Effect of Processing Methods on Microbial Load in Apple Juice (CFU ml⁻¹)

	Control sample	Ultrasonic treatment sample	Pasteurized sample	High pressure process treated sample
Plate Count Agar (PCA)	1.All- 1.7×10 ³ yeast-1.56 ×10 ³ mold-.04 ×10 ² 2.All 3.04 ×10 ³ yeast- 2,93×10 ³ mold-1.6 ×10 ²	1.All -1.24× 10 ³ yeast- 1.03 ×10 ³ mold- 2,1 × 10 ² 2.All 1,36 ×10 ³ yeast-1.10 × 10 ³ mold-2.2× 10 ²	All-2.06 × 10 ² yeast-2.06 ×10 ² All-1.30× 10 ² yeast-1.30 ×10 ²	All-4.34× 10 ² yeast- 1.73×10 ² mold- 2.60×10 ² All-2.17× 10 ² yeast- 8.90×10 ² mold- 0.87×10 ²
Malt Extract Agar (MEA)	All-6.84 × 10 ² yeast- 4,45×10 ² mold- 2.89×10 ² All- 9.45×10 ² yeast- 8.90×10 ² mold- 1,45×10 ²	All-5.75× 10 ² yeast-1.95 ×10 ² mold-3.80 ×10 ²	L1	L1
ENDO e-COLI	23 -CFE e- coli 20 - coliforms	32-E.Coli 10- coliforms	2	<1
VRBD ENTNOBACTERIA	20	<1	<1	<1

Plate Count Agar (PCA) Results

The control sample (CAN) exhibited the highest level of bacterial contamination, with a total microbial count of 3.04×10^3 CFU/mL. This indicates that untreated conditions allow for rapid microbial growth. Ultrasonic treatment (USAE) reduced the bacterial count to a range of $1.24 \times$

10^3 to 1.36×10^3 CFU/mL. The cavitation process created by ultrasonic waves likely disrupted microbial cell structures, lowering their activity. Pasteurization (PAS) was highly effective, reducing the bacterial count to 2.06×10^2 CFU/mL. High-Pressure Processing (HPP) showed similar efficiency, reducing the bacterial, mould and yeast load to 2.17×10^2 CFU/mL by applying extreme pressure to break the cellular walls of microorganisms. These results align with findings in the literature. For example, Prakash Kumar Nayak et al. (2017) demonstrated that HPP could reduce bacterial counts to $\approx 1.8 \times 10^2$ CFU/mL. Similarly, Charles-Rodríguez et al. (2007) reported that pasteurization is highly effective at microbial elimination, often achieving counts below 10^2 CFU/mL.

ENDO Agar Results (E. coli and Coliforms)

The control sample had an E. coli count of 23 CFU/mL, highlighting high contamination in untreated juice. Ultrasonic treatment reduced the count to 32 CFU/mL, showing partial effectiveness in inactivating E. coli. Pasteurization further reduced E. coli to just 2 CFU/mL, while HPP completely eliminated E. coli, with levels falling below detectable limits (<1 CFU/mL). Muhammad Abid et al. (2013) reported that ultrasonic waves can reduce E. coli contamination by approximately 30–35%. Meanwhile, Nayak et al. (2017) highlighted that HPP is capable of achieving near-complete microbial inactivation, consistent with these results.

Malt Extract Agar (MEA) Results (Mold and Yeast)

The mold and yeast levels in the control sample were significantly high, at 6.84×10^2 CFU/mL. Ultrasonic treatment reduced these levels to a range of 5.75×10^2 to 1.95×10^2 CFU/mL. Pasteurization and HPP both eliminated mold and yeast almost entirely, with counts dropping to undetectable levels (<1 CFU/mL). Similar trends are reported in the literature. Charles-Rodríguez et al. (2007) emphasized the effectiveness of pasteurization in reducing mold and yeast, while Nayak et al. (2017) confirmed that HPP achieves comparable results with minimal impact on juice quality.

Table 2.
Color Measurement Results of Apple Juice Samples"

Typ of samples	L Represents lightness	a Represents the green-red axis	b Represents the blue-yellow axis	Delta E- Color difference
Control	31.98±0.01	3.860±0.01	17.594±0.03	0.000
Ultrasonic treatment	32.044±0.05	4.232±0.04	18.110±0.05	0.639
High-Pressure Processing (HPP)	32.164±0.09	3.468±0.01	17.18±0.02	0.597
Pasteurization	46.946±0.01	6.712±0.01	19.678±0.05	15.369

Apple juice color changes were analyzed using ultrasonic treatment, high-pressure processing (HPP), and pasteurization with the ColorFlex EZ Standards Box. The L (lightness), a (green-red axis), b (blue-yellow axis), and Delta E (color difference) values were measured. Delta E was calculated using the formula:

$$\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

Ultrasonic treatment and HPP showed very small color changes. Their Delta E values were 0.639 and 0.597, meaning the difference is almost invisible. The L values (32.044 and 32.164) were close to the control (31.988). The a and b values also did not change much, showing that these methods keep the juice color stable.

Pasteurization caused a big color change. The L value increased to 46.946, making the juice much lighter. The a (6.712) and b (19.678) values also increased, meaning the juice became more yellow and red. The Delta E value was 15.369, which is a strong color difference that is easy to see. This happened because high temperatures affect pigments and chemical compounds in the juice.

The results show that ultrasonic treatment and HPP help keep the juice color close to the original, while pasteurization makes a big change. If keeping the original color is important, ultrasonic treatment and HPP are better. However, pasteurization is still useful for making the juice safe to drink.

Table 3.

Effect of Processing Methods on pH and Brix Levels in Apple Juice

	pH	BRIX %
CONTROL	3.83 $\pm$ 0.2	12.90 $\pm$ 0.1
ULTRASONIC	3.80 $\pm$ 0.1	13.10 $\pm$ 0.2
HPP	3.80 $\pm$ 0.1	12.95 $\pm$ 0.15
PASTER	3.88 $\pm$ 0.1	15.10 $\pm$ 0.2

The pH level of the control sample (CAN) was 3.83, and the (Brix) was 12.9%. Pasteurization increased the pH to 3.88 and the Brix to 15.1%, enhancing the overall sensory quality of the juice. Ultrasonic treatment and HPP preserved the pH at 3.80 but resulted in moderate increases in Brix, to 13.1% and 12.95%, respectively.

These findings align with the work of Charles-Rodríguez et al. (2007), who noted that pasteurization tends to improve sweetness while slightly increasing pH levels. Additionally, (Moaddabdoost Baboli et al. 2020) highlighted that HPP and ultrasonic treatments maintain the natural properties of juice, with minimal changes in acidity and sugar content.

These results demonstrate that pasteurization is the most effective method for improving juice quality, achieving significant microbial reduction while enhancing sweetness and chemical stability. HPP and ultrasonic treatments, while slightly less effective in microbial inactivation, excel at preserving the natural characteristics of the juice. When compared to the literature, these findings support the conclusion that modern processing techniques can be tailored to meet specific quality and safety goals.

Conclusion

This study looked at how High-Pressure Processing (HPP), ultrasonic treatment, and pasteurization affect the quality and safety of apple juice. Each method showed different advantages depending on the goal.

Pasteurization was the most effective method for killing microbes and increasing the Brix percentage, which reached 15.1%. Brix percentage shows the amount of soluble solids in the juice,

mainly sugar, and is important for taste and quality. Pasteurization also slightly increased the pH, making the juice less acidic and more stable. However, it caused small changes in the juice's color and flavor because of the heat used.

HPP reduced bacteria and mold to levels that could not be detected while keeping the juice's natural color, taste, and nutrients. This makes HPP a good method for keeping the juice fresh and extending its shelf life.

Ultrasonic treatment raised the Brix percentage to 13.1%. While it was not as strong as pasteurization or HPP in killing microbes, it kept the juice's natural qualities. This method is also eco-friendly and doesn't need chemicals. Pasteurization made the juice even sweeter and killed microbes. HPP and ultrasonic treatment were better for keeping the juice's natural properties. Choosing the right method depends on the balance between safety, quality, and the natural feel of the juice.

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