

Influence of Certain Parameters on Laboratory Analyses and Verification of Preservatives in Natural Apple and Peach Juices

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ABSTRACT

This study evaluates the influence of selected technological and quality parameters on the physicochemical, microbiological, and organoleptic characteristics of natural apple and peach juices, together with the verification of selected preservatives and artificial sweeteners. Apple and peach purees were subjected to initial quality control upon reception, including pH, °Brix, and organoleptic assessment. The technological process followed modern production standards. Juices were diluted with treated water based on °Brix values, and acidity was adjusted using citric acid or lemon puree. Peach juice received natural flavor to compensate for aroma loss, while apple juice was processed without additional flavoring. After homogenization, juices were pasteurized at 85–95 °C for 15–30 seconds to inactivate microorganisms and enzymes. Packaging materials were sterilized, and filling was performed aseptically. Final tunnel pasteurization (90–95 °C for approximately 30 minutes) ensured microbiological stability and extended shelf life. Samples were collected at three stages: after raw material reception, after formulation and homogenization, and after final packaging. Laboratory analyses included organoleptic evaluation, microbiological testing, physicochemical measurements, and HPLC analysis. HPLC was used to verify the presence or absence of potassium sorbate (E202), sodium benzoate (E211), and selected artificial sweeteners. The chromatographic analysis showed that E202, E211, and the analyzed artificial sweeteners were not detected in the juice samples. The results also indicated that refrigerated storage provided greater physicochemical stability compared with ambient storage. These findings demonstrate the importance of technological processing, appropriate storage conditions, and analytical monitoring in maintaining juice quality and verifying compliance with food safety and labeling requirements.

Keywords: Apple juice, Peach juice, Preservatives, Physicochemical analysis, HPLC

INTRODUCTION

The fruit processing industry and fruit juice production occupy a central position in the global food sector and represent one of the fastest-growing branches, both in terms of consumption and technological development. At the same time, this sector must comply with increasing food safety requirements and international quality standards (AOAC, 2016; ISO, 2018). Fruit juices, in addition to their economic importance, have significant nutritional and dietary value, as they are a rich source of vitamins, minerals, natural sugars, organic acids, and bioactive compounds that positively affect human health (Lee & Kader, 2000; Kaur & Kapoor, 2001; Klein, 1987). In recent decades, growing consumer awareness about healthy nutrition has increased the demand for high-quality and safe juices with appropriate shelf life (Touati et al., 2016; Ampofo-Asiama et al., 2019). As a result, the food industry faces the challenge of maintaining the nutritional and sensory qualities of these products while also meeting food safety requirements and international quality standards (Parveen et al., 2019; Jay et al., 2005). Fruit juices are highly susceptible to chemical and microbiological deterioration. Their high water content and natural sugars create favorable conditions for the growth of microorganisms, especially yeasts, molds, and certain pathogenic bacteria (Parveen et al., 2019). Without the application of proper preservation methods, these products have a relatively short shelf life and may lose their sensory properties (color, aroma, taste) as well as part of their nutritional value (Sogi et al., 2015; Nwaichi & Monanu, 2013). To prevent such deterioration, the food industry has developed a combination of technological approaches, including thermal treatments (pasteurization and sterilization), the use of approved chemical preservatives,

and modern technologies such as aseptic packaging and controlled storage conditions (Villamiel & de Jong, 2000; Polydera et al., 2003). Pasteurization is one of the fundamental processes in the juice industry and can be applied in several forms, such as flash pasteurization, pasteurization using plate or tubular heat exchangers, and tunnel pasteurization after bottling. This process ensures the inactivation of microorganisms and enzymes, preventing spontaneous fermentation. However, pasteurization alone is often not sufficient for long-term storage. Therefore, in industrial practice, thermal treatments are frequently combined with chemical preservatives, which provide additional protection against microbial growth and help maintain product stability during storage.

Among the most commonly used and accepted preservatives in the food industry are potassium sorbate (E202) and sodium benzoate (E211). Potassium sorbate is the salt of sorbic acid and has a broad antimicrobial spectrum against yeasts, molds, and some bacteria. Sodium benzoate, the salt of benzoic acid, is particularly effective as a preservative in acidic environments (Jay et al., 2005). The combination of these two additives is widely applied because their effects complement each other and enhance overall preservation efficiency. However, their use is strictly regulated by law. According to the directives of the European Union and Codex Alimentarius (FAO/WHO), maximum permitted limits are clearly defined, and exceeding these limits may pose potential health risks to consumers (AOAC, 2016; ISO, 2018). The production process of fruit juices includes several standardized stages: reception and quality control of raw materials (apple and peach puree), formulation of the composition by adjusting °Brix through the addition of treated water and acidity through the addition of citric acid or lemon puree, addition of natural flavor in peach juice, high-pressure homogenization, primary pasteurization (85–95 °C for 15–30 seconds), sterilization and filling under aseptic conditions, and tunnel pasteurization of the final product (90–95 °C for approximately 30 minutes) (Pearson, 2018; Villamiel & de Jong, 2000).

During the research, samples were taken at three key stages: after the reception of raw materials, after formulation and homogenization, and after the final packaging stage.

Laboratory analyses include several types of evaluations to assess the overall quality and safety of the juice samples. **Physicochemical analyses** were performed to measure parameters such as pH, °Brix (dry matter content), and total acidity, which are important indicators of the chemical composition and stability of the product (Sogi et al., 2015; Elkins, 1979). In addition, **sensory (organoleptic) analyses** were conducted in order to evaluate key quality attributes, including color, aroma, taste, and consistency, which directly influence consumer acceptance of the product (Kilcast, 1994). Furthermore, **microbiological analyses** were carried out to determine the total microbial count, as well as the presence of yeasts, molds, and potential pathogenic microorganisms, in order to ensure the microbiological safety and quality of the juices (Parveen et al., 2019; Jay et al., 2005).

Special emphasis was placed on verifying the presence or absence of potassium sorbate (E202), sodium benzoate (E211), and selected artificial sweeteners using High-Performance Liquid Chromatography (HPLC). This analytical approach enables the identification and determination of these compounds in juice samples and provides an important tool for verifying compliance with food safety and labeling requirements. The aim of this research was to evaluate selected physicochemical, microbiological, organoleptic, and storage-related quality parameters of natural apple and peach juices, to verify the presence or absence of selected preservatives and artificial sweeteners using HPLC, and to contribute to the improvement of industrial practices for the production of safe and high-quality fruit juices.

METHODS

This study was designed to evaluate the production technology and quality stability of apple (*Malus domestica*) and peach (*Prunus persica*) juices, with particular emphasis on physicochemical, microbiological, organoleptic, and preservative-related parameters. The methodological concept combined industrial processing practice with standardized laboratory control, in order to generate results that are both scientifically relevant and practically applicable for the juice industry. The study followed internationally recognized analytical and safety frameworks, including AOAC, ISO, Codex Alimentarius, HACCP, ISO 9001:2015, and ISO 22000.

Apple and peach puree concentrates were selected as the primary raw materials. Their selection was justified by their high industrial relevance, nutritional value, broad consumer acceptance, and relative technological stability during thermal processing. Apple puree was characterized by 30–32 °Brix, pH 3.3–4.0, and total acidity of 0.4–0.6 g citric acid/100 mL, while peach puree had 30–32 °Brix, pH 3.4–4.2, and total acidity of 0.5–0.7 g citric acid/100 mL. In addition, apple was described as a major industrial fruit in Europe with an estimated juice yield of 550–600 mL/kg fruit, whereas peach was noted for its carotenoid-rich color, aromatic compounds, and higher sensitivity during pasteurization.

Type of puree	Initial °Brix	pH	Total acidity (g citric acid/100 mL)
Apple	30 - 32	3.3 - 4.0	0.4 - 0.6
Peach	30 - 32	3.4 - 4.2	0.5 - 0.7

Table 1. Physicochemical parameters of the initial puree

The technological process was structured as a sequence of standardized industrial operations beginning with raw material reception and ending with final packaging. During formulation, the puree was diluted with reverse osmosis (RO) treated water to standardize the final juice to 11–12 °Brix. RO water was used because it can remove up to 99% of mineral salts, heavy metals, organic compounds, bacteria, and other microorganisms, thereby ensuring chemical neutrality and microbiological safety. The study also notes that, after reverse osmosis, the water additionally passed through activated carbon filtration, water softening, and UV disinfection before being used for dilution.

Acidity was then adjusted using citric acid. For peach juice, additional natural aroma was added to restore organoleptic characteristics lost during heat treatment, whereas apple juice generally did not require extra aroma because of its lower pH and greater microbiological stability.

Homogenization was carried out under high pressure, at 100–200 bar, reducing particle size to approximately 1–2 µm. This step was intended to prevent sedimentation, improve colloidal stability, create a uniform appearance, and enhance the bioavailability of bioactive compounds such as polyphenols and carotenoids. Without homogenization, phase separation would occur, substantially reducing product acceptability.

Primary pasteurization was applied using the HTST approach at 85–95 °C for 15–30 seconds. According to the methodology, this stage served three key purposes: inactivation of pathogenic microorganisms such as *E. coli*, *Salmonella* spp., and *Listeria monocytogenes*; inactivation of oxidative enzymes such as polyphenol oxidase and pectinesterase; and preservation of product quality through minimal nutritional loss, especially of vitamin C. After controlled cooling, the juice was filled aseptically into glass bottles or aseptic packaging materials such as PET or Tetra Pak.

A secondary tunnel pasteurization step was then applied at 90–95 °C for 30 minutes, with the goal of achieving full product and packaging sterility and extending the shelf life to 6–12 months. Final operations included gradual cooling, hermetic sealing, labeling with all legal and technological information, and packaging for storage at 4–20 °C.

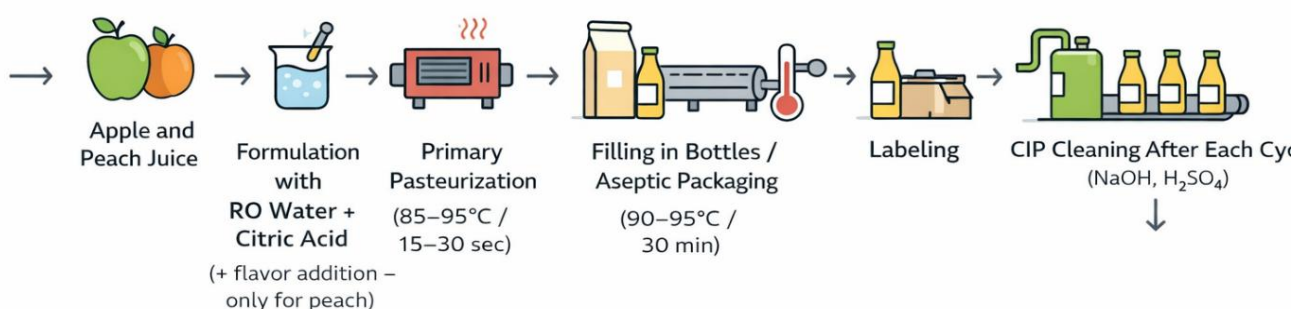


Figure 1. Flow-chart of apple and peach juice production.

The process flow-chart presented in the manuscript summarizes the main production stages as: formulation with puree, RO water and citric acid, addition of aroma in peach juice, homogenization (100–200 bar), primary pasteurization (85–95 °C for 15–30 s), filling into aseptic packaging, tunnel pasteurization (90–95 °C for 30 min), labeling, packaging, and subsequent CIP cleaning.

To maintain hygienic processing conditions, a CIP (Cleaning in Place) system was performed after each production cycle. The recorded CIP sequence included: pre-rinsing with water for 5–10 min at 35–45 °C; alkaline cleaning with 1–2% NaOH for 30 min at 60–80 °C to remove proteins, caramelized sugars, pectins, and fats; intermediate rinsing with water for 5–10 min; acid cleaning with 1–1.5% H₂SO₄ for 20–30 min at 50–60 °C to dissolve mineral deposits of Ca and Mg; and final rinsing with potable water for 5–15 min or until complete clarity. Residual chemicals were checked using phenolphthalein for NaOH residues and methyl orange for H₂SO₄ residues. All parameters were recorded for traceability and audit purposes.

CIP Stages

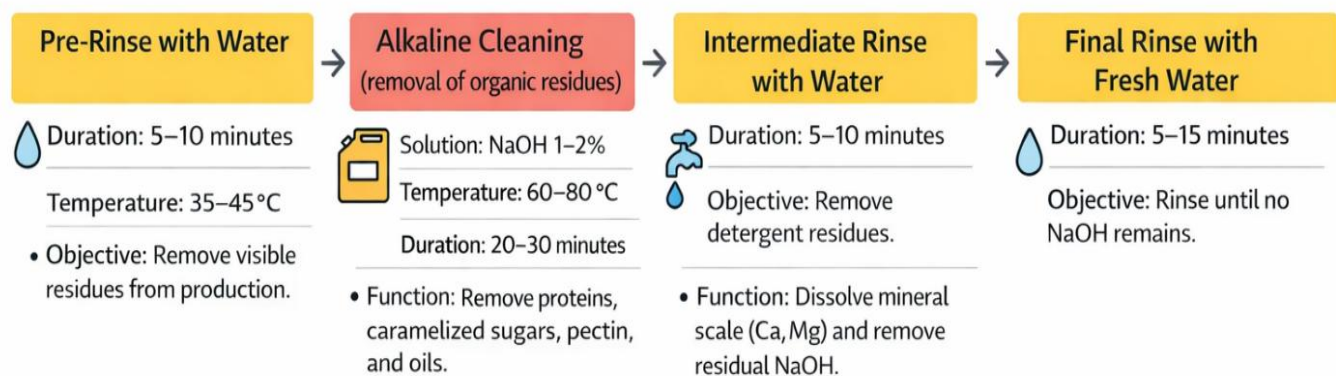


Figure 2. Flow-chart of the CIP process in the juice industry.

The CIP figure in the manuscript illustrates the five-step cleaning protocol and the post-cleaning control of residues with acid–base indicators. Sampling was carried out at three critical stages of production: after puree reception, after formulation and homogenization, and after final packaging. The samples were then stored under two temperature regimes: ambient temperature (20–25 °C), simulating market conditions, and refrigerated storage at 4 ± 1 °C, representing controlled cold storage. This design enabled the study of stability and degradation during storage. The storage monitoring was conducted over 60 days, with measurements on days 0, 15, 30, 45, and 60.

Table 2. Sampling and storage plan for apple and peach juice

Juice type	Stage	Storage condition	Code
Apple	After puree reception	Refrigerator	M1-F
Apple	After puree reception	Ambient	M1-A
Apple	After formulation/homogenization	Refrigerator	M2-F
Apple	After formulation/homogenization	Ambient	M2-A
Apple	After final packaging	Refrigerator	M3-F
Apple	After final packaging	Ambient	M3-A
Peach	After puree reception	Refrigerator	P1-F
Peach	After puree reception	Ambient	P1-A
Peach	After formulation/homogenization	Refrigerator	P2-F
Peach	After formulation/homogenization	Ambient	P2-A

Peach	After final packaging	Refrigerator	P3-F
Peach	After final packaging	Ambient	P3-A

Physicochemical analyses were performed in triplicate ($n = 3$). The pH was measured using a calibrated pH meter according to ISO 2917:1999. Soluble solids ($^{\circ}\text{Brix}$) were determined using a digital refractometer according to AOAC Method 932.12. Titratable acidity was determined by titration with 0.1 N NaOH using phenolphthalein as the endpoint indicator according to AOAC Method 942.15, and the results were expressed as g citric acid/100 mL using a conversion factor of 0.064.

Total sugar content was determined by the Lane–Eynon titrimetric method, based on the reduction of Fehling’s solution by reducing sugars. After appropriate sample preparation and inversion, the sugar solution was titrated against Fehling’s solution using methylene blue as an indicator, and the total sugar content was calculated and expressed as a percentage (%). Vitamin C (ascorbic acid) content was determined by the 2,6-dichlorophenolindophenol (DCPIP) titrimetric method according to AOAC Official Method 967.21. The method is based on the reduction of the DCPIP indicator by ascorbic acid, with the endpoint indicated by the persistence of a light pink color. Vitamin C content was calculated and expressed as mg of ascorbic acid per 100 mL of juice. The analyses were performed in triplicate ($n = 3$).

Table 3. Physicochemical parameters of the final juice

Juice type	Final $^{\circ}\text{Brix}$	Typical pH	Total acidity (g/100 mL)
Apple	11–12	3.3–4.0	0.3–0.5
Peach	11–12	3.4–4.2	0.3–0.5

Organoleptic analyses were conducted by a trained panel of 10 assessors according to ISO 6658:2017 and ISO 8586:2012, using a 1–5 scale. The assessed parameters were color, aroma, taste, and consistency. A separate consumer survey involving 20 citizens was also conducted to estimate product acceptability.

Table 4. Organoleptic evaluation parameters (1–5 scale)

Parameter	Description	Scale
Color	Visual comparison with standard	1–5
Aroma	Intensity and naturalness	1–5
Taste	Sweetness–acidity balance	1–5
Consistency	Uniformity and absence of sediment	1–5

Microbiological analyses included total aerobic microorganisms (ISO 4833-1), yeasts and molds (ISO 21527-1), *Escherichia coli* (ISO 16649-2), *Salmonella* spp. (ISO 6579-1), and *Listeria monocytogenes* (ISO 11290-1). The study states that these tests were used to confirm whether the final products complied with food safety requirements. For preservatives and artificial sweeteners, HPLC analysis was performed using an Agilent Technologies 1260 Infinity Rapid Resolution Liquid Chromatography system equipped with UV-DAD detection and a C18 column. The mobile phase consisted of methanol and water acidified with phosphoric acid. Calibration and identification were performed using a standard mixture of acesulfame K, sodium saccharin, caffeine, aspartame, sodium benzoate (E211), and potassium sorbate (E202). The HPLC analyses were carried out in the Laboratory for Chemical Analyses at the Faculty of Agricultural and Food Sciences, University “St. Cyril and Methodius”, Skopje, under report no. 09-6/731 dated 03.09.2025, with issued results on 10.09.2025.

RESULTS

The obtained results provide a comprehensive evaluation of the quality, safety, and stability of apple (*Malus*

domestica) and peach (*Prunus persica*) juices during processing and storage under different temperature conditions. The results were derived from a combination of physicochemical, microbiological, sensory, and chromatographic analyses, allowing a detailed assessment of product characteristics and their evolution over time. The evaluation of juice quality was carried out through a systematic approach, beginning with the determination of initial physicochemical and microbiological parameters, followed by monitoring changes during storage, sensory evaluation, and finally the identification of preservatives and sweeteners using HPLC analysis. This multi-level analytical approach enabled a comprehensive understanding of both the technological efficiency of the production process and the stability of the final products. The initial results provided essential information on the baseline quality of the juices, reflecting the effectiveness of formulation, homogenization, and pasteurization processes. Parameters such as pH, soluble solids ($^{\circ}\text{Brix}$), total acidity, sugar content, and vitamin C were used as key indicators of product quality and compliance with food standards. In parallel, microbiological analyses were performed to confirm product safety and the absence of pathogenic microorganisms. During the storage period, significant attention was given to the evolution of physicochemical parameters under different temperature conditions. Storage stability is a critical factor in the evaluation of fruit juices, as it directly influences product shelf life, safety, and sensory acceptability. The comparison between ambient storage ($20\text{--}25\text{ }^{\circ}\text{C}$) and refrigerated conditions ($4\text{ }^{\circ}\text{C}$) provided valuable insights into the role of temperature in controlling biochemical changes such as sugar degradation, acid formation, and pH variation.

In addition to physicochemical and microbiological analyses, sensory evaluation was conducted to assess consumer perception of the products. Organoleptic characteristics, including color, aroma, taste, and consistency, represent key quality attributes that determine market acceptance. Furthermore, chromatographic analysis using HPLC was applied to determine the presence or absence of preservatives and artificial sweeteners, ensuring compliance with labeling and food safety regulations.

Organoleptic and consumer acceptance results

The organoleptic evaluation showed high acceptability of both apple and peach juices. Peach juice received higher scores for all evaluated sensory parameters compared with apple juice. The highest score was observed for the taste of peach juice (4.7 ± 0.4), while the overall mean scores were 4.6 for peach juice and 4.3 for apple juice, indicating higher sensory acceptance of peach juice.

Table 5. Consumer acceptance evaluation of apple and peach juices (n = 20).

Parameter	Apple juice (Mean $\pm$ SD)	Peach juice (Mean $\pm$ SD)	Overall evaluation
Color	4.4 ± 0.6	4.6 ± 0.5	Very good
Aroma	4.2 ± 0.7	4.5 ± 0.6	Good–very good
Taste	4.3 ± 0.5	4.7 ± 0.4	Very good
Consistency	4.1 ± 0.6	4.5 ± 0.5	Good–very good
Overall mean	4.3	4.6	High acceptability

Initial physicochemical and microbiological characteristics

The initial analyses showed that both apple and peach juices complied with regulatory requirements for physicochemical and microbiological quality. The measured values of acidity, dry matter, total sugars, and vitamin C were within the permitted limits, indicating proper formulation and effective technological control during production. In addition, no pathogenic microorganisms were detected, while the measured microbial counts remained within acceptable limits, supporting the effectiveness of pasteurization and hygienic processing conditions. As shown in Figure 3, peach juice exhibited higher acidity, dry matter, and total sugar content compared to apple juice, reflecting differences in raw material composition. In contrast, both juices showed identical vitamin C values and microbiological safety, confirming compliance with food quality standards.

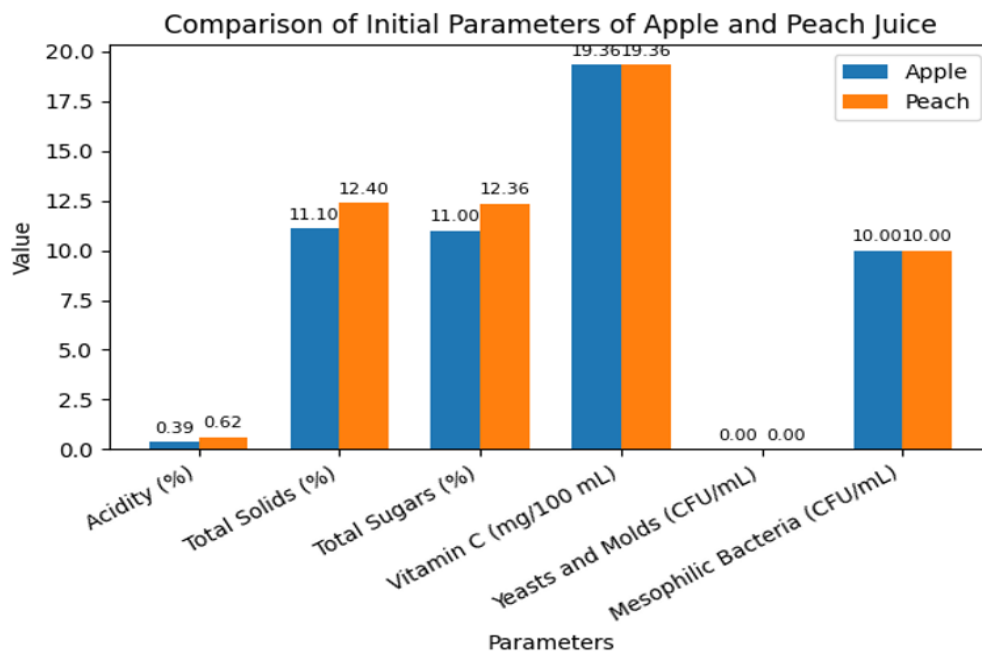


Figure 3. Comparison of initial physicochemical and microbiological parameters of apple and peach juice.

The graphical representation of the initial parameters confirms the differences between apple and peach juice observed in Figure 3. Peach juice showed higher values of acidity (0.62%), dry matter (12.4%), and total sugars (12.36%) compared to apple juice (0.39%, 11.1%, and 11.0%, respectively). In contrast, both juices exhibited identical vitamin C content (19.36 mg/100 mL).

Microbiological analysis indicated that yeasts and molds were not detected in either sample, while mesophilic bacteria remained within safe limits. The graphical comparison clearly illustrates the compositional differences between the two juices, particularly in terms of acidity and sugar content, while confirming their overall safety and compliance with quality standards.

Changes during storage

The evolution of physicochemical parameters during the 60-day storage period revealed clear differences between ambient and refrigerated conditions, as illustrated in Figures 4 and 5.

Figure 4. Apple Juice Storage Changes (0–60 days)

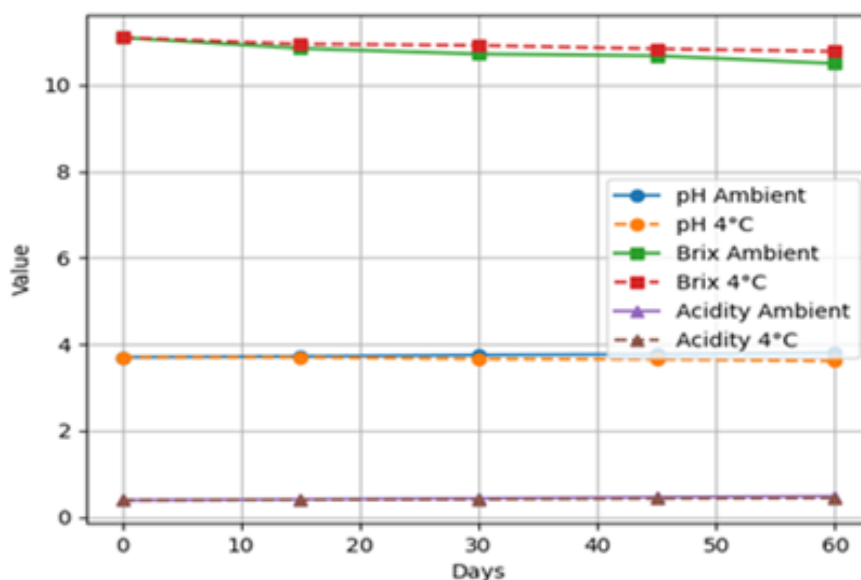


Figure 4. Changes in physicochemical parameters of apple juice during storage (0–60 days)

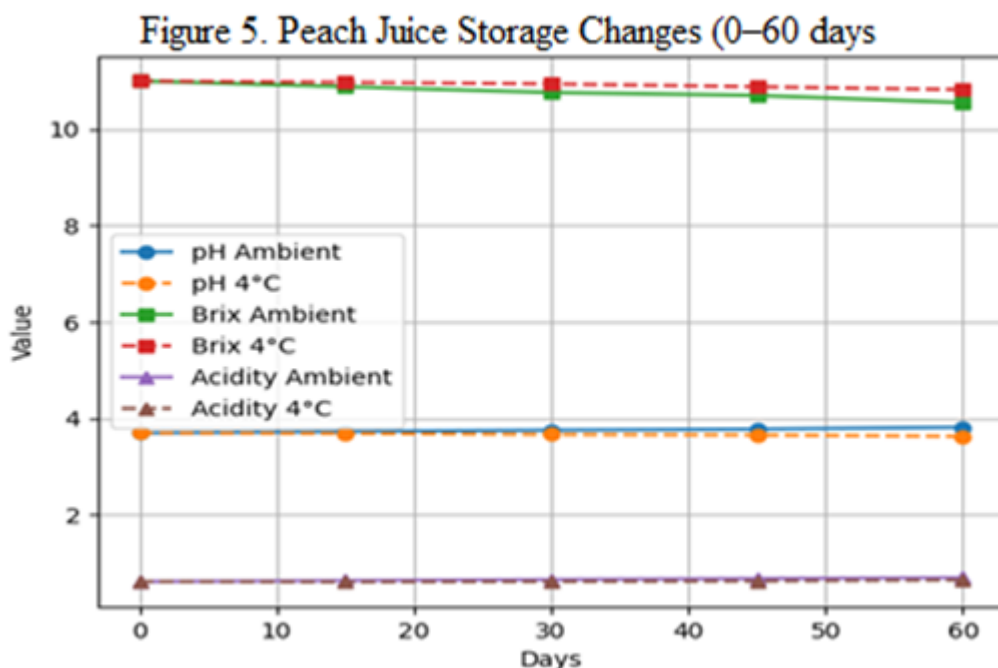


Figure 5. Changes in physicochemical parameters of peach juice during storage (0–60 days)

As illustrated in Figures 4 and 5, storage conditions influenced the physicochemical stability of both apple and peach juices. Under ambient conditions (20–25 °C), both products exhibited changes in pH and total acidity, accompanied by a decrease in °Brix values. These variations indicate ongoing physicochemical and biochemical changes during storage, while the simultaneous changes in pH and titratable acidity may be influenced by the buffering capacity and complex composition of the juice matrix.

In contrast, refrigerated storage (4 °C) slowed down these changes, maintaining greater stability of the juices throughout the 60-day period. The variations observed under cold conditions were less pronounced, confirming the importance of temperature control in preserving product quality.

Comparatively, apple juice showed more pronounced changes during storage, particularly under ambient conditions, suggesting higher sensitivity to physicochemical alterations. This behavior may be associated with its lower initial acidity and specific compositional characteristics. On the other hand, peach juice demonstrated better stability, likely due to its higher initial acidity and compositional characteristics.

Table 6. Changes in physicochemical parameters of apple and peach juice during storage (0–60 days)

Juice	Condition	pH (0 → 60 days)	°Brix (0 → 60 days)	Acidity (g/100 mL) (0 → 60 days)
Apple	Ambient (20–25 °C)	3.70 → 3.80	11.1 → 10.50	0.39 → 0.48
Apple	Refrigerated (4 °C)	3.70 → 3.61	11.1 → 10.78	0.39 → 0.44
Peach	Ambient (20–25 °C)	3.70 → 3.82	11.0 → 10.55	0.62 → 0.70
Peach	Refrigerated (4 °C)	3.70 → 3.63	11.0 → 10.82	0.62 → 0.65

HPLC analysis of preservatives and sweeteners

The chromatographic analysis confirmed the absence of preservatives and artificial sweeteners in both juice samples.

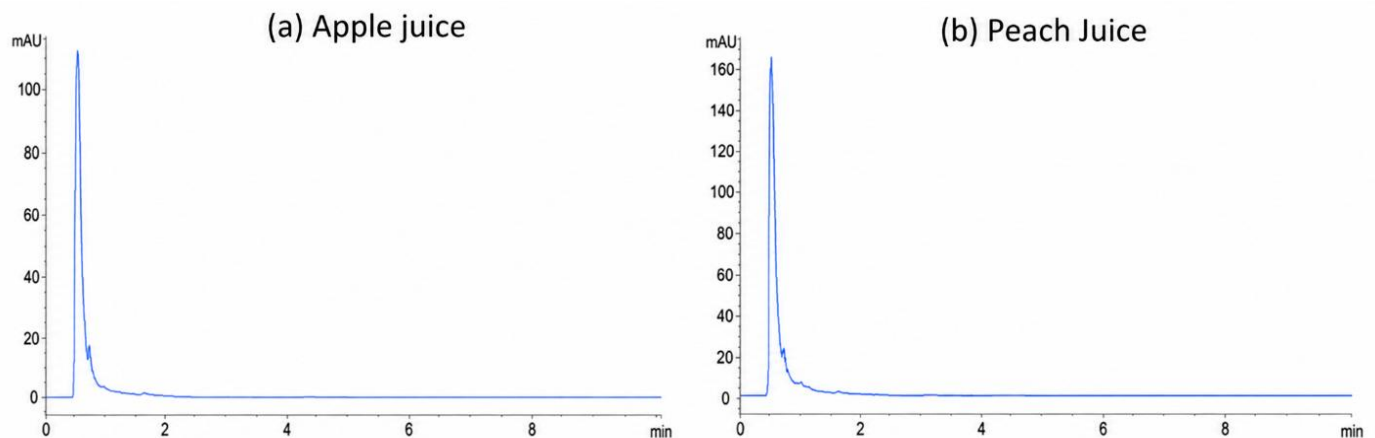


Figure 6. HPLC chromatograms of apple (a) and peach (b) juice samples.

As shown in Figure 6, no characteristic peaks corresponding to preservatives or artificial sweeteners were detected in either apple or peach juice samples. The chromatograms showed that sodium benzoate (E211), potassium sorbate (E202), and the analyzed artificial sweeteners were not detected in the tested samples, consistent with their labeling claims.

CONCLUSION

The results of this study demonstrated that the applied technological process for the production of apple (*Malus domestica*) and peach (*Prunus persica*) juices ensures high product quality, microbiological safety, and compliance with food standards.

The initial physicochemical and microbiological analyses confirmed that both juices met the required regulatory limits, with no pathogenic microorganisms detected and microbial counts remaining within acceptable limits. Peach juice exhibited higher acidity, dry matter, and naturally occurring sugars compared to apple juice, reflecting differences in raw material composition.

During the 60-day storage period, differences were observed between ambient and refrigerated conditions. Under ambient conditions, both juices showed noticeable changes in pH and total acidity, accompanied by a decrease in °Brix values, indicating ongoing physicochemical and biochemical changes during storage. In contrast, refrigerated storage slowed these changes and maintained greater product stability. Apple juice showed more pronounced physicochemical changes during storage, while peach juice demonstrated better stability, which may be associated with differences in initial acidity and compositional characteristics.

HPLC analysis confirmed that preservatives (E202 and E211) and the analyzed artificial sweeteners were not detected in either juice sample. The chromatograms showed no characteristic peaks corresponding to these compounds, supporting the compliance of the analyzed products with their labeling claims.

In conclusion, the study demonstrates that appropriate juice processing technology, combined with controlled storage conditions, contributes to maintaining the safety, quality, and stability of apple and peach juices. Refrigerated storage provided better physicochemical stability during the investigated 60-day period, while analytical monitoring remains essential for verifying product quality, food safety, and compliance with labeling requirements.

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