IMROVING THE NUTRITIONAL QUALITY AND SHELF STABILITY OF WATERMELON JUICE THROUGH ENZYMATIC TREATMENT AND MICROFILTRATION

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ABSTRACT

This study investigates the effectiveness of innovative preservation techniques, specifically enzymatic treatment combined with microfiltration, in improving the nutritional quality and shelf stability of watermelon juice. Watermelon juice is known for its refreshing taste and hydrating properties but faces challenges related to microbial spoilage and nutrient loss. Traditional preservation methods often compromise its sensory attributes and nutritional integrity. Through a comprehensive analysis, this study demonstrates that enzymatic treatment followed by microfiltration improves various aspects of watermelon juice. The treated juice shows improvements in physicochemical properties such as pH, total soluble solids, clarity, and viscosity. Microbiological analysis reveals a substantial reduction in microbial presence following preservation treatment, with treated juice exhibiting markedly lower counts of aerobic plate count, yeast, mold, coliform, and Escherichia coli compared to raw juice. It also exhibits enhanced antioxidant activity while maintaining total phenolic content. Furthermore, nutritional analysis reveals significant improvements in carbohydrate, energy, and potassium content, contributing to the overall nutritional quality of the juice. Microbiological analysis confirms the efficacy of the preservation techniques in ensuring microbial safety and stability over a 6-month storage period at room temperature. Sensory evaluation results indicate that the preservation techniques maintain the sensory attributes of the watermelon juice over time. Overall, this study highlights the potential of enzymatic treatment and microfiltration as effective preservation methods for producing high-quality, nutritionally rich watermelon juice. These techniques offer practical solutions for the beverage industry to meet consumer preferences for freshness, flavor, and health benefits.

Keywords: watermelon juice preservation, enzymatic treatment, microfiltration, shelf stability, nutritional enhancement

INTRODUCTION

Watermelon juice is a popular and refreshing beverage known for its hydrating properties and unique flavor profile. However, preserving watermelon juice poses significant challenges due to its high-water content and susceptibility to various forms of deterioration, including microbial spoilage, flavor degradation, nutrient loss, cloudiness, and polysaccharide-induced separation. The limited shelf life of watermelon juice under diverse storage conditions, as emphasized by Ma et al. (2020) underscores the critical necessity for effective preservation methods.

While traditional preservation techniques like heat treatment and chemical additives have been utilized, they often compromise both the sensory attributes and nutritional quality of watermelon juice. For instance, Aboshi et al. (2020) shed light on the detrimental impact of heat treatment on watermelon juice volatile flavor compounds, emphasizing the necessity for preservation strategies that maintain flavor integrity and nutritional value.

In response to these preservation challenges, innovative non-thermal techniques such as enzymatic treatment and microfiltration, have emerged as promising solutions. Enzymatic treatment involves the application of specific enzymes to catalyze reactions that break down complex components, thereby enhancing texture, flavor, and nutrient retention. Microfiltration, on the other hand, utilizes membranes to remove suspended particles and microorganisms, resulting in clarified and microbiologically stable juices.

However, research in the combined effects of enzymatic treatment and microfiltration on fruit juice preservation is scarce. Despite studies demonstrating benefits such as extending the shelf life and enhancing qualities of prickly pear juice and umbu juice by Kallel et al. (2023) and Ribeiro et al. (2018), the specific synergistic application of these methods in preserving watermelon juice remains underexplored.

To address this gap, this study aims to investigate the synergistic application of enzymatic treatment, particularly utilizing a multi-carbohydrase enzyme, and microfiltration to optimize watermelon juice quality and shelf life. Through a comprehensive assessment encompassing analyses of physicochemical properties, nutritional composition, antioxidant activity and total phenolic content, microbial stability, and sensory characteristics of the treated watermelon juice, this research aims to provide valuable insights into fruit juice preservation, offering practical solutions for producing high-quality, nutritionally rich watermelon juice that aligns with consumer preferences for freshness, flavour, and health benefits.

MATERIALS AND METHODS

2.1. Raw Material

The fresh watermelon juice (*Citrullus lanatus*) used in this study was directly sourced from a local supplier in Jasin, Melaka, to maintain consistency and quality in the raw material. Subsequently, the juice was stored at -20°C until further processing.

2.2. Enzymatic Treatment and Microfiltration Process

The watermelon juice underwent enzymatic treatment using a commercial multi-carbohydrase enzyme (Novozymes, Denmark) under optimized conditions of 0.1% enzyme concentration, 40 minutes of incubation at 49°C in an incubator shaker. Following enzymatic treatment, the process was stopped by subjecting the samples to a temperature of 85°C for 5 minutes. Subsequently, the treated watermelon juice was subjected to microfiltration using a commercial system with a nominal pore size of 0.2 µm. An untreated raw watermelon juice sample was served as the control.

2.3 Physicochemical Analysis

2.3.1 Juice Clarity

The juice samples' absorbance was measured at 660 nm using a UV–VIS spectrophotometer (Shimadzu Corp., Kyoto, Japan) with distilled water as the blank.

2.3.2. pH, Total Soluble Solids, Reducing Sugars

The pH of the juice was assessed with a benchtop pH-meter (Mettler Toledo, Zurich, Switzerland). Total soluble solids were quantified in °Brix using a digital refractometer with automatic temperature compensation (Atago Co., Ltd, Tokyo, Japan). The determination of reducing sugars in juice was conducted using the DNS method (Miller et al., 1959).

2.3.3 Color Measurement

The color properties (CIE L* a* b*) of both untreated and processed juice samples were assessed using a colorimeter (Konica Minolta, Auckland, New Zealand). In this context, the L value represents lightness, a* indicates the red/green scale (+ for red, - for green), and b* signifies the yellow/blue scale (+ for yellow, - for blue).

2.4 Microbial Analysis

Microbial analysis was performed to ensure product safety, evaluating various parameters. Aerobic plate count was determined by plating diluted juice samples on nutrient agar plates and counting colonies to express results as colony-forming units per milliliter (CFU/ml). Yeast and mold count assessments involved using suitable agar media and incubation conditions to quantify colonies as CFU/ml. Coliform and *E. coli* count analyses utilized selective media and incubation settings, with positive colonies confirmed through biochemical tests and results reported in CFU/ml.

2.5 Nutritional Analysis

The nutritional profile of the watermelon juice samples was assessed following the AOAC official method (AOAC, 2016). The carbohydrate content was determined utilizing the differential weight of all compounds, calculated by subtracting the weights of moisture, protein, fat, and ash from the total. The watermelon juice samples were also sent to an accredited outsourced laboratory specializing in nutritional analysis for detailed testing of specific nutrients including vitamin A, vitamin C, potassium, magnesium, calcium, and phosphorus.

2.6 Antioxidant Analysis and Total Phenolic Content

2.6.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant capacity of the juices was evaluated using a spectrophotometric assay with 2,2-diphenyl-1-picrylhydrazyl (DPPH) as the reagent based on the method by Norra et al. (2021). The percentage inhibition of the DPPH free radical by the extracts was calculated as follows: Inhibition (%) = (A blank - A sample / A blank) x 100, where A blank is the absorbance of the control reaction, and A sample is the absorbance of the test compound.

2.6.2 Total Phenolic Content

The total phenolic content in the samples was determined using the Folin-Ciocalteu assay with gallic acid as the standard phenolic compound (Singleton and Rossi, 1965).

2.7. Shelf-life Assessment

2.7.1 Microbial Stability

The assessment of the shelf life of the treated watermelon juice involved the monitoring of microbial stability over a 6-month storage period at room temperature. The samples, packaged in glass bottles, were periodically retrieved at 1-month interval for microbial analysis including aerobic plate count, yeast and mold, coliform, and *E. coli*.

2.7.2 Sensory Evaluation

Sensory evaluation was conducted at the initiation and culmination of the shelf-life study. Sensory panel consisting of 35 panelists evaluated the sensory attributes using a 7-point hedonic scale, ranging from 1 (dislike extremely) to 7 (like extremely). Panelists provided ratings based on their perception of the juice's color, clarity aroma, viscosity, clarity, sweetness, and overall acceptability of the treated watermelon juice samples. This sensory analysis aimed to evaluate the juice's sensory attributes comprehensively while considering changes over the 6-month storage.

2.8. Statistical Analysis

Experiments were conducted in triplicate, and data were expressed as mean ± standard deviation. Statistical analysis was performed using Minitab Statistical Software (version 18) and significant differences between means were determined using analysis of variance (ANOVA) followed by Tukey's test.

RESULTS AND DISCUSSION

3.1. Physicochemical Analysis

Table 1 presents the physicochemical analysis results comparing untreated and treated watermelon juice. Physicochemical parameters such as pH and total soluble solids are pivotal in determining the quality and storage stability of fruit juices due to their impact on taste, microbial stability, and shelf life (Adedeji et al., 2020). In our study, the treated watermelon juice displayed a higher total soluble solids content and a slightly lower pH compared to the untreated juice. These alterations can be attributed to the enzymatic breakdown of complex carbohydrates, leading to the release of galacturonic acid from pectin and the degradation of carbohydrate polymers, thereby increasing solubility and potentially enhancing flavor (Bora et al., 2017).

Moreover, the treatment resulted in a notable increase in reducing sugar content, enhancing the natural sweetness and flavor profile of the juice without the need for additional sweeteners. This aligns with consumer preferences for cleaner ingredient labels. Furthermore, the observed decrease in viscosity of the treated juice is a promising finding, indicating a smoother mouthfeel and improved overall texture. This finding is particularly noteworthy as consumers often prefer juice products with relatively lower viscosity (Salehi, 2020). Moreover, the treated juice displayed markedly higher clarity with an absorbance value of 0.003 compared to 2.112 in the raw juice, likely a result of particulate removal through microfiltration, enhancing the visual appeal of the product.

Additionally, the treated watermelon juice exhibited excellent qualities, free of suspended solids with no haze or sedimentation, clear and slightly yellowish. The color parameters of the treated watermelon juice showed notable changes, with higher L* and b* values but a lower a* value. The increase in L* value and b* value was also observed in a recent study on the combined effects of enzymatic treatment and microfiltration on physicochemical quality of feijoa juice (Schmidt et al., 2021). These shifts in brightness and yellowness, respectively, signify alterations in the juice's color profile due to enzymatic reactions and impurity removal during processing. Many consumers prefer brighter or lighter juices, hence, the color (L* value) and clarity of beverages are usually considered as standard quality indicator (Gonzalez Viejo et al., 2019).

Table 1: Physicochemical analysis of raw and treated watermelon juice

Analysis	Raw Watermelon Juice	Treated Watermelon Juice
pH	5.52 ± 0.002^a	5.41 ± 0.004^a
Total soluble solid (°Brix)	8.9 ± 0.00^a	9.5 ± 0.00^b
Reducing sugar content (mg/mL)	4.1 ± 0.00^a	6.2 ± 0.04^b
Viscosity (mPa.s)	3.18 ± 0.01^a	1.51 ± 0.06^b
Clarity (abs)	2.112 ± 0.008^a	0.003 ± 0.000^b
Colour (L* value)	28.75 ± 0.02^a	44.89 ± 0.06^b

Colour (a* value)	2.77 ± 0.03^a	-4.48 ± 0.05^b
Colour (b* value)	4.37 ± 0.08^a	6.9 ± 0.007^b

Note: Values within the same row followed by different letters (a, b) are significantly different at $p < 0.05$.

3.2 Microbiological Analysis

Microbial counts, crucial indicators of product safety, were assessed in both untreated and treated watermelon juice samples. The results, presented in Table 2, highlight a significant reduction in microbial presence following the preservation treatment. The treated watermelon juice exhibited markedly lower microbial counts compared to the raw juice, indicating the efficacy of the preservation techniques in inhibiting microbial growth and enhancing microbiological stability.

Specifically, the aerobic plate count, yeast and mold count, coliform, and *E. coli* levels were substantially reduced in the treated watermelon juice. The aerobic plate count decreased from 1.3 x 10^3 CFU/ml in the raw juice to less than 1.0 x 10 CFU/ml in the treated juice. Similarly, yeast and mold counts were reduced from <15 x 10 CFU/ml to <1.0 x 10 CFU/ml, indicating a significant decrease in fungal presence. Furthermore, the coliform and *E. coli* counts also showed a notable reduction to levels below the detection limit in the treated samples.

These results demonstrate the effectiveness of the preservation techniques in ensuring microbial safety, thereby minimizing the risk of spoilage, and extending the shelf life of the watermelon juice. It is worth noting that the microbial content of the treated juice complies with the standards set forth by the Food Act 1983 and Food Regulation 1985. The substantial reduction in microbial counts affirms the product's enhanced microbiological stability, aligning with industry standards and consumer expectations for safe and high-quality fruit beverages.

Table 2: Comparison of microbial count in raw and treated watermelon juice

Microbial count (CFU/ml)	Raw Watermelon Juice	Treated Watermelon Juice
Aerobic plate count	1.3×10^{3a}	$<1.0 \times 10^b$
Yeast and mold	$<15 \times 10^a$	$<1.0 \times 10^b$
Coliform	1.7×10^{3a}	$<1.0 \times 10^b$
E. coli	$<1.0 \times 10^a$	$<1.0 \times 10^a$

Note: <1 x 10 indicate the microorganisms tested were not detected in the analyzed samples. Values within the same row followed by different letters (a, b) are significantly different at $p < 0.05$.

3.3 Nutritional Analysis

The nutritional composition of watermelon juice is an essential aspect of its quality and health benefits. Table 3 presents the comparative nutritional analysis of untreated and treated watermelon juice samples, revealing significant improvements in the treated juice's carbohydrate, energy, and potassium content. Specifically, the treated juice displayed a higher energy content (39 kcal/100 ml) and carbohydrate content (9.0 g/100 ml), indicating an increase in nutritional density following the preservation treatment. The enhanced carbohydrate and energy levels align with the objectives of the preservation techniques, aiming to enrich the nutritional profile of the watermelon juice. Additionally, the potassium content of the preserved juice also showed a notable increase, providing consumers with a nutrient essential for various physiological functions such as muscle contraction and nerve function.

Furthermore, the treated juice maintained a consistent protein and ash content similar to the raw watermelon juice, reflecting the preservation methods' effectiveness in preserving essential nutrients during processing. However, a notable reduction in total dietary fiber content was observed in the treated juice, indicating a slight alteration in fiber composition post-treatment. The treatment process also led to variations in the vitamin and mineral content of the watermelon juice. While the treated juice showed decreased levels of vitamin A, vitamin C, magnesium, calcium, and phosphorus compared to the untreated juice, it exhibited a substantial increase in potassium content, reflecting an enrichment in essential minerals crucial for overall health. Comparatively, Ribeiro et al. (2018) also noted a significant decrease in vitamin C content in umbu juice following enzymatic treatment and microfiltration.

Overall, the nutritional analysis highlights the positive impact of the preservation techniques on enhancing the carbohydrate, energy, and potassium content of watermelon juice while maintaining key nutritional components. These findings demonstrate the potential of the treated watermelon juice to offer improved nutritional value and health benefits, catering to consumer preferences for nutrient-rich and high-quality beverages.

Table 3: Nutrient comparison between raw and treated watermelon Juice

Nutrient	Raw Watermelon Juice	Treated Watermelon Juice
Energy (kcal/100 ml)	34 ± 0.3^a	39 ± 0.6^b
Carbohydrate (g/100 ml)	7.7 ± 0.99^a	9.0 ± 1.21^b
Fat (g/100 ml)	nd	nd
Protein (g/100 ml)	0.7 ± 0.00^a	0.7 ± 0.00^a
Ash (g/100 ml)	0.3 ± 0.01^a	0.2 ± 0.00^a
Moisture (%)	91.5 ± 1.61^a	90.4 ± 1.86^a

Total Dietary Fiber (g/100 ml)	1.1 ± 0.03[a]	0.6 ± 0.01[b]
Vitamin A	569 ± 8.1[a]	127.6 ± 3.6[b]
Vitamin C	8.1 ± 0.4[a]	5.4 ± 0.1[b]
Potassium	112 ± 3.1[a]	346.3 ± 2.6[b]
Magnesium	10 ± 0.9[a]	5.0 ± 0.5[b]
Calcium	7 ± 0.2[a]	2.9 ± 0.1[b]
Phosphorus	11 ± 0.5[a]	2.8 ± 0.3[b]

Note: Values within the same row followed by different letters (a, b) are significantly different at $p < 0.05$.

3.4 Antioxidant Activity and Total Phenolic Content

Antioxidant activity and total phenolic content are essential indicators of the health-promoting properties of watermelon juice. Studies have shown the detrimental effects of various treatment processes, such as thermal, mechanical and irradiation on the desirable antioxidant benefits of fruits and their products (Al-Juhaimi et al., 2018). In the assessment of these parameters, the innovative preservation techniques' impact on antioxidant capacity and phenolic compounds is evaluated and presented in Table 4. Results showed that the treated watermelon juice exhibited higher DPPH radical scavenging activity (28.2%) compared to the untreated juice (22.5%), indicating an enhancement in antioxidant activity following preservation. While the total phenolic content of the treated juice was slightly lower (6.5 mg GAE/100 ml) than the untreated juice (7.2 mg GAE/100 ml), the difference was not significant. This minor reduction suggests that the preservation techniques did not significantly impact the juice's total phenolic content. Therefore, despite the marginal decrease, the treated watermelon juice still maintains a considerable level of phenolic compounds, contributing to its antioxidant properties. These findings emphasize the positive impact of the preservation methods on enhancing the watermelon juice's antioxidant activity without significantly affecting the total phenolic content. Such enhancements align with consumer preferences for nutrient-rich and health-enhancing beverages. A similar trend in antioxidant activity and total phenolic content was also observed in clarification of pear juice by Amobonye et al. (2022). Besides, Kallel et al. (2023) also demonstrated the effectiveness of combined enzymatic treatment and microfiltration in preserving the quality and antioxidant content of prickly pear juices.

Table 4: Comparison of antioxidant activity and total phenolic content in raw and treated watermelon juice

Analysis	Raw Watermelon Juice	Treated Watermelon Juice
DPPH radical scavenging activity (%)	22.5 ± 3.00[a]	28.2 ± 0.42[b]
Total phenolic content (mg GAE/100 ml	7.2 ± 0.04[a]	6.5 ± 0.03[a]

Note: Values within the same row followed by different letters (a, b) are significantly different at $p < 0.05$.

3.5 Shelf-life Assessment and Sensory Evaluation

The shelf-life microbial assessment of the watermelon juice was conducted over a 6-month storage period, as summarized in Table 5. Microbial counts, including aerobic plate count, total yeast and mold, coliform, and *E. coli*, were monitored monthly to evaluate the juice's microbiological stability. The results reveal consistently low microbial counts throughout the storage period, with all values remaining below 1.0 x 10 CFU/ml. This indicates that the watermelon juice maintained microbiological safety and stability, with no significant microbial growth observed during the 6-month storage period at room temperature.

A comparative analysis with the study by Ribeiro et al. (2018) which applied the same preservation method to umbu juice, showed that their juice remained microbiologically stable for 90 days when stored at 6°C. Similarly, Kallel at al. (2023) also showed that at 4°C storage, the clarified prickly pear juice was remained microbiologically safe for 90 days. In contrast, the current study demonstrated superior microbial stability, as the watermelon juice remained safe and microbiologically stable for a longer period of 6 months at room temperature. The consistent low levels of aerobic plate count, yeast and mold, coliform, and *E. coli* in the preserved watermelon juice highlight the effectiveness of the preservation techniques in preventing microbial contamination and sustaining the microbiological quality of the product.

These findings signify the robustness of the preservation methods employed in the study, indicating that the watermelon juice can maintain its microbiological safety and integrity over an extended shelf-life period. The results serve as essential indicators of the product's quality and safety, offering consumers confidence in the microbiological stability of the preserved watermelon juice even under ambient storage conditions.

Table 5: Microbiological analysis of treated watermelon juice during storage period (months)

Storage period (months)	Aerobic plate count	Total yeast & mold	Coliform	*E. coli*
0	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10
1	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10
2	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10
3	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10
4	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10
5	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10

6	<1.0 x 10	<1.0 x 10	<1.0 x 10	<1.0 x 10

Note: <1 x 10 indicate the microorganisms tested were not detected in the analyzed samples

The sensory evaluation of the watermelon juice was also conducted to assess its sensory attributes at 0 months and after 6 months of storage, as summarized in Table 6. The sensory evaluation included attributes such as color, clarity, aroma, viscosity, sweetness, and overall acceptance to gauge the juice's sensory quality over time. The results showcase the evolution of key attributes in the watermelon juice over the storage period. The color intensity slightly increased from 5.4 to 5.7, indicating a subtle enhancement in visual appeal. Moreover, the clarity of the juice improved significantly from 5.9 to 6.3, reflecting enhanced transparency and visual quality. While the aroma experienced a slight decline from 5.6 to 5.3, the overall aroma profile remained favorable. The viscosity of the juice decreased slightly from 6.0 to 5.5, suggesting a minor change in texture. Additionally, the perceived sweetness level showed a marginal reduction from 5.9 to 5.5, indicating a subtle shift in taste. Despite these minor variations, the overall acceptance rating decreased slightly from 6.0 to 5.5, implying a modest decline in consumer preference. These attribute results collectively demonstrate the preservation techniques' ability to maintain the watermelon juice's sensory attributes and overall quality over the 6-month storage period, ensuring a consistent and satisfactory consumer experience.

Table 6: Sensory evaluation scores of treated watermelon juice attributes at 0 and 6 months

Attributes	0 month	6 months
Color	5.4 ± 0.2	5.7 ± 0.3
Clarity	5.9 ± 0.4	6.3 ± 0.1
Aroma	5.6 ± 0.1	5.3 ± 0.5
Viscosity	6.0 ± 0.3	5.5 ± 0.3
Sweetness	5.9 ± 0.2	5.5 ± 0.1
Overall acceptance	6.0 ± 0.2	5.5 ± 0.1

CONCLUSION

In conclusion, this study investigated the efficacy of innovative preservation techniques, namely enzymatic treatment combined with microfiltration, in improving the nutritional quality and shelf life of watermelon juice. The results demonstrate the effectiveness of these techniques in improving various aspects of the juice, including physicochemical properties, microbiological stability, nutritional composition, antioxidant activity and total phenolic content, and sensory attributes.

Enzymatic treatment, followed by microfiltration, resulted in significant improvements in the clarity, stability, and overall quality of watermelon juice. The treated juice exhibited lower pH, higher total soluble solids, increased reducing sugar content, and improved viscosity and clarity compared to the untreated juice. Additionally, the preservation techniques led to enhanced antioxidant activity while maintaining the juice's total phenolic content. Microbiological analysis revealed consistently low microbial counts throughout the 6-month storage period, indicating the preserved juice's microbiological safety and stability. Moreover, the nutritional analysis demonstrated significant improvements in the treated watermelon juice's nutritional composition, including elevated levels of carbohydrates, energy, and potassium, while maintaining essential nutrients such as protein and ash. These enhancements contribute to the overall nutritional quality of the juice, making it a healthier beverage option for consumers. Sensory evaluation results indicated that the preservation techniques maintained the watermelon juice's sensory attributes over time, with only minor variations observed in color, aroma, viscosity, sweetness, and overall acceptance.

Overall, the findings of this study highlight the potential of enzymatic treatment and microfiltration as promising approaches for enhancing the shelf life and nutritional quality of watermelon juice. These techniques offer practical solutions for the fruit juice industry to produce high-quality, nutritionally rich beverages that meet consumer preferences for freshness, flavor, and health benefits. Further research could explore alternative enzymes or enzyme combinations to enhance specific nutritional components or sensory attributes. Additionally, investigating the effects of different microfiltration membranes and operating conditions on juice quality could provide insights into further optimization possibilities. Moreover, exploring the impact of these preservation techniques on other fruit juices or juice blends could broaden their application in the beverage industry.

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