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Valorization of watermelon (*Citrullus lanatus*) rind as a substrate for vinegar production

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Abstract: *Vinegar is a widely used acidic product obtained through the aerobic oxidation of ethanol. To reduce production costs, alternative raw materials such as agricultural by-products are being explored. Watermelon rind, often discarded as waste, contains fermentable sugars and natural antimicrobial compounds, making it a potential substrate. Four treatments were formulated using watermelon rind with 0% (control), 12% sugar, 12% honey, and 12% watermelon flesh. Alcoholic fermentation proceeded for 21 days, followed by 20 days of acetic fermentation using Acetobacter aceti at 28–32°C. Key parameters including pH, Brix, ethanol content, and color were monitored. Sensory evaluation focused on aroma, acidity, aftertaste, and overall acceptability using a 9-point hedonic scale. The sugar-enriched treatment showed the highest ethanol production and strongest acidification, indicating efficient substrate conversion. It ranked highest in sensory quality. Statistical analysis (ANOVA, LSD) confirmed significant differences ($p < 0.05$), supporting the feasibility of using sugar-enriched watermelon rind in vinegar production.*

Keywords: Watermelon Rind, Alcoholic Fermentation, Acetic Fermentation, Antimicrobial Compound.

1. INTRODUCTION

Vinegar is one of the oldest known cooking ingredients and food preservation agents in the world [1]. It is composed of varying amounts of fruit acids, coloring agents, salts, and other byproducts formed during the fermentation process. Vinegar production begins with alcoholic fermentation, where yeasts convert fermentable sugars into ethanol. This is followed by acetic fermentation, during which acetic acid bacteria oxidize ethanol into acetic acid [2]. During fermentation, a complex community of microorganisms contributes to the release of numerous bioactive compounds. These substances have been shown to interact with various biochemical pathways in the body, leading to a range of health-promoting effects [3]. As a natural product rich in bioactive molecules and characterized by distinctive physicochemical properties, vinegar has long been used in both traditional medicine and culinary practices. Its significance is increasingly recognized worldwide, driven by growing awareness of its functional and therapeutic benefits [4]. Previous research studies have demonstrated the positive impacts of vinegar on various aspects of health, including blood glucose control, blood pressure regulation, cholesterol reduction, lipid metabolism regulation, antioxidant and antimicrobial effects, as well as anti-infection properties.

Vinegar is a product derived exclusively through biotechnological processes, specifically via double fermentation, which involves both alcoholic and acetic fermentation of liquids or other agricultural materials. Various types of vinegar are produced from a wide range of sources, including wine, fruits and berries, cider, alcohol, grains, malt, beer, and honey. Beyond its common use as a food additive, vinegar is also widely recognized for its preservative properties, helping to prevent food spoilage.

Extensive research has been conducted on vinegar production using diverse raw materials such as fruits, fruit peels, and other agricultural feedstocks [5]. A local study in the Philippines explored the potential of fermenting underutilized fruits for alcohol production, a key step in the vinegar-making process [6].

Among the many potential raw materials, watermelon (Citrullus lanatus) presents a promising option. Belonging to the cucurbit family (Cucurbitaceae), the name "Citrullus" is derived from the Greek word citrus, referring to the fruit, while "lanatus" describes the plant's fine-haired stems and leaves, which give it a woolly texture. The fruit is aptly named "watermelon" due to its high-water content—approximately 93%. Structurally, a watermelon is composed of about 55.3% juice, 31.5% rind, and 10.4% pomace [7] making it a viable candidate for fermentation-based applications. In the Philippines, watermelon is cultivated across approximately 6,500 hectares, with major production areas including Iloilo (27%), Pangasinan (16%), and Nueva Ecija (11%). Western Visayas stands out as one of the country's leading regions for watermelon cultivation [2].

Despite the high volume of watermelon production, a substantial amount of waste is generated, as consumers typically consume only the red fleshy part of the fruit. One of the primary waste components is the rind the white inner layer which is often discarded. Notably, watermelon rind contains a significant concentration of citrulline, an amino acid with various health benefits [8]. Watermelon rind constitutes approximately 40% of the total watermelon mass [9]. Furthermore, more than 90% of watermelon rind waste is disposed of as residue, posing environmental challenges [10]. In 2013 alone, around 36 million tons of watermelon rind were discarded into the environment. The direct disposal of rind waste contributes to environmental issues [11]. Thus, to address the environmental concerns and promote a circular economy, it is essential to find ways to utilize agricultural by-products such as watermelon rind.

In addition, watermelon rinds can undergo different processes to be transformed into value-added products. These processes include drying [12], fermentation, extraction, and enzymatic treatment. Each process offers unique opportunities to create value-added products such as watermelon rind chips, pickles [13] jams, and even bioactive compounds for pharmaceutical applications [14]. These processes help reduce waste and unlock the

potential of watermelon rind, turning it into valuable and sustainable products.

By converting waste into resources, these processes not only reduce environmental impact but also create opportunities for innovation and economic gain, unlocking the full potential of watermelon rind as a sustainable and valuable material. This study explores the potential of valorizing watermelon rind through its utilization as substrate for vinegar production.

2. MATERIALS AND METHODS

2.1 Sourcing of Watermelon samples

The watermelon (*Citrullus lanatus*) samples used in this study were freshly harvested from Gigaguit, Surigao del Norte province in the Philippines. The harvested fruits were then transported to Ipil, Gigaguit, Surigao del Norte, for further processing.

To ensure consistency and minimize variability in the results, all samples were of the same variety and exhibited uniform properties, including similar levels of ripeness and maturity. This standardization was implemented to maintain homogeneity and ensure the reliability of the experimental data.

2.2 Alcoholic Fermentation

2.2.1 Preparation of Watermelon Rind

The watermelon rind was carefully separated from the flesh and thoroughly washed with distilled water to remove any dirt or impurities. It was then cut into small pieces and blended into a smooth mixture. A total of 700 grams of the blended rind was transferred into clean, sterilized containers. These containers were sealed and stored at room temperature (approximately 28–32 °C) until further use.

2.2.2 Treatment and Yeasts Activation

To initiate the fermentation process, sugar, honey, and watermelon flesh were each added to the watermelon rind mixture at a concentration of 12% [15]. A control group, which received no additive, was also included for comparison. These concentrations ensured an adequate supply of fermentable sugars necessary for efficient ethanol production without inducing osmotic stress.

A standardized 10% yeast concentration (*Saccharomyces cerevisiae*) was applied to all samples to ensure efficient alcoholic fermentation. It supports steady sugar conversion into ethanol while avoiding issues related to excessive yeast, such as ethanol toxicity or nutrient depletion [16]. Additionally, each mixture was supplemented with 300 mL of distilled water to maintain a controlled and uniform fermentation environment, as detailed in Table 1. The fermentation process was allowed to proceed for 21 days under consistent room temperature conditions.

Table 1. Different Treatment Proportion per Sample

Treat-ment	Composition			Yeast (%)	Distilled Water (mL)
	Sugar (%)	Honey (%)	Water-melon Flesh (%)		
T0	0	0	0	10	300
T1	12	0	0	10	300
T2	0	12	0	10	300
T3	0	0	12	10	300

2.3 Acetic Fermentation

After 21 days of alcoholic fermentation, the mixture was strained to separate the liquid from solid watermelon residues. This clarified the liquid by removing pulp and other particles that could affect the next stage. The strained liquid was transferred to clean containers in preparation for acetic fermentation. The strained liquid was placed in containers covered with cloth to allow oxygen exposure, essential for acetic fermentation. *Acetobacter aceti* was introduced using Bragg's Apple Cider Vinegar with the mother as the inoculant [17]. The containers were kept under sunlight to encourage bacterial activity. Since *Acetobacter* requires oxygen to convert ethanol into acetic acid, sealing the containers was avoided to ensure proper fermentation.

2.4 Chemical Parameter Analysis

2.4.1 pH Value

The pH of watermelon rind vinegar was measured using a digital pH meter (Apera Instruments, PH800 Laboratory Benchtop pH Meter Kit). pH indicates the concentration of hydrogen ions in a solution and reflects its acidity or alkalinity.

2.4.2 Total Soluble Solid

Total Soluble Solids (TSS) content was measured using a Digital Brix and Sucrose Refractometer, calibrated with distilled water. For each reading, 1–3 drops of the vinegar sample were placed on the prism. Measurements were taken both before and after fermentation to monitor changes in sugar content.

2.4.3 Alcohol Content

Alcohol content is measured by an alcohol meter, which assesses the density of the liquid to determine ethanol concentration. The meter is placed directly into the vinegar, and readings are taken both before and after fermentation.

2.4.4 Color

The color values of watermelon rind vinegar were measured directly using a colorimeter (LS172) and the CIELAB color space system. Values for L* (lightness), a* (redness), b* (yellowness), and chroma (C) were recorded to evaluate its color properties.

2.5 Sensory Analysis

2.5.1 Sensory Parameters

Sensory evaluation was conducted based on specific parameters including appearance, aroma, acidity, flavor complexity, aftertaste, mouthfeel, and overall acceptability. Both trained and untrained panelists participated in the evaluation, offering a combination of expert insights and consumer-oriented perspectives to comprehensively assess the sensory attributes of the product.

2.5.2 Hedonic Scale

Affective testing was carried out using the 9-point hedonic scale, a widely used tool for measuring product acceptance. This scale ranges from "extremely disliked" to "extremely liked," with a neutral midpoint, allowing for balanced consumer feedback. The hedonic scale enables the quantification of consumer preferences, supports statistical analysis, and provides valuable insights for product development and optimization [18].

3. RESULTS AND DISCUSSIONS

3.1 pH

In vinegar fermentation, especially using fruit-based substrates such as watermelon rind, achieving a final pH within the 2.0–3.0 range indicates successful acidification and proper microbial fermentation [19].

Table 2. pH Values before and after fermentation

TREATMENTS	pH	
	Day 0 (Before)	Day 41 (After)
T0	5.98[b]	3.67[a]
T1	6.15[a]	2.8[d]
T2	5.84[b]	3.51[b]
T3	5.65[c]	3.33[c]

Table 2 shows the initial pH values (day 0) varied across treatments, with T1 having the highest pH (6.15), which supports the activation of glycolytic enzymes like invertase and pyruvate decarboxylase [20]. T3 had the lowest initial pH (5.65), indicating higher acidity that could inhibit enzymatic function and delay the initiation of glycolysis [21]. By Day 41, all treatments showed a significant decline in pH, indicating the accumulation of organic acids from ethanol oxidation. T1 had the largest pH decrease (from 6.15 to 2.80), suggesting efficient fermentation and high acetic acid production through ethanol oxidation by acetic acid bacteria [22]. T3, with the lowest initial pH, had the smallest reduction (from 5.65 to 4.75), indicating limited fermentation and lower metabolic activity. T0 and T2 had moderate pH decreases, with final values of 3.67 and 3.51, indicating balanced fermentation processes.

3.2 Total Soluble Solid

During fermentation, *Saccharomyces cerevisiae* converts sugars into ethanol and carbon dioxide. The process begins with the hydrolysis of sucrose into glucose and fructose by the enzyme invertase. These sugars are then metabolized through glycolysis to produce pyruvate. Pyruvate is then decarboxylated to form acetaldehyde, which is reduced to ethanol by alcohol dehydrogenase. The overall chemical reaction can be summarized as:

$$C_6H_{12}O_6 \text{ (glucose)} \rightarrow 2\ C_2H_5OH \text{ (ethanol)} + 2\ CO_2 \text{ (carbon dioxide)}$$

Table 3. Total soluble solid before and after fermentation

TREATMENTS	Total Soluble Solid	
	Day 0 (Before)	Day 41 (After)
T0	4.40[d]	1.43[c]
T1	5.63[a]	2.00[a]
T2	6.75[b]	1.10[c]
T3	7.88[c]	1.45[b]

*Treatment means with the same letter are not significantly different using Least Significant Difference Test ($p<0.05$)

Table 3 shows that T1 had the highest initial total soluble solids (TSS) at 5.63°Brix, followed by T3 (7.88°Brix), T2 (6.75°Brix), and T0 (4.40°Brix). After 41 days of fermentation, T1 retained the highest residual TSS at 2.00°Brix, indicating a relatively less efficient conversion of sugars into ethanol and organic acids. In contrast, T2 showed the greatest reduction in TSS, decreasing to 1.10°Brix, suggesting the most efficient fermentation. T3 and T0 had moderate reductions, reaching 1.45°Brix and 1.43°Brix, respectively.

The observed decrease in TSS reflects the metabolic activity of *Saccharomyces cerevisiae*, which ferments sugars into ethanol, subsequently converted to acetic acid by acetic acid bacteria. Treatments with higher initial TSS, such as T1 and T3, showed less complete fermentation, likely due to sugar overload. T2, formulated with honey containing simpler sugars, exhibited more efficient fermentation. Overall, the extent of TSS reduction appears to be influenced by the initial sugar composition and its fermentability.

3.3 Alcohol Content

Higher initial sugar concentrations significantly enhance ethanol biosynthesis during alcoholic fermentation due to their stimulatory effect on *Saccharomyces cerevisiae* metabolism. Ethanol production efficiency is determined by substrate concentration.

Table 4. Alcohol content before and after fermentation

TREATMENTS	Alcohol Content	
	Day 0 (Before)	Day 41 (After)
T0	0.00%	6.78[c]
T1	0.00%	10.45[a]
T2	0.00%	7.73[b]
T3	0.00%	6.78[c]

Table 4 shows that watermelon rind, with its low sugar content, is not naturally suitable for fermentation. On Day 0, all treatments (T0, T1, T2, T3) started with 0% alcohol, confirming the absence of alcohol before fermentation. After 21 days of alcoholic fermentation, Treatment T1, with added sugar (12%), achieved the highest alcohol content of 9.98%, followed by T3 (6.18%) and T2 (5.60%), with T0 reaching only 4.37%. The difference in alcohol content was attributed to the varying sugar sources, which directly influenced ethanol yield. During acetic fermentation (Day 22 to Day 41), ethanol was converted to acetic acid by acetic acid bacteria. On Day 41, T1 maintained the highest alcohol content (10.45%), suggesting incomplete oxidation, while T2 increased to 7.73%, and both T0 and T3 stabilized at 6.78%, indicating more efficient ethanol conversion.

3.4 Color Values

Table 5 shows the color measurements of watermelon rind vinegar before fermentation. T1 is the lightest (L* = 25.15), while T3 is the darkest (L* = 22.19). T3 also has the highest redness (A* = 3.53), while T0 and T2 are similar (A* = 1.81 and 1.79). T0 has the highest yellowness (B* = 12.14), and T3 the lowest (B* = 9.91). For overall color difference (E*), T1 has the highest value (27.75), followed by T2 (27.06), T0 (26.23), and T3 (24.55), with T3 being closest to the reference color.

Table 6 shows that T1 (12% sugar) and T2 (12% honey) produced the brightest and most visually appealing vinegar, with high L*, a*, and b* values. T3 (12% watermelon flesh) was darkest with the lowest a* and b* values due to pigment degradation and browning. T2 had the greatest total color change (ΔE = 44.77), while T3

showed the least (ΔE = 25.78), indicating early pigment loss. Sugar and honey helped preserve color by reducing browning, while watermelon flesh accelerated it, resulting in poorer visual quality.

Table 5. Color values before alcoholic fermentation

Samples	L±SD*	A±SD*	B±SD*
T0	24.35±0.06	1.81±0.05	12.14±0.13
T1	25.15±0.08	2.05±0.04	11.57±0.04
T2	24.23±0.09	1.79±0.04	11.91±0.05
T3	22.19±0.13	3.53±0.11	9.91±0.04

Table 6. Color values after acetic fermentation

Treatment	L±SD*	A±SD*	B±SD*
T0	35.10±0.03	5.47±0.05	20.17±0.13
T1	36.08±0.05	6.08±0.05	23.08±0.09
T2	35.92±0.05	5.78±0.07	26.11±0.07
T3	23.18±0.04	1.13±0.03	11.23±0.08

3.5 Sensory Evaluation

Sensory analysis is essential in product development, guiding improvements in taste, texture, and appearance based on consumer preferences. It includes descriptive testing, using trained panelists to detail sensory attributes, and affective testing, which measures consumer acceptance through tools like hedonic scales. These methods help refine products to better meet market demands [23].

3.5.1 Appearance

The appearance scores of the vinegar samples T0, T1, T2, and T3 shown in Figure 2 were derived from a panel of eight evaluators, including both trained (3) and untrained (5) individuals, providing a comprehensive view of both expert and consumer perspectives. Among the treatments, T2 (12% honey) achieved the highest mean appearance score (7.89), followed closely by T1 (12% sugar) with 7.86, and T0 (control) at 7.68. T3 (12% watermelon flesh) recorded the lowest score of 7.60.

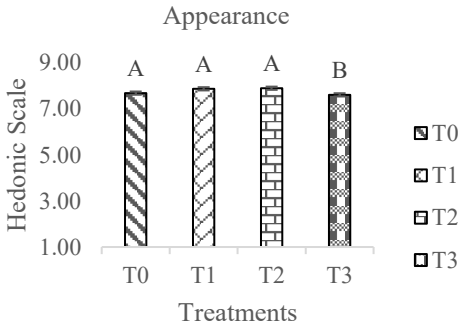

Figure 2. Sensory analysis result for appearance.

T2 and T1 produced vinegar with superior visual attributes, likely due to higher clarity and brightness, as reflected by their L* values in the CIELAB color system. The lower appearance score for T3 corresponds with its significantly reduced L* value (23.18), indicative of a darker product caused by browning reactions and pigment degradation during fermentation. This darker coloration may have negatively influenced visual appeal. The technical implication is that additives such as sugar and honey contribute to better preservation of visual quality, while watermelon flesh may enhance enzymatic and non-enzymatic browning, reducing appearance acceptability.

3.5.2 Aroma

The aroma scores of the vinegar samples shown in Figure 3, based on evaluations T2 (12% honey) achieved the highest aroma score at 5.19, suggesting enhanced aromatic appeal, likely due to the volatile compounds and natural fragrances present in honey. T3 (12% watermelon flesh) followed with a score of 4.76, which may be attributed to residual fruit notes contributing positively to the aroma profile.

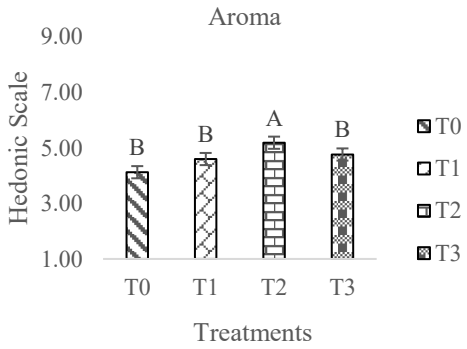

Figure 3. Sensory analysis result for aroma.

T1 (12% sugar) had a moderate score of 4.60, indicating acceptable aroma but lacking the complexity introduced by honey or fruit components. T0 (control) received the lowest score at 4.13, reflecting a more neutral or less pleasant aroma, possibly due to the absence of additive-derived volatiles.

3.5.3 Acidity

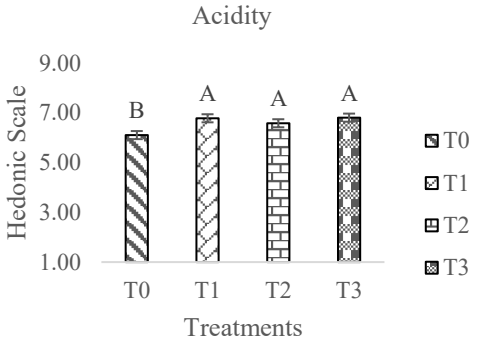

Figure 4. Sensory analysis results for acidity.

Acidity ratings shown in Figure 4 as evaluated by a panel, reflect the perceived sourness and balance of the vinegar samples post-fermentation. T3 (12% watermelon flesh) recorded the highest acidity score at 6.80, closely followed by T1 (12% sugar) at 6.78, indicating a strong but well-accepted acidic profile. Both watermelon flesh and sugar facilitated effective acetic acid production during fermentation, enhancing the vinegar's sourness and contributing positively to its sensory acceptance.

T2 (12% honey) also performed well, scoring 6.58. The slightly lower rating compared to T1 and T3 may be due to honey's inherent sweetness, which can mask or mellow acidity perception. The control sample, T0, received the lowest acidity rating of 6.10, indicating a milder acidic taste that might be perceived as less flavorful or less characteristic of typical vinegar.

3.5.4 Flavor Complexity

Figure 5 shows that flavor complexity refers to the depth, balance, and richness of taste perceived in the vinegar samples. Among the treatments, T1 (12% sugar) scored the highest at 6.74, indicating a more layered and appealing flavor profile. Sugar does not only support efficient fermentation but also enhance the development of desirable flavor compounds.

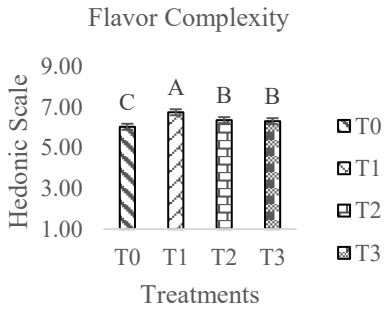

Figure 5. Sensory analysis results in flavor complexity.

Treatment 2 (12% honey) and Treatment 3 (12% watermelon flesh) followed with close scores of 6.35 and 6.30. Honey contributed additional aromatic and sweet notes, while watermelon flesh may have introduced fruity undertones. However, these did not surpass the flavor complexity provided by sugar. The control sample, T0, received the lowest score at 6.03, indicating a simpler flavor profile, possibly due to the absence of added fermentable sugars or flavor-enhancing compounds. Sugar as an additive promoted a more complex and favorable flavor profile in the vinegar product.

3.5.5 Aftertaste

Figure 6 shows that aftertaste, the lingering flavor sensation following ingestion, varied notably among treatments. T0 (control) received the highest score of 6.81, suggesting a clean and pleasant residual taste despite lacking added sweeteners. T2 (12% honey) followed with a score of 6.57, indicating a moderately favorable aftertaste likely enhanced by honey's natural aromatic compounds. T1 (12% sugar) scored slightly lower at 6.40, suggesting a less distinct or shorter-lasting aftertaste compared to the control and honey-based samples.

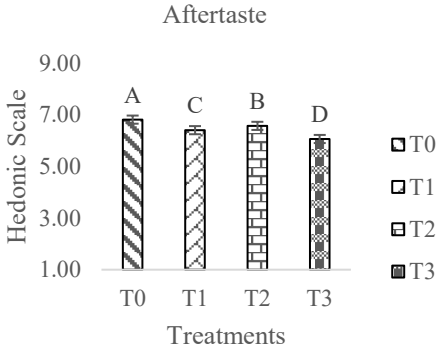

Figure 6. Sensory analysis result for aftertaste.

T3 (12% watermelon flesh) had the lowest score at 6.06, potentially due to residual off-flavors or increased browning compounds resulting from fruit degradation during fermentation.

3.5.6 Mouthfeel

Figure 7 shows the result for mouthfeel which encompasses the tactile sensations experienced in the mouth, showed distinct variation across treatments. T3 (12% watermelon flesh) had the highest score at 6.78, suggesting a fuller or smoother texture, potentially due to natural pectins and fibers present in the fruit.

Treatment 1 (12% sugar) and Treatment 2 (12% honey) followed with scores of 6.71 and 6.58, respectively. The added sweeteners likely contributed to a more rounded and viscous mouthfeel compared to the control. Honey may have slightly enhanced mouth-coating properties due to its complex sugar and antioxidant content. T0 (control) received the lowest score of 6.11, indicating a thinner or less developed mouthfeel, which is expected in the absence of texture-enhancing additives.

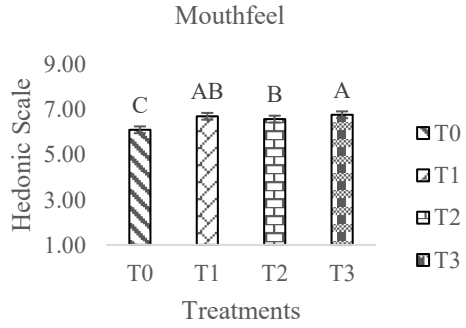

Figure 7. Sensory analysis results for mouthfeel.

3.5.7 Overall Acceptability

Figure 8 shows the overall acceptability of watermelon rind vinegar varied across treatments, influenced by multiple sensory factors including aroma, acidity, flavor complexity, aftertaste, and mouthfeel. Treatment 1 (12% sugar) received the highest score of 7.34, indicating a strong positive response from evaluators. Its balanced taste, enhanced sweetness, and smoother mouthfeel, which improved the overall sensory experience.

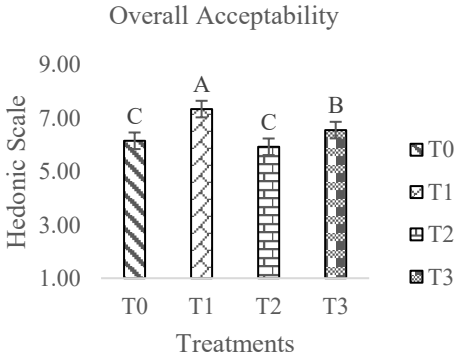

Figure 8. Sensory analysis results for overall acceptability.

4. CONCLUSION

Watermelon rind was utilized as a substrate for vinegar production, assessing the impact of four treatments control (no additive), sugar, honey, and watermelon flesh

on the fermentation process. The sugar-enriched treatment exhibited the highest ethanol production and acetic acid concentration during fermentation, along with optimal physicochemical parameters such as total soluble solids, pH, and color. Sensory analysis indicated superior acceptability in terms of aroma, acidity, aftertaste, and overall quality. The control treatment, relying on the natural sugars of the rind, also yielded vinegar with favorable physicochemical properties, demonstrating the inherent fermentation potential of watermelon rind. Statistical analysis (ANOVA and LSD test) confirmed significant differences across treatments. The sugar-based formulation showed the highest commercial viability for vinegar production.

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