Article

Dynamic Changes in Physicochemical and Microbiological Qualities of Coconut Water during Postharvest Storage under Different Conditions

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Abstract: Coconut is naturally sealed with coconut water inside. Microbial contamination occurs only after the seal is broken during extraction. This study evaluated changes in the microbiological, physicochemical, and chemical properties of coconut water during postharvest storage at ambient and refrigerated temperatures. Initial microbial counts ranged from 2 to 5 log CFU/mL, while physicochemical factors, including total soluble solids (TSs), pH, and sugar content (4–7 °Brix, 5.0–5.5, 4–6% g/100 mL), were consistent. The dynamic changes in the physicochemical properties of coconut water stored under both conditions exhibited a clear correlation with the increased microbial populations. Fructose was the primary sugar, with citric and malic acids as major acids, while the predominant volatile compounds were ethanol, ethyl acetate, ethyl ester, acetic acid and octanoic acid. Storage conditions led to similar microbial and physicochemical changes, but ambient temperature accelerated spoilage 10 times faster than refrigeration. Sucrose decreased steadily, whereas fructose and glucose remained stable until a precipitous decline coincided with lactic acid bacteria (LAB) reaching >6 log CFU/mL on the final day of storage. Weissella cibaria and Leuconostoc spp. are the main species in coconut water. The presence of specific volatile compounds, including octanoic acid, acetic acid, ethyl acetate, and butyl phenol, is associated with the activities of Lactobacillus, particularly Weissella. There was a clear relationship among microbial groups and populations, total titratable acidity (TTA), and sensory criteria. Remarkably, TTA was closely correlated with total plate count (TPC) (>5 log CFU/mL) and an unacceptable sensory rating.

Keywords: fresh coconut water; microbial; physicochemical quality; spoilage indication

1. Introduction

Coconut water, commonly found in tropical countries, is one of the most popular low-calorie beverages, with a delicate, mildly sweet flavor and a unique fresh essence, especially when freshly extracted from immature fruits. Consumer demand for coconut water in the form of natural therapeutic refreshment and sport drinks has drastically broadened the market opportunities of the product. The World Health Organization (WHO) recommends drinking coconut water as a rehydration remedy in cases of acute diarrhea. As a result, this beverage has recently been described as a sport beverage and
has piqued the interest of manufacturers as a natural functional drink and a natural isotonic [1]. The global market value of coconut water was projected to reach a compound annual growth rate of 17.12% (12.13 billion US dollars) during 2021–2026.

Coconut is naturally hermetically sealed with clear coconut water inside. Microbial contamination occurs only after the natural seal is broken during extraction. The major constituent of coconut water, i.e., sugar, provides suitable conditions and nutrients for microbial growth, while minor constituents, including vitamins and minerals, play different roles in the metabolisms of pathogenic and spoilage microorganisms. Characteristics of low pH (4.6–5.6) with high water activity (approximately 0.99) support rapid growth of microbes. Hence, proper storage of the water is critical for minimizing the effect of microbial spoilage on the quality of the final product. Bacteria and yeasts are the main microbes found in fresh coconut water. Elevated temperature during storage causes rapid multiplication of microorganisms and product spoilage. Therefore, coconut water should be stored at 0 to 4 °C and kept away from direct sunlight during storage and transportation to prolong its shelf life and product quality [2,3]. Ensuring the safety and suitability for consumption of coconut water without changing its nutritional value or sensory quality is a challenge.

The chemical composition [2,4,5], and physicochemical properties of coconut water under various processing and storage conditions [6] have been widely studied. Coconut water consists of about 96% water and 6% soluble solids, including sugars, proteins, and minerals. The composition highly depends on the coconut variety and its maturity stage [2]. For example, sugar, protein, fat, and mineral content is 3.42–5.00%, 0.12–0.52%, 0.07–0.15%, and 0.47–0.87%, respectively [4]. Hence, coconut water is very susceptible to spoilage, which is a major concern during production and handling. Improper preharvest and postharvest practices, such as inappropriate storage conditions, can adversely affect the quality of coconut water.

Reports of illness due to coconut water are rare, and none has been attributed to chemical contamination. A primary concern is contamination by microorganisms that enter coconut water as a result of improper postharvest handling and processing. Only coconuts at an appropriate developmental stage (9 months) that are sound and free of cracks should be used for bottling of coconut water [3]. If certain minimum requirements are not met, the coconut should be rejected. Rolle (2007) recommended that coconuts be washed with potable water to remove soil and reduce the risk of cross-contamination during the process of obtaining coconut water [3]. Furthermore, the washed coconuts should be soaked in a dilute bleach solution for about 15 min to further reduce microbial numbers on the surface. Reducing the initial microbial load on the coconut surface is crucial, since any contaminants on the surface may come into contact with coconut water when the fruit is cut open. After obtaining the coconut water, it is normally filtered instead of heat-treated in order to preserve the natural flavor and aroma. For small-scale processors, a coarse filter followed by a finer filter is generally used. The filtered coconut water is then cooled to 4 °C before bottling.

In the manufacture of coconut beverages, the quality of fresh coconut water prior to the production process is the key to obtain a product with high quality. The quality of fresh coconut water or incoming raw materials is inspected based on acceptance criteria using visual inspection and/or other sensory evaluations by quality assurance personnel. The screening method used in raw material inspection is based on the experience of personnel and subject to different variables. With several factors involved during the sensory evaluation, the result may not represent the quality of the lot and sometimes causes major inconsistency in the final product. To reduce the risk of microbial spoilage before processing, an effective and simple inspection method should be applied. The obtained results should sufficiently indicate the presence of microorganisms and/or their activity levels, e.g., physicochemical properties and some metabolite profiles. Ready-to-drink coconut water should not contain more than 1 × 10⁴ CFU/mL of total viable count, while Staphylococcus aureus should not be detected in 1 mL. Additionally, Escherichia coli as well as
yeast and mold (YM) should not be over 2.2 MPN/100 mL and 100 CFU/mL, respectively. The physicochemical and microbiological properties of fresh coconut water should be inspected, and the quality should be maintained prior to manufacturing on a daily basis. Frequent monitoring could help increase the quality control efficiency and reduce the loss of an entire batch due to quality defects in raw material. This problem is frequently found in the coconut water industry.

The physicochemical properties of fruit juices can be used to assess their freshness, purity, and nutritional content. The pH, titratable acidity (TTA) and total soluble solids (TSSs) are generally used. pH and TTA that deal with acidity of a fruit juice can be used to determine microbial spoilage, and TSS content can be used to determine the sweetness of a juice [7,8]. TSSs play an important role in shelf-life studies, particularly in relation to the quality and stability of food and beverage products. TSSs primarily consist of sugars, organic acids, and other soluble components that contribute to the flavor and perceived sweetness of the juice. In shelf-life studies, monitoring TSSs helps determine the optimal range that maintains the desired flavor and sensory attributes throughout the juice’s shelf life. pH is a critical factor in shelf-life studies of various products, particularly those susceptible to microbial spoilage. By studying the impact of pH on chemical stability, shelf-life studies can identify pH ranges that ensure the preservation of desired compound characteristics. The decrease in pH can be attributed to various factors, including microbial activity, enzymatic reactions, chemical reactions, and carbon dioxide dissolution. Coconut water, despite undergoing preservation techniques, may still contain residual microorganisms that metabolize the nutrients in the beverage, leading to the production of organic acids. Titratable acidity is another key physicochemical property of fruit juices that deals with acidity. While pH is important to assess the ability of a microorganism to grow in a specific food, titratable acidity is a better predictor than pH of how organic acids in the beverage impact quality and/or flavor [9]. Color is another primary attribute that consumers use to assess the freshness and quality of fruit juices. In shelf-life studies, monitoring color changes helps evaluate the visual quality of fruit juices over time and determine the shelf life based on acceptable color standards.

The objectives of this study were to evaluate spoilage indicators of fresh coconut water through investigation of microbial communities, physicochemical properties and volatile metabolite profiles associated with deterioration. The data were also compared to the screening methods of fresh coconut water conducted by the manufacturers, in order to find correlations and propose a potential new indication for inspecting the quality of raw materials.

2. Materials and Methods

2.1. Fresh Coconut Water Collection and Storage Conditions

Six samples of fresh coconut water (5 L for each sample) were used in the experiment. Three samples were collected using a manual and aseptic preparation technique of cutting the fruit open and the other three samples were from three different industrial manufacturers, including one young coconut water sample with manual cut to open (L1), two mature coconut water samples with manual cut to open (L2 and L3), as well as three supplied coconut water samples (F1, F2 and F3) as showed in Table 1. A total of 150 mL from each sample was transferred into two sets of 250 mL aseptic flasks. The samples were stored (i) under 4 °C and (ii) around ambient temperature (25 to 32 °C). Microbiological, physicochemical, chemical (sugar and organic acids) and metabolite volatile profiles of coconut water stored under 4 °C were collected for analysis on days 0, 1, 2, 3 and 4 of the storage, while samples stored at ambient temperature were collected after 0, 2 and 5 hours of storage. Each new flask was taken to analyze, and each analysis performed had replicates undertaken.
### Table 1. Bacterial communities by culture-independent methods observed in fresh and spoiled coconut water under refrigerated and ambient temperatures.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial Microbes</th>
<th>Temperature</th>
<th>Main Microbes at the End of Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young coconut water (street food-type preparation)</td>
<td>Weissella cibaria</td>
<td>AT (5 h)</td>
<td>Weissella cibaria</td>
</tr>
<tr>
<td>L1</td>
<td>Weissella spp.</td>
<td></td>
<td>Weissella spp.</td>
</tr>
<tr>
<td></td>
<td>Leuconostoc spp.</td>
<td></td>
<td>Leuconostoc spp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 °C (4 days)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weissella cibaria</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weissella spp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leuconostoc spp.</td>
</tr>
<tr>
<td>Mature coconut water (street food-type preparation)</td>
<td>Weissella cibaria</td>
<td>AT (5 h)</td>
<td>Weissella cibaria</td>
</tr>
<tr>
<td>L2</td>
<td>Weissella spp.</td>
<td></td>
<td>Weissella spp.</td>
</tr>
<tr>
<td></td>
<td>Leuconostoc spp.</td>
<td></td>
<td>Leuconostoc spp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 °C (4 days)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weissella cibaria</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weissella spp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leuconostoc spp.</td>
</tr>
<tr>
<td>* with low-intensity band</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mature coconut water (street food-type preparation)</td>
<td>Weissella cibaria</td>
<td>AT (5 h)</td>
<td>Weissella cibaria</td>
</tr>
<tr>
<td>L3</td>
<td>Weissella spp.</td>
<td></td>
<td>Weissella spp.</td>
</tr>
<tr>
<td></td>
<td>Klebsiella pneumoniae</td>
<td></td>
<td>Leuconostoc spp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 °C (4 days)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weissella cibaria</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weissella spp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leuconostoc spp.</td>
</tr>
<tr>
<td>Industrial supplied coconut water F1</td>
<td>Weissella cibaria</td>
<td>AT (5 h)</td>
<td>Weissella spp.</td>
</tr>
<tr>
<td></td>
<td>Weissella spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leuconostoc spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Klebsiella pneumoniae</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 °C (1 day)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weissella spp.</td>
</tr>
<tr>
<td>Industrial supplied coconut water F2</td>
<td>Weissella cibaria</td>
<td>AT (5 h)</td>
<td>Weissella cibaria</td>
</tr>
<tr>
<td></td>
<td>Weissella spp.</td>
<td></td>
<td>Weissella spp.</td>
</tr>
<tr>
<td></td>
<td>Leuconostoc spp.</td>
<td></td>
<td>Leuconostoc spp.</td>
</tr>
<tr>
<td></td>
<td>Escherichia coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 °C (4 days)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weissella cibaria</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Weissella spp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Leuconostoc spp.</td>
</tr>
<tr>
<td>Industrial supplied coconut water F3</td>
<td>Weissella cibaria</td>
<td>AT (5 h)</td>
<td>Weissella spp.</td>
</tr>
<tr>
<td></td>
<td>Weissella spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leuconostoc spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Klebsiella pneumoniae</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 °C (4 days)</td>
<td></td>
</tr>
</tbody>
</table>

2.2. Microbial Profile and Population Determination

Microbial ecology was determined by both traditional and culture-independent methods. Traditional plating was conducted for total viable count and yeast and mold count using Compact Dry TC and Compact Dry YM, respectively. LAB was determined using de Man, Rogosa and Sharpe (MRS) agar (Himedia, India) at 35 ± 2 °C for 48 h. The representative isolates were then identified by DNA-sequencing analysis. The nucleic acid sequences of bacteria were chosen from the conserved regions of 16S rRNA V3. Polymerase chain reaction (PCR) was performed using primer set 338 F/518 R [10]. Yeast isolates were from the D1/D2 domain of the 26S rDNA with the forward primers NL1/LS2, as described by Cocolin et al. (2000) [11].
For the culture-independent method, nested PCR–denaturing gradient gel electrophoresis (n-PCR-DGGE) was used. The bacterial RNA was directly extracted from samples and purified using a HyReverse™ Genomic DNA mini kit (RBC, Taiwan). Nested PCR started with the first universal bacterial primer set of 27 F and 1492 R [12], followed by the second nested PCR primer set targeting the 16S rRNA V3 region and 357 F with GC clamp attached and 517 R [13] for DGGE analysis. Amplification was achieved using Taq DNA polymerase (Vivantis, Selangor, Malaysia) with a DNA thermal cycler (BioRad T100 TM, Jurong East, Singapore). DGGE analysis was performed following electrophoresis as per Chahorm and Prakitchaiwattana (2017) [14]. DNA on DGGE bands was extracted and reamplified using primer set 357 F/517 R [10]. The PCR amplicon was sent to a commercial sequencing facility (Macrogen, Seoul, Korea) after cleaning, and derived sequencing data were analyzed by BLAST program of NCBI.

2.3. Physicochemical and Compound Analysis

Total soluble solid (°Brix) in coconut water was measured by a hand refractometer (Atago, Master-a, Bellevue, WA, USA), while a pH meter (Mettler Toledo, EFP20 FiveEasy Plus™, Greifensee, Switzerland) was used to determine pH value. Titratable acidity (TTA) was analyzed according to AOAC standards [15]. CIELAB color space values (L*, a*, b*) were determined by a CIE color system using a Chroma Meter (Minolta CR 400, Konica Inc., Tokyo, Japan) in order to evaluate the browning index. Turbidity (degree of light scattering by particles) was detected at a wavelength of 610 nm using a UV spectrophotometer (Thermo Spectronic, GENESYS 10 UV, Waltham, MA, USA) according to the method described by Tan et al. (2014) [16]. Furthermore, total nitrogen in coconut water was determined by the standard Kjeldahl method, and reducing sugar was quantitatively analyzed by the Nelson–Somogyi method [17].

For analysis of ethanol, sugars and organic acid content by high-performance liquid chromatography (HPLC) (Waters™ 717 plus Autosampler with Waters™ 600 Controller, Waters Associates Inc., Milford, MA, USA), the coconut water samples were prepared by filtration using a 0.45 μm syringe filter (Micro Analytix Pty. Ltd., Caringbah, NSW, Australia), and 1 mL of the filtrates was transferred to a vial for injection. The samples were then analyzed using an HPX-87 H 300 7.8 mm ion exclusion column (Bio-Rad, Hercules, CA, USA) under conditions described by Chanpratsartskul et al. (2010) [18]. Ethanol and sugars were detected by a Waters 2414 refractive index detector, while a Waters™ 996 photodiode array detector was used for organic acid detection (Waters Associates Inc., Milford, MA, USA). The data were analyzed by the Millennium software program.

2.4. Analysis of Volatile Metabolites Using Headspace SPME-GC/MS

Metabolite profiles in term of volatile compounds were detected by GC/MS following a protocol used in our previous work [19]. SPME fiber (50/30 μm DVB/CAR/PDMS, Supelco, Bellefonte, PA) was used to extract volatile compounds, while detection was achieved by gas chromatography–mass spectrometry (Agilent 7890 A GC-7000 Mass Triple Quad) equipped with a capillary column (DB-WAX, 60 m × 0.25 mm × 0.25 μm, J&W Scientific, Santa Clara, CA, USA) and a quadrupole mass detector. The analysis software was Agilent Mass Hunter Qualitative Analysis B.04.00 prior to volatile compound identification by comparison-derived mass spectra with NIST mass spectral libraries (National Institute of Standards, 2011 version). The quantity of volatile compounds was calculated from the peak area.
2.5. Comparison between Sensory Criteria and Laboratory Indexes for Fresh Coconut Water Quality

The coconut water samples were graded using sensory criteria of the coconut beverage manufacturer. Coconut water was graded based on visual and odor inspection by experienced inspectors. Fresh coconut water quality was divided into 6 levels of 5, 4, 3, 2, 1 and 0, which represented very good, good, fair, acceptable 1, acceptable 2, and rejected, respectively. A detailed description of sensory quality attributes (sense-based spoilage) for each grade can be found in Table 2. The correlations among the sensory criteria and physicochemical characteristics from laboratory analysis were analyzed and compared.

<table>
<thead>
<tr>
<th>TPC</th>
<th>Turbidity</th>
<th>TTA</th>
<th>LAB</th>
<th>Turbidity</th>
<th>TTA</th>
<th>YM</th>
<th>Turbidity</th>
<th>TTA</th>
<th>Grade Range</th>
<th>Sense Based Spoilage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.97±0.29 a</td>
<td>19.91±3.08 b</td>
<td>0.08±0.02 c</td>
<td>2.50±0.64 d</td>
<td>19.91±3.08 e</td>
<td>0.07±0.01 f</td>
<td>2.33±0.15 g</td>
<td>20.24±3.45 h</td>
<td>0.07±0.00 i</td>
<td>2–5</td>
<td>Clear to slightly turbid and coconut-like aroma</td>
</tr>
<tr>
<td>4.04±0.27 d</td>
<td>12.87±7.71 b</td>
<td>0.08±0.01 e</td>
<td>0.08±0.50 f</td>
<td>5.05±0.28 g</td>
<td>0.08±0.03 h</td>
<td>3.18±0.25 i</td>
<td>23.50±3.12 j</td>
<td>0.08±0.02 k</td>
<td>2–5</td>
<td>Slightly turbid and less coconut-like aroma</td>
</tr>
<tr>
<td>5.09±0.25 c</td>
<td>14.62±10.20 b</td>
<td>0.09±0.03 e</td>
<td>5.01±0.28 f</td>
<td>12.89±6.85 g</td>
<td>0.08±0.02 h</td>
<td>3.98±0.21 i</td>
<td>5.07±0.26 j</td>
<td>0.17±0.09 k</td>
<td>2</td>
<td>Turbid and ferment odor</td>
</tr>
<tr>
<td>5.72±0.15 b</td>
<td>11.14±6.93 b</td>
<td>0.11±0.04 e</td>
<td>5.94±0.29 f</td>
<td>13.71±8.95 g</td>
<td>0.09±0.02 h</td>
<td>5.17±0.28 j</td>
<td>11.44±8.37 k</td>
<td>0.11±0.04 l</td>
<td>0</td>
<td>Turbid and ferment odor</td>
</tr>
<tr>
<td>7.81±0.55 b</td>
<td>58.66±26.50 b</td>
<td>0.21±0.10 e</td>
<td>7.78±0.57 f</td>
<td>41.44±27.37 g</td>
<td>0.11±0.04 h</td>
<td>5.86±0.25 i</td>
<td>32.98±32.98 j</td>
<td>0.15±0.10 k</td>
<td>0</td>
<td>Turbid and ferment odor</td>
</tr>
<tr>
<td>8.83±0.00 e</td>
<td>82.58±0.00 e</td>
<td>0.34±0.00 e</td>
<td>9.25±0.3 e</td>
<td>70.85±23.94 e</td>
<td>0.29±0.08 e</td>
<td>7.94±0.00 e</td>
<td>82.58±0.00 e</td>
<td>0.34±0.00 e</td>
<td>0</td>
<td>Turbid and ferment odor</td>
</tr>
</tbody>
</table>

* Different superscript letters in the same column indicate significant differences at the level of 0.05 (n = 3).

2.6. Statistics

All statistical analyses were carried out in triplicate using the statistical package XLSTAT™ Trial statistical software for Windows. Significant difference at a p-value of less than 0.05 was analyzed by one-way analysis of variance, followed by Tukey’s test to compare means. Principal component analysis (PCA) was performed on the data of microbes and their metabolites, along with key physicochemical properties. It was used to derive the first 24 principal components from those normalized datasets, including acid and the 10 most abundant volatile compounds, in order to examine the relationship among variables from various samples. The web-based tool ClustVis was used for the PCA. The principal components were calculated using the singular value decomposition method with imputation (https://biit.cs.ut.ee/clustvis/, accessed on 9 August 2023) [20].

3. Results and Discussion

3.1. Changes in Microbial and Physicochemical Properties of Fresh Coconut Water under Different Storage Conditions

Microbial and chemical properties of fresh coconut water from various preparation techniques under different storage conditions were monitored to evaluate spoilage characteristics. Samples from young (L1) and mature coconut (L2, L3) were obtained from manual and aseptic preparation of cutting the fruit open and industrial production (F1, F2 and F3). They were subjected to microbial and chemical analyses during storage under refrigerated (Figure 1) and ambient (Figure 2) temperatures. Results in Figures 1 and 2 show that initial microbial populations of samples including TPC, LAB and YM ranged from 2 to 5 log CFU/mL, except for F1 from an industrial manufacturer containing initial populations up to 8 log CFU/mL. The parameters used to test the microbiological quality of coconut water were TPC (4.98–6.00 log CFU/mL) and YM (2.45–4.06 log CFU/mL). It was previously reported that traditional and manual extraction of coconut water may
introduce microbial contamination up to 6.0 log CFU/mL, which may include both spoilage- and disease-causing microorganisms [21]. Thus, based on the parameters used, the values demonstrated that microbiological quality of coconut water industrially supplied in this study were acceptable. The microbial profile and population of coconut water obtained from all conditions at both storage temperatures were relatively similar, as shown in Figure S1 and Table 1.

Figure 1. The microbial and chemical properties of fresh coconut water from various preparation conditions and under refrigerated storage conditions: (a) young, L1; (b) mature, L2; (c) mature, L3; (d) industrial production, F1; (e) industrial production, F2; (f) industrial production, F3.
Figure 2. The microbial and chemical properties of fresh coconut water from various preparation conditions and under ambient conditions: (a) young, L1; (b) mature, L2; (c) mature, L3; (d) industrial production, F1; (e) industrial production, F2; (f) industrial production, F3.
Initially, TSSs, pH, and sugar content of all samples were 4–7 °Brix, 5.0–5.5, and 4–6% g/100 mL (HPLC analysis, except for F1, containing 3% g/100 mL; Figure 3), respectively, while TTA was close to 0.00 (Figures 1 and 2). Under refrigerated conditions, significant change was not observed in microbial populations or physicochemical profiles during the first 3 days (Figure 1). However, pH was lower than 5 and the TTA of all samples was relatively higher on the fourth day of storage. In line with the change in pH and TTA, the LAB number significantly increased from 2–5 log CFU/mL to 6–9 log CFU/mL, compared to those of TPC and YM. The change in physicochemical properties, particularly pH, primarily demonstrated the spoilage of coconut water, since the normal pH range of the product is 5.0–5.4. When pH was less than 5, it indicated potential or likely spoilage of coconut water [22]. In addition, a change was found in another physicochemical property, i.e., turbidity, which was associated with an increase in LAB. Depending on the stage of maturity, the turbidity of mature samples significantly rose when the LAB population reached >6 log CFU/mL, while in young samples, this relation was not observed. Similarly, a previous report demonstrated that endogenous and microbial enzymatic activity substantially contributed to the spoilage of coconut water. Turbidity was greatly affected by the bacterial activity, particularly LAB, TPC and TTA of the coconut water [2].

Coconut water samples stored under ambient temperatures (25–32 °C) were subjected to analysis every two hours. According to Figure 2, the changes in microbial and physicochemical profiles were relatively similar to the refrigerated condition, but deteriorations were observed in a much shorter time span. During the first two hours, significant change was not observed in LAB, TPC or YM, but rapid growth was observed after two hours. LAB, in particular, had reached >6 log CFU/mL by 5 hours. Changes in physicochemical properties were also similar to samples stored at low temperature, but the changes occurred at a much faster rate. Similarly, turbidity of all samples significantly changed when LAB reached >6 log CFU/mL. Interestingly, the physicochemical and microbial properties of samples from young coconut (Figures 1 and 2) under both storage conditions were not different from those of the mature samples.

The dynamic changes in the physicochemical properties of coconut water stored under both conditions, especially the alteration rates, exhibited a clear correlation with the increased microbial populations, particularly the growth in lactic acid bacteria (LAB).
Thus, identification of the key microbes with both culturable and non-culturable techniques and their volatile metabolites (headspace SPME-GC/MS), as well as analysis of ethanol, sugar and organic acid content (HPLC) in storage coconut water, were conducted. These analyses provided comprehensive information to better understand the key metabolites associated with spoilage characteristics, as well as key microbes and their roles.

In this study, rapid growth in LAB in the other groups of microbes was consistently observed in all coconut water samples in relation to the significant change in spoilage indicators, including pH, TTA, TSSs and turbidity. Thus, LAB could be the key microbe associated with coconut water deterioration. Representative colonies from all cultural plates were statistically selected and further identified by sequencing analysis. It was found that microbes in all samples during both storage conditions were relatively similar. For LAB, Lactobacillus fermentum was found in all samples. For TPC, Klebsiella pneumoniae, Staphylococcus arlettae, Kluyvera cryocrescens, Proteobacteria, Enterobacter spp. and Enterococcus faecium were found in different samples (Table S1), while Candida tropicalis was primarily found in all samples from YM.

 Communities of microorganisms associated with coconut water deterioration were also investigated by rRNA analysis. Results from rRNA in Table 1 indicate that Weissella cibaria, Weissella spp. and Leuconostoc spp. of the LAB group were prevalent throughout storage times in all samples. Even though coconut samples were collected from different sources and stored in different conditions, similar profiles of bacterial expression were observed. It was noted that the presence of this bacterial group was not influenced by manufacturing practices or geographical conditions.

From this investigation, the LAB group of Leuconostoc spp., particularly W. cibaria and Weissella spp., being the key bacteria associated with deterioration of fresh coconut water was well supported. Deterioration characteristics of coconut water, such as becoming more viscous and turbid were found, could be a result of these Leuconostoc proliferations. W. cibaria and Weissella spp. have also been reported as potential probiotics that could produce bacteriocins with inhibitory effects on Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhimurium, Vibrio cholera, Pseudomonas aeruginosa and Shigella spp. [23,24]. Through rRNA, pathogens such as K. pneumoniae, Escherichia coli and Erwinia spp. were initially observed in the samples from industrial manufacturers (F1, F2, F3), but their expression was not found at any temperature towards the end of storage. This could be due to an inhibitory effect of the secondary metabolites of W. cibaria and/or Weissella spp. on the other microbial groups (Table 1).

LAB grows by converting single sugar molecules to lactic acid and other metabolites. Coconut water contains a low amount of sugar with low acidity, which supports the rapid growth in LAB and suppresses the growth of yeast and the other microorganisms. In addition, LAB requires a small amount of sugar to proliferate and dominate the system. In this case, TSSs of coconut water after deterioration by LAB would not be significantly changed. Thus, TSSs might not be suitable as a freshness indicator for coconut water.

Furthermore, results obtained from the samples at different maturity stages were unlikely associated with the deterioration rate. In this study, the browning index of the samples under both storage conditions was also monitored. Unlike the other physicochemical properties, inconsistency in browning index data was observed among samples. However, from our preliminary study, the data demonstrated that the browning index of samples under refrigeration was lower than that at ambient temperature. This may suggest that low temperature can help reduce browning reactions in coconut water. The enzymatic browning that occurred during processing and storage could result in undesirable changes in the color, flavor, texture, and nutritional content of coconut water. Polyphenol oxidase (PPO) and peroxidase (POD) are responsible for enzymatic browning, which is a common process in fruit juice and can have a significant impact on the quality and visual appeal of the product [25,26]. Polyphenols are a class of bioactive substances that are abundantly present in various plant sources, including coconuts. These compounds have
a tendency to undergo oxidative reactions upon exposure to atmospheric oxygen. Peroxidase, an enzyme present in coconut water, catalyzes this oxidation reaction.

When growth kinetics of LAB were evaluated, the slopes of a linear graph of the initial population to $>6$ log CFU/mL from the samples stored at 4 °C and ambient temperature were 0.425 and 0.523, respectively. From our preliminary study, the rates of increase in the microbial population under 4 °C and room temperature were 0.025 and 0.251 log CFU/hour, respectively. This demonstrated that the LAB could grow at room temperature up to 10 times faster than that at refrigeration temperature. The TTA and turbidity were significantly changed and were able to be quantified when the LAB population reached $>6$ log CFU/mL.

LAB was found to be the main microbe responsible for deterioration, which was mostly affected by storage temperature and time. Also, TTA and turbidity were likely to be the potential indicators of the freshness of coconut water. To verify the deterioration characteristics of fresh coconut water, chemical composition (sugars and organic acids), volatile metabolite compounds, and organoleptic properties were further investigated.

3.2. Changes in Sugars, Organic Acids and Volatile Metabolite Compounds under Different Storage Conditions

Based on HPLC analysis (Figure 3), total sugars in all fresh samples ranged from 4% to 6% (g/100 mL), which corresponded to TSS values. In coconut water, mainly fructose was found, followed by glucose and sucrose. Under refrigeration, total sugar in all samples had significantly reduced by day 3 of storage. Fructose and glucose did not show any significant change until day 3, but drastically reduced on the last day of storage, where LAB reached $>6$ log CFU/mL. Interestingly, sucrose, a minor sugar in coconut water compared to the others, changed throughout storage. This change was found in all samples and corresponded with the key metabolic characteristics of W. cibaria, the prevalent bacterium from rRNA results. For growth of W. cibaria, it was reported that sucrose was preferred along with the activation of exopolysaccharide (EPS) rather than glucose and lactose. The EPS surface property of W. cibaria, as induced by sucrose, would be more hydrophobic with an inhibitory effect on the other bacteria. The metabolic compounds of the bacterium were lactic acid, acetic acid and ethanol [24]. This evidence supported that W. cibaria could be the key microbe associated with the deterioration in fresh coconut water and responsible for increased turbidity, viscosity, and “off” odors.

Organic acid changes in all samples are shown in Figure 4. The amount of the main acids, including citric and malic, was relatively stable under all storage conditions. Lactic and acetic acids were increased throughout the storage period of both conditions. In particular, lactic acid was significantly increased and responsible for changes in TTA in coconut water during storage. It was also found that an increase in TTA in all samples was related to an increase in LAB population (Figures 1 and 2). LAB could be the main microorganisms causing a rapid deterioration in coconut water under refrigeration and room temperatures. However, when spoilage was visually observed, the TTA was significantly decreased. This might be a result of the other groups of microbes that used organic acids as a carbon source for producing metabolites causing “off” odors.
Results from GC-MS analysis of volatile compounds in coconut water are shown in Figure S2. It was found that the abundant volatile groups were alcohol, ester, acid and phenol. Among these, ethanol (Alc1), ethyl acetate (Est2), ethyl ester (Est2) acetic acid (Ac1) and octanoic acid (Ac2), were predominantly detected as the major metabolites. Alcoholic metabolite, i.e., ethanol, was largely detected as the main volatile metabolite in coconut water [27–29]. Similarly, ethanol was the most abundant volatile identified in this study. However, the reduction in ethanol was generally observed in all samples during storage at refrigerated and ambient temperature. This might be related to the dynamic change in organic acid as a function of microbial activity. In contrast, acetic acid was generally increased during storage. In association with the growth in LAB and yeast in the matrix, the change in acetic acid and ethanol could be a result of dynamic balance between utilization and reproduction schemes in the tricarboxylic acid cycle of microorganisms in the system [27,30]. This relationship was also supported by rRNA-based metabolic profiling, in which W. cibaria, a heterofermentative bacterium, was strongly expressed, as shown in Table 1 and Figure S1. In addition, the presence of specific volatile compounds like octanoic acid, acetic acid, ethyl acetate, and butyl phenol was found, especially when associated with the activities of Lactobacillus, particularly Weissella (Figure S2, Table 1) [31,32]. In addition, one study confirmed that these key metabolites, particularly octanoic acid, were not associated with yeast activities when fermenting coconut water [33]. This observation strongly suggests a potential role of these lactic acid bacteria in contributing to the volatile profiles observed in spoiled coconut water. This insight into the metabolites produced by these bacteria highlights their potential significance in the spoilage process. It could be valuable information for understanding the mechanisms of spoilage and developing strategies to prevent or mitigate it.

Interestingly, 2-heptanol, decanoic acid and ethyl ester were not detected in any samples from manual and aseptic preparation technique of cutting the fruit open, but they were dominant in those from industrial manufacturers. A metabolite presenting a pleasant and overripe aroma, 2-heptanol was reported as one of volatile alcohols naturally found in coconut water [4] and related products, including coconut meat [34,35] and coconut milk [36,37]. Since this metabolite is transformed from triglyceride at high temperatures [38], the absence in all samples obtained using the manual technique of cutting the fruit open could be due to its lack of exposure to high temperatures compared to those from industrial manufacturers. Microorganisms such as LAB and yeast can utilize acid and alcohol as a precursor [39,40] and produce the fruity and floral-flavor ester compounds in various fermented food, such as fermented coconut water [30] and desiccated coconut [34]. Also, the substrate of decanoic acid was not detected in the samples obtained.
from cutting the fruit open (Table S2), and hence an absence of its ester product was expected.

In order to predict a relationship of parameters involved in a coconut water grading system, PCA of all normalized physicochemical properties that might be affected by metabolic activity of dominant microbes were studied (Figure 5). Analysis output using Clustvis revealed a scatterplot with axes corresponding to the principal components (PCs) 1 and 2, and showed the largest variance of 68.3% and 14.5%, respectively, with a relatively high cumulative value of 82.8%. The plots clearly noted a strong cluster of TTA (as percentage of lactic acid), sugar content, turbidity, and the most volatile metabolites of 1-butanol (Alc2), 2-heptanol (Alc3), ethyl acetate (Est1), decanoic acid, ethyl ester (Est3), dodecanoic acid, ethyl ester (Est4), acetic acid (Ac1), octanoic acid (Ac2) and 2,4-di-tert-butylphenol (Phe1). Another cluster nearby consisted of microorganisms on negative PC1. Nevertheless, the sensory criteria used by manufacturers seemed to be outliers from the others, as shown in the top-left corner of the plot.

Figure 5. PCA score plot derived from microbial and physicochemical profiles, including key metabolites and grading scores of coconut water.

Furthermore, the results from the analysis showed that the sensory criteria were highly related to pH value on the vertical axis (PC2). Previous studies indicated that an organoleptic perception of many foods containing various organic acids might be related to hydrogen ion concentration (pH). Salmerón et al. (2015) indicated the importance of final pH value in the final acceptance of the LAB-fermented cereal beverages [41]. Great influence of pH on food flavor was also reported in LAB fermented products such as soy-based yogurt [42] and milk-based yogurt [43]. This matches a previous report of Hahn (2012) in which a high correlation of pH value with maturity stage was observed. He also noted the difficulty of trained panelists in coconut water grading, since the differences in key properties, such as transparency and freshness of coconut water, were not detected among various stages of coconut samples [44].

With evidence from previous research, a sensory grading system using well-trained personnel might not fully reflect the microbiological or physicochemical qualities, since
the sensory evaluation can be masked by the pH effect. A possible approach for coconut 
water grading by detection of other key physicochemical properties that precisely reflect 
microbial quality was studied and further discussed.

Microbiological tests, along with the examination of volatile metabolites using head-
space SPME-GC/MS and the analysis of ethanol, sugars, and organic acid content through 
HPLC, offer a detailed understanding of the product’s characteristics. These determinations go beyond basic parameters like pH and acidity, providing a more comprehensive 
assessment of product quality. However, implementing these tests on an industrial scale 
requires careful consideration of factors such as cost, time, and the level of technical 
expertise available. It should be noted that although the advanced analytical techniques 
mentioned may not be practical for routine use in small- or large-scale industries, their 
application for validation purposes is crucial for ensuring product quality and meeting 
regulatory standards. The decision to implement these tests on an industrial scale should 
be based on a careful evaluation of the benefits they provide against the practical chal-
 lenges associated with their use in a particular manufacturing context.

3.3. Properties of Fresh Coconut Water before and after Storage as Compared to Sensory Grading System

Fresh coconut water was initially contaminated with TPC, YM and LAB, ranging 
from to 5 log CFU/mL (except for the spoiled F1 sample, with 8.5 log CFU/mL). The TSSs, 
pH, and TTA were 4–7 °Brix, approximately 5, and 0.07–0.09%, respectively. Based on 
maturity, these characteristics meet the quality of the 5- to 12-month-old coconut (“The 
chemistry of coconut water”, Coconut Handbook, Tetra Pak). The main microbial species 
obtained from cutting the fruit open and industrial manufacturers were relatively similar. 
W. cibaria and Leuconostoc spp. were mainly observed. When spoilage was visually ob-
served, the TPC reached > 5 log CFU/mL, while LAB increased to >9 log CFU/mL. Com-
pared to laboratory analysis of coconut properties with a sensory grading system, the 
samples with TPC close to 5 log CFU/mL were consistently graded as level 2. At this level, 
the coconut water would be marginally accepted at the lowest level. Also, the samples 
with TPC > 5 log CFU/mL were consistently evaluated as level 0 or unacceptable.

In this section, the population of each microbial group is arranged based on level of 
detection and compared to the key physicochemical properties, such as TTA and turbid-
ity, as shown in Table 2. It was found that the TTA consistently reached > 0.1% when TPC 
was > 5 log CFU/mL. The TTA changes also corresponded to an increase in total acid and 
reduction in sugars detected by HPLC. The results demonstrated that TTA was linearly 
related to microbial population, so TTA has the potential to be an alternative indicator for 
rapid grading and quality screening of fresh coconut water prior to further processing. 
When the range of 1 log CFU/mL data on TPC, YM and LAB was compared with physi-
occhemical properties, some significant correlations were observed. It was found that tur-
bidity was statistically different when there was an increase of 5–6 log CFU/mL in TPC. 
Also, turbidity was statistically different when LAB and YM in coconut water increased 
to 7–8 and 5–6 log CFU/mL, respectively. In summary, TTA would be statistically changed 
when TPC, LAB and YM increased to ranges of 5–6, 7–8 and >4 log CFU/mL, respectively.

4. Conclusions

The spoilage profiles of fresh coconut water from different conditions observed were 
relatively similar, as were the main microbial species associated with fresh coconut water. 
Weissella cibaria and Leuconostoc spp. were the predominant microbes associated with 
spoilage of coconut water. The physicochemical properties of coconut water stored under 
both conditions, especially the alteration rates, displayed a clear correlation with the in-
creased microbial populations. This approach involves using microbial groups and pop-
ulations, total titratable acidity (TTA), and sensory criteria as alternatives to screening for 
spoilage quality. The correlation between TTA and the total plate count (TPC), along with 
an unacceptable sensory rating, suggests that TTA could serve as an important indicator
of spoilage, potentially linked to microbial activity and sensory changes. This integrated approach provides a comprehensive way to assess and predict the spoilage status of the product.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae9121284/s1. Figure S1: Bacterial communities in coconut samples under different storage conditions through DGGE analysis; Figure S2: GC-MS analysis of dominant volatile compounds in coconut juice from processing sites: (a) street vendor L1—fresh fruit; (b) street vendor L3—mature fruit; (c) street vendor L2; (d) industrial supplied F1; (e) industrial supplied F2; (f) industrial supplied F3. Table S1: Main microorganisms observed in every fresh and spoiled coconut water sample under chilling and room temperature conditions by cultural plating.

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**References**


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