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The Effect of Temperature and Storage Time on Chemical and Sensory Properties of Coconut Water Kefir

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Abstract. *Coconut water kefir is a processed food product, derived from young coconut water. On this product, chemical and sensory properties are maintained through proper post-harvest handling. Therefore, this study aimed to determine the optimal temperature and storage time for coconut water kefir to produce the best sensory properties acceptable to the panelists. The effect of temperature and storage time treatments on chemical parameters was assessed through measurements of pH, °Brix, and alcohol content. Sensory analysis included both hedonic and hedonic quality testing. Furthermore, the experimental treatments applied were variations in storage temperature, both in the freezer (-25℃) and refrigerator (3℃), and storage time of 0, 1, 7, 14, and 21 days. The results showed that the interaction between temperature and storage time had no significant effect on the pH value and °Brix of coconut water kefir. All samples had low alcohol content, with values below 1%. The optimum sensory properties, as perceived by the panelists, were observed in the sample stored at a refrigerated temperature for 1 day. Further statistical analysis using the Kruskall-Wallis Test showed that refrigerator storage had a significant effect on hedonic and hedonic quality testing. Conversely, freezer storage had a significant effect on only the hedonic quality test.*
Keywords: *coconut water kefir; storage temperature; storage time.*

Type of the Paper: Regular Article.

1. Introduction

Interest in the consumption of healthy foods and artisanal homemade preparations of fermented products, such as water kefir, is on an increasing trend. Water kefir is an ancient drink of uncertain origin, consumed practically worldwide [1]. The drink is a cheaper and more popular alternative to developing milk kefir beverages, as it can be made with fruit juices, coconut water, organic sugar water, or filtered water [2]. Among these substrates, coconut water offers several better advantages. It has nutritional properties and is a good source of fiber, vitamin C, and various essential minerals [3]. Furthermore, coconut water *in extremis* has been used as a short-term intravenous hydration fluid [4].

Indonesia ranks first in coconut production worldwide, followed by the Philippines and India [5]. The country also exports coconut and derivative products to destinations including Malaysia, China, India, Netherlands, Korea and Japan [2]. From 2019 – 2021, Indonesia experienced an increasing trend in the number of harvests. Based on data from the Central Statistics Bureau (BPS),

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coconut commodities in the country reached 28,530,000 tons in 2021, reflecting a significant production. As a result, the ten largest producers of this tropical fruit are dominated by countries from the Asian region with tropical climates [6].

Coconut fruit comprises 35% fiber, 12% shell, 25% water, and 28% flesh. Coconut water is a liquid endosperm formed in small quantities during the third month of the fruit ripening [7]. It is one of the main products and is widely consumed as a health drink. According to the 2017 Indonesian Food Consumption Data [8], 100 g of coconut water contains 17 calories of energy, 0.2 g, 0.1 g, 3.8 g, 15 mg, 8 mg, 0.2 mg, 1 mg, and 149 mg of protein, fat, carbohydrates, calcium, phosphorus, iron, sodium and potassium, respectively.

Coconut water is an alternative fermentation medium with well-documented nutritional content and benefits. It can be processed into different forms including coconut water kefir. The type of sugar contained in coconut water are sucrose, fructose, and glucose [6]. These sugars are used by microorganisms during metabolic activities and the fermentation process to produce a probiotic drink known as coconut water kefir. The probiotic drink has no milky content and is included in the group of water kefir obtained by fermenting water-based solutions, including fruit juices, vegetables, and other sources of sugar [9]. *Bacillus paracasei, Lactobacillus hilgardii, Lactobacillus nagelii,* and *Saccharomyces cerevisiae* are some of the microorganisms that play a role in the fermentation of this type of drink [10]. Water kefir is known to contain lactic acid bacteria, acetic acid bacteria, and yeast. Meanwhile, coconut water kefir has cholesterol-lowering benefits due to the isolation of lactic acid bacteria [11].

Effective fermentation handling under favorable conditions can maximize the capacity of the product obtained. In this context, extensive studies have been conducted on post-harvest handling of kefir, with a primary focus on milk kefir of cow [12]. These analyses explored the effect of temperature and storage time on alcohol content in milk kefir. In the same year, a study examined the impact of temperature and storage time on properties of milk kefir to estimate shelf life [13]. Additionally, there is a publication on kefir with coconut water as the medium, which primarily investigates the influence of fermentation time, without observing aspects of temperature or storage time [14]. Another study by Hecer et al. [15], examined the effect of different fermentation conditions on the composition of kefir. The changes in physical, chemical, and microbial parameters with a different incubation temperature and time was also observed [15].

The handling of fermented food impacts sensory properties of the resulting products, as supported by various publications [16,17]. Several factors influence these sensory properties of fermented food, including temperature and storage time. Appropriate application of the factors is essential for achieving desirable sensory characteristics. Furthermore, storage condition affects

several physical and chemical properties of the final product, which are closely related to consumer acceptance [18].

A study needs to be conducted on the analysis of pH, °Brix, alcohol content, and sensory properties of coconut water kefir with variations in temperature and storage time. The process of developing fermented kefir drinks from coconut water requires testing post-harvest handling methods. The results of this study are intended to serve as a reference for enhancing the production capacity of fermented food, thereby increasing the economic value of coconut water. The primary focus was on chemical and sensory analysis parameters of coconut water kefir subjected to handling variations in temperature and storage time post-harvest. This study aimed to determine the optimal temperature and storage time for coconut water kefir, ensuring sensory properties acceptable to panelists. The effect of temperature and storage time on pH, °Brix, and alcohol content of this product was examined.

2. Materials and methods

2.1. Equipment

The study was conducted in the Dietetics and Culinary Laboratory and Laboratory Food Chemistry-Biochemistry at Al Azhar University of Indonesia. It comprised several steps including preparation of equipment and material, followed by coconut water kefir production, harvesting, storage treatment, and analyzing chemical and sensory analysis properties. These stages of coconut water kefir are shown in Fig. 1. The equipment used were a pan, closed glass container, air container, knife, silicone rubber filter, plastic spoon, PET bottle, label, stove, cooking thermometer, freezer and refrigerator thermometer, digital scale, analytical scale, refrigerator, freezer, containers sample presentation, sensory test assessment form, beaker glass, tissue, pH meter (pH meter Lab Bench pH/mV meter AMT20 bench top), refractometer (Nohawk Portable Digital Sugar Brix Meter 0-35%), series of distillation tools, round bottom flask, hot plate, erlenmeyer, pycnometer, spray bottle, and pipette.

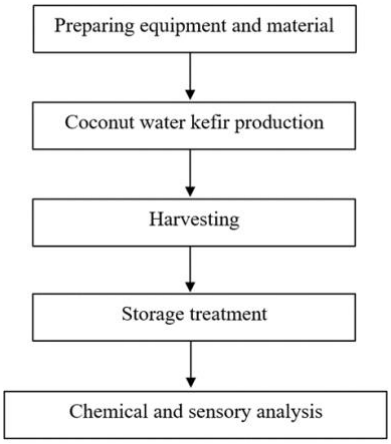

Fig. 1. Stages of coconut water kefir study

2.2. Material

The materials used in this study include young coconut water obtained from a seller in the area around Al Azhar University of Indonesia, water kefir grains from the commercial shop (Aracaki), granulated sugar (Gulaku), mineral water, distilled water, Vaseline, and pH buffer powder.

2.3. Coconut water kefir production

During the production of kefir, 5% (w/v) water kefir grains were added to young coconut water. The process of coconut water kefir production is shown clearly in Fig. 2. Water kefir grains contain microbes such as *Lb. harbinensis, Lb. hilgardii, Lb. nagelii, Lb. paracasei, Lb. hordei, B. aquikefri, Candidatus O. aquakefiri, S. cerevisiae,* and *D. bruxellensis* [19]. A total of 50 g of this material was added to 1500 mL of coconut water. The mixture was then fermented at room temperature for 48 hours. Following fermentation, coconut water kefir was filtered to separate the liquid product from water kefir grain. This liquid product was placed into PET bottles and stored at varying temperature and storage time.

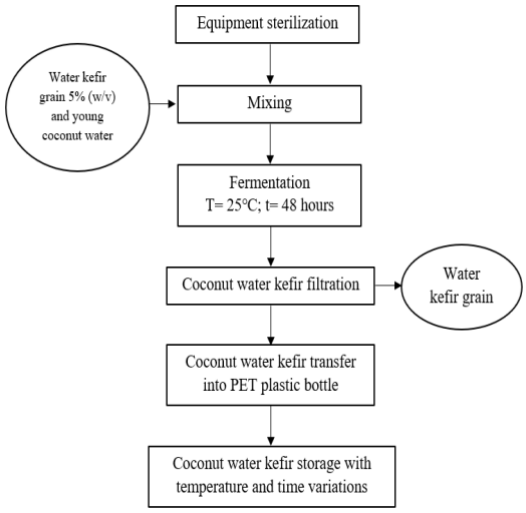

Fig. 2. Coconut water kefir production [20]

Table 1. Variation of temperature and storage time of coconut water kefir

Storage Time (day)	Storage Temperature	
	Freezer (A)	Refrigerator (B)
0	AB0	
1	A1	B1
7	A2	B2
14	A3	B3
21	A4	B4

2.4. Harvesting and storage treatment

After 48 hours of fermentation at 25℃, coconut water was harvested and various storage treatments were applied. Storage time treatment variations in this study were based on the procedures of Irigoyen et al. [20] with modifications. Meanwhile, the variety of storage

temperature treatments was based on the procedures of Setyawardani [21] and Setiawati and Yunianta [22] with modifications. These studies examined kefir sensory properties by applying storage treatment at freezer temperature (-25℃) and refrigerator (3℃). Variations of temperature and storage time treatment are shown in Table 1.

2.5. pH

Before measurements were taken, the pH meter was calibrated with buffer solutions of 4.0, 7.0, and 10.0. The pH was measured by dipping the electrode into the sample and waiting a few moments until a stable reading was obtained. Every time the solution was changed, the electrode was rinsed with distilled water and dried before further usage [23]. The measurements were conducted in triplicate [24], and similar to a previous study, the pH of coconut water kefir was observed directly without any dilution [25].

2.6. Degree of brix

Degre of Brix testing was conducted using a hand refractometer, where the prism was first rinsed with distilled water and cleaned using a soft fabric. The sample was dripped onto the refractometer prism and covered with a cover. Finally, the start button was pressed [26] and °Brix measurements were made in triplicate [27].

2.7. Alcohol content

The alcohol content was measured using the pycnometer method. A sample of 100 ml was put into a round bottom flask, then 100 ml of distilled water was added and distilled to 80℃. The distillate product was collected in a 50 ml Erlenmeyer flask. Furthermore, the outside surface of the pycnometer was dried with tissue and the instrument was weighed in the empty state (a). The distillate was put in a pycnometer at 10 mL, then weighed (A). The same procedure was conducted for distilled water as a comparison, such that aquades were put into a pycnometer and weighed (B). Furthermore, the specific gravity of alcohol was calculated and converted to a table of alcohol percentages. This allowed for the determination of the alcohol content of the sample [28].

2.8. Sensory properties

Sensory analysis of coconut water kefir, with variations in temperature and storage time, was conducted using 30 non-trained panelists. Each panelist evaluated 8 samples of coconut water kefir from each treatment. The hedonic test in sensory analysis assesses the level of delight for a product [29]. The parameters evaluated in this test included color, taste, aroma, and overall acceptance. A hedonic scale was used, sourced from [30], and ranged between (1) really dislike, (2) dislike, (3) slightly like (4) like, to (5) really like. Meanwhile, the quality hedonic test provided more specific and typical judgments aiming to determine the panelists' responses to a common organoleptic quality characteristic [31]. Parameters observed were color, aroma, sour taste, sweet taste, bitter taste, and soda sensation. The scale for the hedonic quality test, similar to the hedonic test, ranged

from 1 to 5, with detailed explanations for each parameter provided in Table 2. Mineral water was available during both tests as a neutralizers to avoid sensory biases caused by the residual sensations of previously served samples, also known as carryover effect [32].

Table 2. Hedonic quality parameters

Scale	Hedonic Quality Parameters					
	Color	Aroma	Sour Taste	Sweet Taste	Bitter Taste	Soda Sensation
1	Not very murky	Not very pungent	Not very sour	Not very sweet	Not very bitter	Not very strong
2	Not murky	Not pungent	Not sour	Not sweet	Not bitter	Not strong
3	Slightly murky	Slightly pungent	Slightly sour	Slightly sweet	Slightly bitter	Slightly strong
4	Murky	Pungent	Sour	Sweet	Bitter	Strong
5	Very murky	Very pungent	Very sour	Very sweet	Very bitter	Very strong

2.9. Data analysis

Data analysis was conducted using statistical applications SPSS 27.0. Chemical parameter data are recapitulated and analyzed statistically using ANOVA analysis at the significance level α = 0.05 or 95% confidence interval. In cases when there is a significant difference, the Duncan Test was applied for further analysis. Meanwhile, data obtained from sensory parameter testing are recapitulated and analyzed statistically using the Kruskall Wallis Test at a significance level of α = 0.05 or 95% confidence interval. The Kruskal–Wallis test is a nonparametric test for more than two independent samples. It assumes that the observations in each group come from populations with distribution and the samples are random and independent [33]. In cases when there is a significant difference, the U-Mann Whitney Test was applied for further analysis. Subsequent testing was intended to determine the possible differences in each chemical parameter and sensory properties from the sample with 2 factorials, namely "storage temperature" and "storage time".

3. Results and Discussion

3.1. pH

The pH of coconut water kefir based on the interaction of temperature factors and storage time is shown in Fig. 3. The values were between 3.71 to 3.93, which falls within the ideal range for water kefir products. Furthermore, the ANOVA test on pH for the interaction of temperature and storage time factors showed no significant difference (P>0.05) across all sample treatments. The values experienced a decreasing trend but were not significantly different.

The analysis results in this study showed that the longer storage time, the lower the pH value. The decreasing trend is caused by the activity of lactic acid bacteria, originating from water kefir grain. This signified the ability of coconut water to support the growth of the fermentation culture [34]. Total bacteria lactic acid was maintained in water kefir through the filtered process of kefir

grains and varying time treatments storage [35]

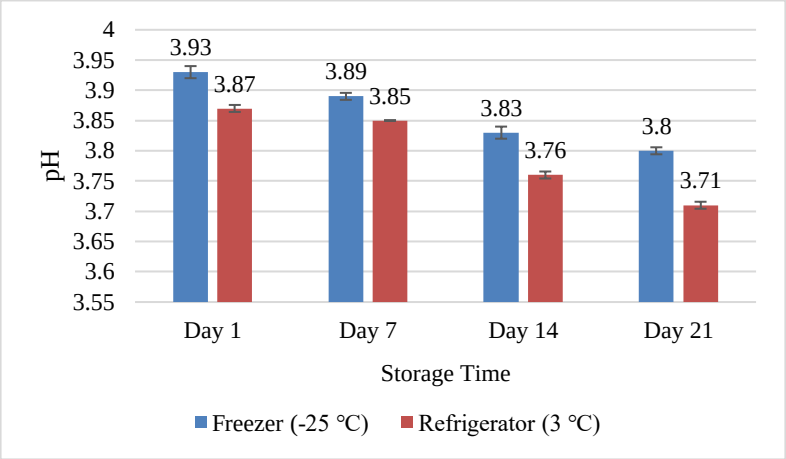

Fig. 3. The effect on the interaction between temperature and storage time on pH

Higher temperature causes the pH of coconut water kefir to decrease. The yeast population experienced significant growth during kefir storage under the influence of refrigerated temperature. This has an impact on the synthesis of many metabolites at a refrigerated temperature. The phenomenon causes low pH value [22]. Meanwhile, storage at freezer temperature led to the inhibition of bacterial growth [36].

3.2. Degree of Brix

The results of °Brix analysis of coconut water kefir based on the interaction of temperature and storage time factors are shown in Fig. 4. °Brix value of kefir ranged between 2.16 – 3.43, falling within the ideal category for water kefir product [37]. The publication of Dwiloka et al. [37] stated that the degree of brix of coconut water kefir was between 1.04 – 3.80. The ANOVA test conducted showed results that were not significantly different ($P>0.05$) across all sample treatments.

The longer storage time, the higher the quantity of carbohydrates that broke down into simple particles, serving as a nutrition source for microorganisms. This process impacts the °Brix value, leading to a decrease [38]. During the harvesting process of coconut water kefir, the remaining microorganisms from water kefir grains are separated to continue metabolism [35]. The longer storage time, the more content of the nutrients in the media decreases due to continuous usage by bacteria and yeast. Consequently, the sugar or nutrient content in the media is reduced, leading to lower °Brix values. Sugar, a nutritional component in products, is also used by lactic acid and acetic acid bacteria in the body to produce metabolites [39]. The longer storage, the more nutrients are needed to conduct metabolic processes. Furthermore, the total soluble solids amount in the production of water kefir varies depending on the carbohydrate concentration and fruit variety. This variation results from the use of carbon sources in the environment during fermentation, which converts into different metabolites and decreased the total soluble solids amount [40].

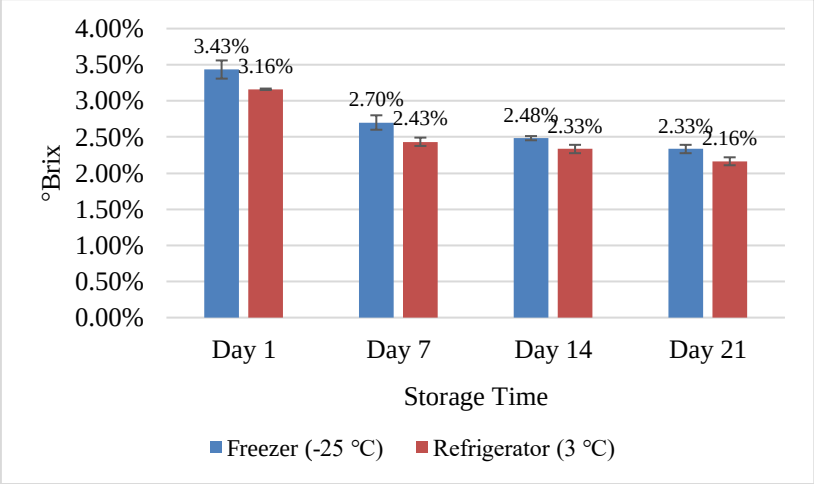

Fig. 4. The effect on the interaction between temperature and storage time on °Brix

A higher temperature caused a decreasing trend of °Brix of coconut water kefir. This is because at a freezing temperature, bacterial activity was inhibited and the use of sugar for metabolic processes was decreased. As a result, the sugar content to remain constantly preserved in kefir products in frozen condition.

Brix measurement and sample temperature are two interrelated variables. A handled refractometer needs temperature correction while performing the measurement [41]. This instrument consisted of focus adjustment, calibration screw, daylight plate, eyepiece, rubber grip, and the main prism assembly. A charge-coupled device (CCD) was used as sensor to precisely measure the intensity of the reflected light. Before taking measurements, the refractometer was calibrated with the help of a calibration screw and distilled water for temperature compensation. The thermometer for temperature compensation was built within the instrument [42].

3.3. Alcohol content

Alcohol was present at varying levels within the final product due to the fermentation process. Therefore, it is necessary to ensure that ethanol levels within the product meet regulatory, quality, and safety standards [43]. This product was detailed as a fermentation process made from sugar, water, and one or more fruit or vegetable extracts or infusions, and was declared to contain no more than 1% alcohol by volume [44].

The results of the analysis of the alcohol content in coconut water kefir are shown in Table 3. The alcohol content of all samples was below 1%, and this was in line with a study conducted by [21], where the ethanol content in kefir varies widely, from 0.01 to 1.00%. The alcohol level is affected by several factors such as the culture, starter concentration, fermentation time, storage time, and temperature.

The optimum temperature for the growth of alcohol-forming microbes was 25°C, leading to optimal production of alcohol, while at low temperature, the growth was inhibited [22]. Freezing

conditions changed the nutritional composition of the fermentation media. It also influenced the production of alcohol bacteria [21].

Table 3. The Effect on the interaction between temperature and storage time on alcohol content

Storage Temperature	Time Temperature	Specific Gravity of Alcohol	Alcohol Content (%)
Room temperature (25 ℃)	Day 0	0.9981	<1%
Refrigerator (3 ℃)	Day 1	0.9954	<1%
	Day 7	0.9946	<1%
	Day 14	0.9941	<1%
	Day 21	0.9938	<1%
Freezer (-25 ℃)	Day 1	0.9957	<1%
	Day 7	0.9949	<1%
	Day 14	0.9946	<1%
	Day 21	0.9939	<1%

According to Setiawati and Yunianta [22], storage time had a direct proportionality influence on the alcohol content of kefir. Based on the measurement method using a pycnometer, a lower specific gravity signified a higher content of alcohol in the sample. As the shelf life increases, the specific gravity becomes lower. This is caused by increased yeast growth with each increase in storage time.

3.4. Hedonic test

The symbiotic metabolic activity of several bacterial and yeast species, which constitutes the natural starter of water kefir grains, imparts a unique flavor and aroma to water kefir. Its distinctive flavor results from being a self-carbonated product subjected to lactic acid and alcoholic fermentations. The process produces lactic acid, CO2, ethanol, and other flavour-forming products. Consequently, water kefir is well-known as a low-alcoholic beverage with a soured, frothy, and mildly acidic taste [45].

The results of the hedonic test performed in this study include several parameters such as color, aroma, taste, and overall acceptance presented in Fig. 5 and Fig. 6.

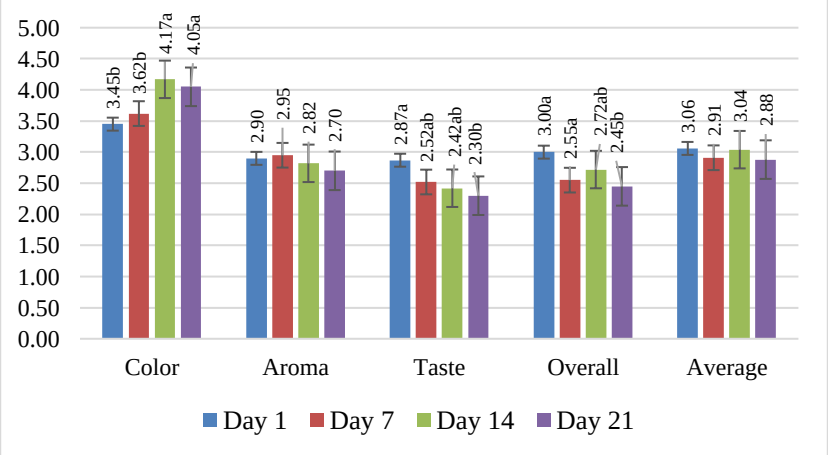

Fig. 5. Result of sensory analysis for hedonic test on coconut water kefir stored in a refrigerator

Fig. 5 explains the results of the hedonic test on coconut water kefir stored at a refrigerated temperature (3 °C). Kruskal Wallis Test on coconut water kefir samples stored in a refrigerator for color, taste, and overall parameters showed a significant difference ($P<0.05$). Meanwhile, in the aroma parameters, the results were not significantly different ($P>0.05$) across all treatments. The U Mann-Whitney test was then conducted on color, taste, and overall parameters across storage treatments refrigerator.

The average of color sensory value for coconut water kefir stored at refrigerated temperature ranged from 3.45 – 4.17 (slightly like to like). Panelists did not like the aroma of almost all samples with storage in refrigerated temperature. During fermentation, there is the formation of flavor components, namely acetaldehyde and lactic acid. This gives kefir a distinctive aroma, which tends to be less liked by some people [46]. The average of taste sensory value ranged from 2.30 – 2.87 (dislike). The fermentation process in drinks changed the physical and chemical characteristics which had an impact on the appearance of new aroma, tastes, and colors [47]. In line with this, Wardhana et al. [38] stated that temperature, humidity, oxidation, and microorganism activity changed the molecular content of ingredients as well as taste.

The panelists' assessment is on a scale of dislike to slightly like for the overall parameter of coconut water kefir stored at refrigerated temperature (3 °C). The average overall sensory value for this treatment ranged from 2.45 – 3.00. Based on the panelists' assessment, the best sample was coconut water kefir sample stored for 1 day. Each sensory parameter data was calculated and averaged to obtain the highest rating based on preferences. Meanwhile, coconut water kefir samples stored for 21 days had the lowest assessment from the average of all parameters and were the least interested in the panelists. Data shown in Fig. 5, were obtained from calculating the average of each scale on each parameter with a total of 30 data from 30 panelists. Furthermore, the highest and the lowest values are emphasized to determine the range of average values.

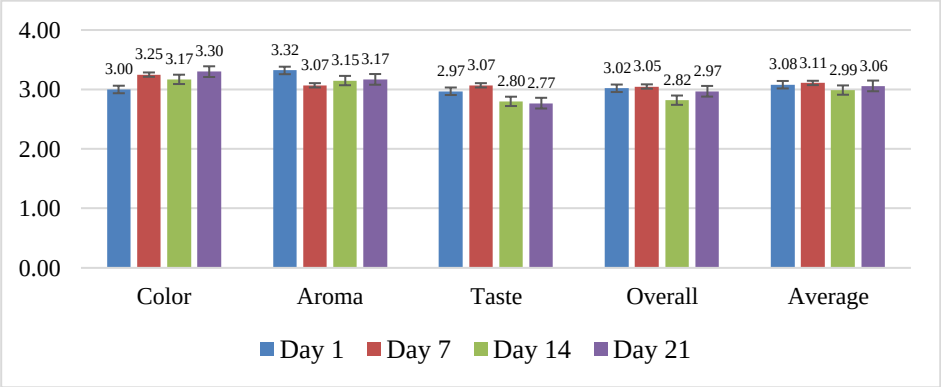

Fig. 6. Result of sensory analysis for hedonic test on coconut water kefir stored at freezer

Fig. 6 explains the results of the hedonic test on coconut water kefir stored at freezer temperature (-25°C). Kruskal Wallis Test for color, aroma, taste, and overall parameters showed

no significant difference (P>0.05) for all treatments. This kind of temperature does not influence the panelists' acceptance of all parameters tested.

3.5. Hedonic quality Test

The hedonic test aimed to obtain a response on quality specific to variations in coconut water kefir with a different temperature and storage time. The results, which include several parameters such as color, aroma, sweetness, sourness, bitterness, and sensation of soda are presented in Fig. 7 and Fig. 8.

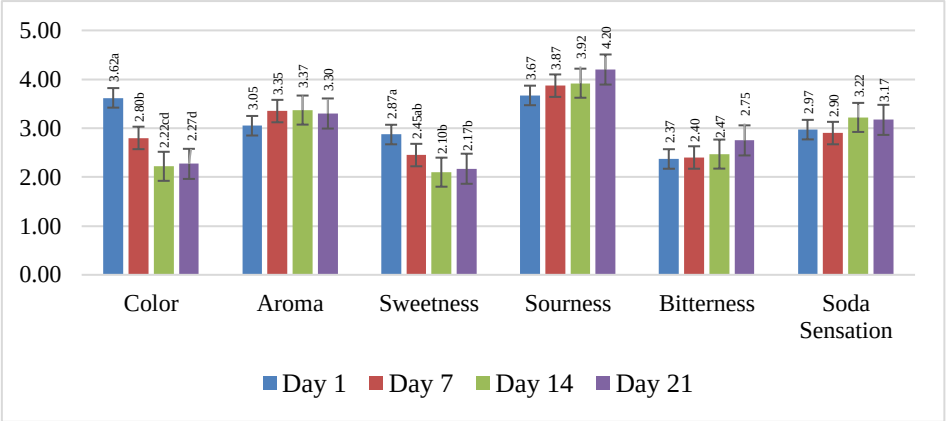

Fig. 7. Result of sensory analysis for hedonic quality test on coconut water kefir stored at refrigerator

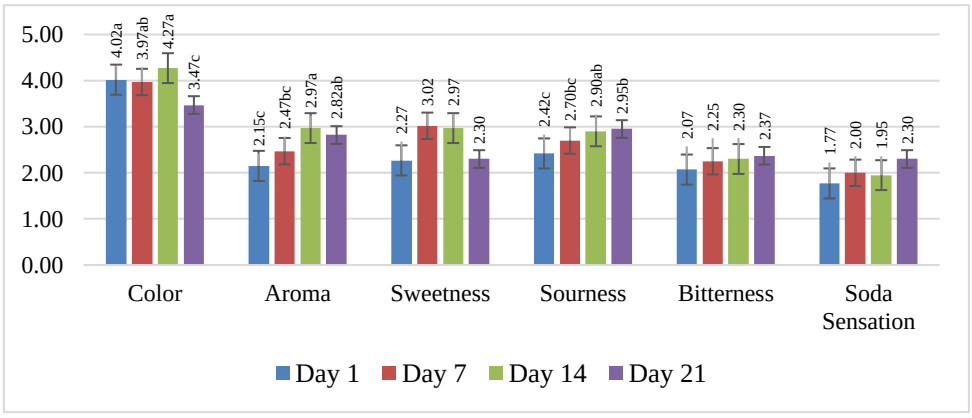

Fig. 8. Result of sensory analysis for hedonic quality test on coconut water kefir stored at freezer

Fig. 8 explains the results of the hedonic quality test on coconut water kefir stored at a refrigerated temperature (3°C). Kruskal Wallis Test on coconut water kefir samples stored at refrigerated temperature for color and sweetness parameters showed a significant difference (P<0.05). Meanwhile, for aroma, sour taste, bitter taste, and soda sensation parameters, the results were not significantly different (P>0.05) for all treatments. The U Mann Whitney Test was then conducted on the color and sweetness parameters across storage treatments refrigerator.

The average sensory value for the murky color of coconut water kefir stored at refrigerated temperature ranged from 2.22 – 3.62 (not murky to slightly murky). A lower color rating signified that the turbidity level of coconut water kefir is decreasing. The murky color observed was

produced from metabolites due to the fermentation of lactic acid bacteria [48]. All samples had a medium rating and showed a slightly strong aroma. The typical aroma of probiotic products such as coconut water kefir was obtained from lactic acid due to bacterial metabolism. Furthermore, the average sensory value for the sweetness of product stored at refrigerated temperature ranged from 2.10 – 2.87 (not sweet). The sweetness was decreased due to reduced sugar content and breakdown by lactic acid, acetic acid, and yeast bacteria during the fermentation and storage processes [49]. In contrast, all samples have medium to high ratings for sourness parameters. On the bitter taste parameter, almost all samples have low ratings. The sour taste of fermented drinks is caused by the metabolism of lactic acid bacteria and yeast, while the fermentation process, namely the breakdown of glucose into alcohol, leads to a bitter taste [50]. Fermentation metabolites, such as esters, peptides, aldehydes, acetaldehyde, and higher alcohols are also associated with bitterness [51]. On the soda sensation parameter, all samples had low to medium ratings, signifying not strong to slightly strong soda sensation. The sensation of soda was attributed to the reaction between carbonate and acid which then forms CO2 [52].

Fig. 7 explains the results of the hedonic quality test on coconut water kefir stored at freezer temperature (-25℃). Kruskal Wallis tests on coconut water kefir samples stored at freezer temperature for the parameters of color, aroma, and sour taste showed significant differences ($P < 0.05$) across all treatments. The U Mann-Whitney test was then conducted on the parameters of color, aroma, and sour taste in all freezer storage treatments.

The average sensory value of murky color, aroma, and sourness parameters on coconut water kefir stored at freezer temperature ranged from 3.47 – 4.27 (slightly murky to murky), 2.15 – 2.97 (not strong aroma), and 2.42 – 3.92 (not sour to slightly sour), respectively. The parameters of sweetness, bitterness, as well as soda sensation, are no different between treatments, making it difficult to sort the assessments. However, on the sweetness parameter, almost the entire sample had a low rating to an intermediate score. This signified that coconut water kefir showed a taste ranging from not sweet to slightly sweet. On the bitterness parameter, the entire sample had a low rating, reflecting a not bitter taste. In contrast, the assessment for sweetness and bitterness showed very low ratings on the parameter of soda sensation. This suggested that the sensation of soda in coconut water kefir is very not strong to not strong.

4. Conclusions

In conclusion, the interaction between temperature and storage time did not significantly affect the pH and °Brix value of coconut water kefir. Furthermore, the alcohol content of all samples was in the range below 1%. The optimum temperature and storage time of coconut water kefir for obtained sensory properties acceptable to the panelists were achieved at refrigerated

temperature for 1 day. This assessment was based on the average of all parameters for hedonic testing. Further statistical analysis results showed that refrigerator storage had a significant influence on hedonic and hedonic quality assessment. Freezer storage did not significantly influence hedonic assessment. However, it had a significant influence on hedonic quality assessment. This study on coconut water kefir could be improved by considering the addition of various types of sugar.

Abbreviations

Not applicable.

Data availability statement

Data will be made available on request.

Credit authorship contribution statement

Sefira Putri, Maryam Jameelah, Lukman Azis: Conceptualization, Data curation, Methodology, Writing – review and editing. **Sefira Putri**: Formal analysis, Investigation, Resources¸ Writing – original draft, Visualization. **Maryam Jameelah:** Conceptualization for study methodology, Supervision of each step (especially the fermentation process), Validation of the data, review and editing the manuscript, and Research Project Administration. **Lukman Azis:** revise the manuscript, and validate of analytical steps.

Declaration of Competing Interest

The authors declared the absence of competing financial interests or personal relationships that could have appeared to influence this study.

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