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Physicochemical and Sensory Characteristics of Green Coconut (*Cocos nucifera* L.) Water Kefir

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Abstract

This research aims to examine the effects of fermentation time on the physicochemical and sensory characteristics of green coconut water kefir in order to determine the optimal fermentation time based on the resulting sensory attributes. There were four fermentation time treatments (12, 24, 36, and 48 hours), each with five replications. The materials used were green coconut water and 5 % kefir grains. Physical analyses included pH and viscosity, while the chemical analyses included total dissolved solids (TDS), alcohol content, water content, protein content and fat content. Sensory attributes included sourness, soda sensation, sour aroma, viscosity and turbidity. The results showed that fermentation time had significant effects on pH, TDS, alcohol content, water content, protein content and the sensory attributes of green coconut water kefir. Viscosity and fat content were not affected by fermentation time. The ideal fermentation time was 12 hours resulting in a pH level of 4.6, viscosity of 0.09, TDS of 3.8^o Brix, alcohol content of 1.16%, water content of 97.14 %, protein content of 6.64 % and fat content of 1.17%. Sensory evaluation found a low level of sourness, low soda sensation, high sour aroma, high viscosity and low turbidity.

Keywords: Fermentation; Green coconut; Physicochemical; Sensory; Water kefir

1 Introduction

Coconut (*Cocos nucifera*) is one of the palm tree species which is widely cultivated in tropical regions, especially in areas near beaches (Chidambaram, Singaraja, Prasanna, Ganesan & Sundararajan, 2013). There are many varieties of coconut such as green dwarf, yellow dwarf and red dwarf. Green dwarf or green coconut (*C. nucifera* L.) is the most utilized variety of coconut due to its high content of total phenols and ascorbic acid (Santos et al., 2013). Indonesia is the largest producer of green coconut in the world, with the highest diversity (Kailaku, Syah, Risfaheri, Setiawan & Sulaeman, 2015; Pandin, 2015). The edible part of the fruit consists of coconut meat and coconut water (Yong, Ge, Ng & Tan, 2009). Recently, the green coconut water market has grown rapidly in the functional beverages category due to its hydration qualities (Marsh, Hill, Ross & Cotter, 2014). Furthermore, green coconut water contains micronutrients such as inorganic ions and vitamins that are beneficial in promoting the human body's antioxidant system (Evans & Halliwell, 2001; Yong et al., 2009), antimicrobial peptides (Mandal et al., 2009), catechins and epicatechins (Chang & Wu, 2011). Green coconut water is also a rich

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source of cytokinin that has antiaging properties in human skin cells (Ge et al., 2006).
Green coconut water is widely consumed in its natural form (Franco, Yamamoto, Tadini & Gut, 2015) or in a processed, ready-to-drink beverage form (Santana, Ribeiro & Iguti, 2011). Heat treatment is used in commercial coconut water manufacture so as to prevent microbial spoilage and oxidative enzymatic (Tan, Cheng, Bhat, Rusul & Easa, 2014). However, heat treatment often leads to changes in the product's organoleptic and nutritional quality (Cappelletti et al., 2015). Therefore, an innovative approach is required to develop new products based on green coconut water.
Fermentation is one of food preservation methods which can improve the nutritional value of food (Marsh et al., 2014). Green coconut water contains sugars, proteins, free amino acids and minerals (Flávera et al., 2015) thus it is possible to process it into a fermented non-dairy beverage such as water kefir. Water kefir is obtained by fermenting water sucrose with kefir grains containing lactic acid bacteria and yeast (Marsh, O'Sullivan, Hill, Ross & Cotter, 2013), resulting in a beverage with effervescent characteristics (Ismaiel, Ghaly & El-Naggar, 2011; Liu & Lin, 2000).
The characteristics of a fermented product are influenced by fermentation time. As there was no reported work, it was necessary to analyze the effect of fermentation time on the physicochemical and sensory properties of green coconut water kefir. Therefore, this research aims to establish the optimal fermentation time for green coconut water kefir by assessing the physicochemical and sensory characteristics.

2 Materials and Methods

2.1 Materials

Five liters of commercially available coconut water was obtained at a Mulawarman Street store in Semarang, Central Java. Kefir grains were obtained from the beadsnik online shop located in Denpasar. Selenium, sulfuric acid, 4 % H_3BO_3, Methyl Red (MR) and Methyl Blue (MB), Aquadest, 45 % NaOH, 0.1N HCl, 91 % H_2SO_4, and amyl alcohol were used. Porcelain dishes, an oven (Memmert, Germany), Kjeldahl flasks (Pyrex, Japan) were also used.

2.2 Kefir preparation from green coconut water

The method used to produce green coconut water kefir was adapted from Lestari, Bintoro and Rizqiati (2018). The green coconut water was pasteurized for 30 seconds at 60 ^{o}C, poured into jars and then cooled to 28 ^{o}C. Kefir grains were added to the jars at 5 % (w/v) to begin the fermentation process. The kefir samples were treated with different fermentation times, which were 12 hours (T1), 24 hours (T2), 36 hours (T3) and 48 hours (T4). The coconut kefir was then filtered to separate the grains from the coconut water before the specified testing.

2.3 Physicochemical properties

pH analysis

The sample pH was measured using a pH meter (AOAC, 2013). The pH meter was calibrated with standard buffers (pH 4.0 and pH 7.0) just prior to use.

Viscosity analysis

The Ostwald viscometer was calibrated with deionized water. The mass of the pycnometer was weighed with an analytical balance, then again containing 10 mL of water and finally containing 10 mL of each sample. The time taken for each sample to drain by gravity between two etched marks of the Ostwald viscometer was measured. The viscosity of each sample was calculated according to the equation below (Fathima, Devi, Rekha & Dhathathreyan, 2009):

$$\eta_s = \eta_w \cdot \frac{t_s}{t_w} \cdot \frac{m_{p+s} - m_p}{m_{p+w} - m_p} \quad (1)$$

$\mathbf{m}_p$: Mass of pycnometer (g)

$\mathbf{m}_{p+s}$: mass of pycnometer + filled volume of the sample (g)

$\mathbf{m}_{p+w}$: mass of pycnometer + filled volume of water (g)

η_w : water viscosity (cP)

η_s : sample viscosity (cP)

$\mathbf{t}_s$: drain time for sample (s)

$\mathbf{t}_w$: drain time for water (s)

Alcohol content

The alcohol content was measured by distillation and a pycnometer (AOAC, 2013). The samples (50 mL) were placed in a Kjeldahl flask and 100 mL aquadest was then added. The distillation process occurred at 80 ^{o}C and the distillate was collected in an Erlenmeyer flask. Fifty mL of the distillate was transferred to a pycnometer. Excessive distillate was removed from the top of the capillary tube of the pycnometer. The distillate-filled pycnometer was then weighed. The same procedure was repeated for aquadest. The density of alcohol was calculated using the formula below:

$$\rho = \frac{m_{p+d} - m_p}{m_{p+a} - m_p} \quad (2)$$

ρ ; Alcohol density (g/cm^3)

$\mathbf{m}_p$: Mass of pycnometer (g)

$\mathbf{m}_{p+d}$: mass of pycnometer + filled volume of the distillate (g)

$\mathbf{m}_{p+a}$: mass of pycnometer + filled volume of aquadest (g)

The alcohol content was then obtained using the conversion table for alcohol.

Total Dissolved Solids (TDS)

Total Dissolved Solid (TDS) was measured by a hand-held refractometer (AOAC, 1995). Three drops of aquadest were added to the prism of the refractometer and then wiped off with tissue paper. Three drops of a sample were then added to the cleaned prism, and the lid shut properly. The scale was read at a bright room condition. It showed the percentage of Total Dissolved Solids according to the International Sugar Scale of 1936 in ^{o}Brix unit. The prism of the refractometer was rinsed off again with tissue paper, before the next sample was measured.

Water content

Water content was measured by oven-drying. Empty porcelain dishes were dried in the oven at 105 ^{o}C for 4 hours, and then weighed using an analytical balance. Two grams of each sample was weighed out onto each dish, which were placed in the oven and dried at 105 ^{o}C for 4 hours. The dishes were then transferred, with partially covered lids, to the desiccator to cool down. The dishes and their dried samples were reweighed. The water content was calculated using the following formula based on AOAC (2005):

$$\text{Water content}(\%) = \frac{B - (C - A)}{B} \times 100 \quad (3)$$

A : container's weight (g)

B : sample's initial weight (g)

C : container's and sample's weight after drying (g)

Fat content

Fat content was measured using the Gerber method (AOAC, 2002). A butyrometer was filled with 10 mL of sulfuric acid. Eleven mL of a sample and 1 mL of amyl alcohol were placed into the butyrometer. The tube was sealed with a rubber stopper and shaken until the sample was dissolved. The solution was then centrifuged for 15 minutes at 1200 rpm and transferred from the butyrometer into a water bath at 60-63 ^{o}C. The

solution was immersed, leaving only the small bulb exposed. The fat column was equilibrated for 5 minutes or longer. The scale on the tube of the butyrometer was read to indicate the fat content of the sample.

Protein content

The protein content of a sample was determined based on the total nitrogen content using the Kjeldahl method. A half gram of sample was weighed and placed into the Kjeldahl flask. A half mL of selenium and 10 mL of sulfuric acid were then added to the flask. The resulting solution was digested until a clear-green color was achieved. The digested sample was then distilled. The trap which contains 5 mL 4 % H_3BO_4, two drops of MR and two drops of MB was placed below the distiller. The sample along with 100 mL aquadest and 40 ml 45 % NaOH were added sequentially into the distillation flask. The stove was switched on and the distillation process was allowed to proceed until the trap changed its color from purple to green. Forty mL of distillate was obtained. For the blank control, the same procedure was repeated using 200 mL of aquadest. The distillate was titrated using 0.1 N HCl until the color turned to purple. The protein content was calculated using the following formula based on AOAC (2000):

$$Protein(\%) = \frac{(titrant - blank) \cdot NHCL \cdot 14.008 \cdot 6.25}{\text{Mass of the samples} \cdot 1000} \cdot 100\% \quad (4)$$

2.4 Sensory evaluation test

Sensory quality was evaluated by the rank test (Lawless & Heymann, 1999). Twenty-five semi-trained panelists (fifteen women and ten men) were used in this study. The age of the panelists were between 22 and 25 years. Panelists were given questionnaires containing name, test date, the names of the test samples and instructions. The sensory attributes assessed in this test were the level of sourness, sour aroma, soda sensation, turbidity and viscosity. Panelists evaluated five samples and ranked each attribute on a 1-4 scale. They were also instructed to cleanse their palate with mineral water between evaluating each sample.

2.5 Statistical analysis

The parameters of pH, viscosity, Total Dissolved Solids, alcohol content, protein content, fat content and water content were analysed statistically by Analysis of Variance (ANOVA) using SPSS V22.0. Duncan's multiple range test was then used to determine significant differences amongst the results. Non-parametric data arising from sensory evaluation was analysed by the Kruskal-Wallis test. The significant results obtained by sensory evaluation were investigated using the Mann Whitney u-test to determine significant differences from each treatment.

3 Results and Discussion

3.1 Physicochemical properties

pH analysis

Acidity level, denoted by pH, is commonly used to determine the quality of fermented products as it influences the texture and flavour of the product. As shown in Table 1, pH was affected by the fermentation time. There was a significant difference in the fermentation duration of 12 hours, while in other treatments the difference was not significant. The pH after 12 hours of fermentation time was 4.6; and was 3.4 after 24 hours, 3.6 after 36 hours, and 3.68 after 48 hours. Generally, the pH of water kefir ranges between 3.5 and 4 (Randazzo et al., 2016).

The decrease in pH that occurred in kefir green coconut water after 24 hours of fermentation was due to the growth of bacteria that will convert sugar into lactic acid and acetic acid, thereby decreasing the pH of the product. This was consistent with the findings of Delgado-Fernandez, Corzo, Olano, Hernandez-Hernandez and Javier Moreno (2019) which stated that the longer the fermentation time, the more active the bacteria and the greater the accumulation of organic acids resulting in increased sourness. The presence of too many free hydrogen ions (H^+) may affect the survival of the bacteria after 36 and 48 hours of

fermentation. A longer fermentation time will lead to the death of microorganisms present in kefir due to increasing alcohol levels and decreasing nutrients available for growth (Laureys & De Vuyst, 2014). This result showed that pH can be used as a reference to determine the optimal time to end the fermentation process.

Viscosity analysis

As shown in Table 1, the viscosity of green coconut water kefir throughout the fermentation period ranged from 0.08 to 0.1 cP and was not affected by fermentation time. According to Zannini, Waters, Coffey and Arendt (2016), the viscosity is low if it is less than 2 cP for a 5% w/w solution in water. Viscosity in a fermented beverage was affected by the nutrient content of the raw material and the production process.
Green coconut water contains about 0.72 g/100 g of protein which is a low amount (Yong et al., 2009). Protein content in raw materials is one of the most important factors in determining kefir viscosity. A low protein content in raw materials results in a low viscosity of water kefir since there is insufficient energy for the growth of microbes (Dimitreli, Petridis, Akakiadou & Chrysalidou, 2014). Sabokbar, Moosavi-Nasab and Khodaiyan (2015) also reported that the viscosity values of kefir are related to exopolysaccharide (EPS) or kefiran production by the kefir grain during the fermentation. Therefore, sufficient nutrient content and optimal fermentation conditions are needed to obtain the desired viscosity.
Gul, Atalar, Mortas and Dervisoglu (2018) also observed that kefir viscosity increases with higher fat content as the interaction of fat globule membranes in the protein network improves water holding capacity (WHC) and results in the formation of a more stable gel. Green coconut water kefir contains 0.33 g/100 mL of fat which a low amount (Prades, Dornier, Diop & Pain, 2012). Other factors that may affect the viscosity of kefir are the state of the protein in the main ingredients, total solids and the ability of microbes to produce acid during fermentation (Yoo, Seong & Yoon, 2013).

Total Dissolved Solids (TDS)

In the present study, there were significant differences in TDS across different fermentation times ($P < 0.05$). Significant differences were found in the fermentation periods of 12, 24 and 48 hours; but 36 hours was not significantly different from 24 hours of fermentation. The TDS values of the treatments were 3.8 ^{o}Brix, 2.16 ^{o}Brix, 2.04 ^{o}Brix and 1.04 ^{o}Brix, respectively.
The TDS values reduced with increasing fermentation time. TDS indicates the amount of sugar dissolved in coconut water which mostly consists of glucose, fructose and sucrose. According to Yong et al. (2009), the amount of TDS in green coconut water is 21.68 mg/mL, consisting of 9.18 mg/mL sucrose, 7.25 mg/mL glucose and 5.25 mg/mL fructose. The TDS values of T1 to T4 decreased due to the fermentation process. Yeast in kefir grain can hydrolyze sucrose into monosaccharides, namely glucose and fructose through the action of invertase enzymes. Glucose that is produced from this activity is subsequently transformed into organic acids. This is consistent with Gulitz, Stadie, Wenning, Ehrmann and Vogel (2011) who stated that generally all species of yeast contained in water kefir along with lactic acid bacteria (LAB) produce organic acids from glucose. The accumulation of acid as a product of LAB activity can also trigger a decrease in sugar content, as shown by the results of T4. The process of breaking down sugar by microbes from water kefir grain continuously reduces the availability of sugar and increases the acids. Jeong, Lee, Jung, Choi and Jeon (2013) reported that a decrease in nutrient availability and the accumulation of organic acids produced by LAB occurs with the increasing fermentation duration. Furthermore, these nutrients will deplete and cause an increase in alcohol accumulation which results in microbes entering the death phase.

Alcohol content

The observed results showed that fermentation time significantly affected the alcohol content of green coconut water kefir, where alcohol content increased with longer fermentation periods. The average alcohol content produced in samples fermented for 12, 24, 36 and 48 hours

Table 1: Physical Characteristics of Green Coconut Water Kefir

Parameters	Treatments (hours)			
	12	24	36	48
pH	4.6 ± 0.27^{a}	3.4 ± 0.07^{b}	3.6 ± 0.27^{b}	3.68 ± 0.07^{b}
Viscosity (cP)	0.09 ± 0.03^{ns}	0.1 ± 0.01^{ns}	0.1 ± 0.04^{ns}	0.08 ± 0.01^{ns}

Data shown as the mean of repetitions ± standard deviation (SD). Different superscript letters on the same horizontal line show significant differences ($p<0.05$).

Table 2: Chemical Characteristics of Green Coconut Water Kefir

Parameters	Treatments (hours)			
	12	24	36	48
Total Dissolved Solids (^{o}Brix)	3.8 ± 0.14^{a}	2.04 ± 0.09^{b}	2.16 ± 0.48^{b}	1.04 ± 0.09^{c}
Alcohol content (%)	1.16 ± 0.16^{a}	1.96 ± 0.18^{b}	2.80 ± 0.93^{c}	$4.1\ 4\pm 0.87^{d}$
Water Content (%)	97.14 ± 0.09^{a}	97.19 ± 0.07^{a}	97.1 ± 0.06^{a}	97.35 ± 0.12^{b}
Protein Content (%)	6.04 ± 0.94^{a}	5.46 ± 0.39^{a}	5.05 ± 0.92^{ab}	4.05 ± 0.84^{b}
Fat Content (%)	1.7 ± 0.38^{ns}	1.95 ± 0.31^{ns}	1.75 ± 0.27^{ns}	1.67 ± 0.39^{ns}

Data shown as the mean of repetitions ± standard deviation (SD). Different superscript letters on the same horizontal line show significant differences ($p<0.05$).

Table 3: Sensory Test of Green Coconut Water Kefir

Sensory Attributes	Treatments (hours)			
	12	24	36	48
Level of Sourness	3.52 ± 0.96^{a}	2.28 ± 0.68^{b}	1.60 ± 1.04^{c}	2.60 ± 0.87^{b}
Soda Sensation	2.40 ± 1.50^{ns}	2.40 ± 0.76^{ns}	2.28 ± 0.94^{ns}	2.92 ± 1.12^{ns}
Sour Aroma	3.52 ± 1.00^{a}	2.56 ± 0.87^{b}	2.24 ± 0.72^{b}	1.68 ± 1.03^{c}
Viscosity	2.52 ± 1.26^{ns}	2.76 ± 1.05^{ns}	2.00 ± 1.12^{ns}	2.76 ± 0.97^{ns}
Turbidity	3.12 ± 1.30^{a}	2.16 ± 1.07^{b}	2.48 ± 0.92^{b}	2.24 ± 0.97^{b}

Data shown as the mean of repetitions ± standard deviation (SD). Different superscript letters on the same horizontal line show significant differences ($p<0.05$). Sensory test scores from 1 to 4 represent: very high, high, low, very low

were 1.16 %, 1.96 %, 2.80 % and 4.14 %, respectively. Longer fermentation periods were associated with the higher activity of yeast and alcohol-producing microbes. The microbes that are primarily responsible for producing alcohol in kefir grain is yeast (*Saccharomyces cerevisiae*) (de Melo Pereira, Ramos, Galvao, Souza Dias & Schwan, 2010). Some *Lactobacillus* strains also have the ability to produce alcohol because they have alcohol-dehydrogenase that can convert substrates into ethanol (Magalhães-Guedes, Pereira, Campos, Dragone & Schwan, 2011).
A study on pomegranate and orange juice kefir by Kazakos et al. (2016) found that the alcohol level was below 1 %. Similar results were also found in brown sugar (Magalhães-Guedes, Pereira, Dias & Schwan, 2010) and cow's milk kefir (Zajsek & Gorsek, 2010). In general, the alcohol content of kefir usually ranges from 0.5 to 2% depending on the substrate used (Setyawardani, Rahardjo, Sulistyowati & Wasito, 2014). The higher alcohol content observed in green coconut was possibly due to the higher sugar content of 21 mg/mL, consisting of sucrose, glucose and fructose, in green coconut water (Yong et al., 2009). These sugar matrices stimulate the metabolism of kefir yeast, resulting in increased concentrations of ethanol, glycerol and esters in the final product. These metabolites provide the distinct sensory characteristics of kefir such as refreshing flavor, fruity aroma and texture (Fiorda et al., 2017). In conclusion, fermentation for 12 hours gave the best alcohol contentamong the treatments.

Water content

It was found that the average water contents of green coconut kefir, with a fermentation time of 12, 24, 36 and 48 hours were 97.14 %, 97.19 %, 97.1 % and 97.35 %, respectively. Fermentation time had a significant effect on water content but there was no significant difference between green coconut water kefir of treatments T1, T2 and T3. Magalhães-Guedes et al. (2010) and Rocha-Gomes et al. (2018) also found similar results in Brazilian sugary water kefir and brown sugar water kefir within a range of 95-98%. The high water content of kefir in the current study was also caused by the largest component of the medium which consists of 95% water (Yong et al., 2009), hence the name water kefir.
The water content tends to increase with longer fermentation period. Currently, research on physicochemical properties of water kefir, especially water content, is still limited. However, the increase of water content that occurred after 48 hours of fermentation was allegedly due to the decreasing ability of kefir grains to retain moisture. Kefir grain is a matrix of exopolysaccharide (EPS) which is capable of binding with water in aqueous solution (Wang, Zhao, Tian, Yang & Yang, 2015). The reduction of EPS or kefiran might also give rise to this finding, where more moisture is available in the product. As reported by Kok-Tas, Seydim, Ozer and Guzel-Seydim (2013), enzymatic degradation of EPS occurs during fermentation and the storage period of kefir, and leads to a decrease in EPS content.

Protein content

Based on the results, fermentation time affected the protein content in green coconut water kefir. Protein levels significantly decreased from T1 to T4, although T2 was not significantly different to T3. This is consistent with the results of Mechmeche, Kachouri, Ksontini, Setti and Hamdi (2018) who found that kefir can reduce protein levels and increase antioxidant activity during fermentation through the production of bioactive peptides. Protein content of samples throughout the fermentation period ranged between 4.05% and 6.04%. These results were higher than the protein content of Brazilian sugary water kefir fermented for 24 hours which was 0.4% (Magalhães-Guedes et al., 2010) and brown sugar water kefir which was 0.27% after 48 hours fermentation (Rocha-Gomes et al., 2018).
The protein content of a fermented product usually increases with fermentation time due to the increase of microbial biomass and secretion of protein molecules (Magalhães-Guedes et al., 2011). A different result was obtained in this study where the protein content decreased with increasing fermentation time. This is supposedly due to an inadequate supply of nutrients after 24 hours or a medium acidity level that is incompatible with the microbes' survival and leads to their death. The type and amount of protein con-

tained in the main ingredient may also affect the quality of kefir where the protein may coagulate during fermentation due to lactic acid accumulation and produce different functional peptides (Shi, Chen, Li, Huang & He, 2018). Overall, T1 (with 12 hours of fermentation time) had the best result among the treatments.

Fat content

As shown in Table 2, there was no significant differences in the fat content of water kefir with different fermentation times ($P > 0.05$). The fat content produced at 12, 24, 36 and 48 hours of fermentation were 1.7 %, 1.95 %, 1.75 % and 1.67 %, respectively. These findings suggest the duration of fermentation did not affect fat content of water kefir. However, there was a decrease if fat content from 24 hours to 48 hours of fermentation. This is possibly due to the lipases produced by the kefir grain (Gonzalez-Sanchez, Azaola, Gutierrez-Lopez & Hernandez-Sanchez, 2010). Another explanation is the production of invertase enzyme by microbes in kefir grain, which hydrolyze sucrose into glucose and fructose that are subsequently transformed into organic acids by yeast and LAB (Fiorda et al., 2017).
Fat is a minor component of green coconut water kefir, with a fat content lower than that in milk kefir (2.34%) fermented for 24 hours (Magalhães-Guedes et al., 2011). This is in accordance with the observations of Prades et al. (2012) who reported the fat content of coconut water to be around 0.33 g/100 mL. A low fat content makes water kefir a good alternative for those with cholesterol issues who seek a low calories beverage with similar health benefits as milk kefir. Fat content affects the texture of kefir. A higher fat content will increase water holding capacity (WHC) of the product and cause a firmer consistency and higher viscosity (Gul et al., 2018).

3.2 Sensory evaluation

Sensory test results for green coconut water kefir included level of sourness, soda sensation, sour aroma, viscosity and turbidity (Table 3).

Level of sourness of green coconut water kefir

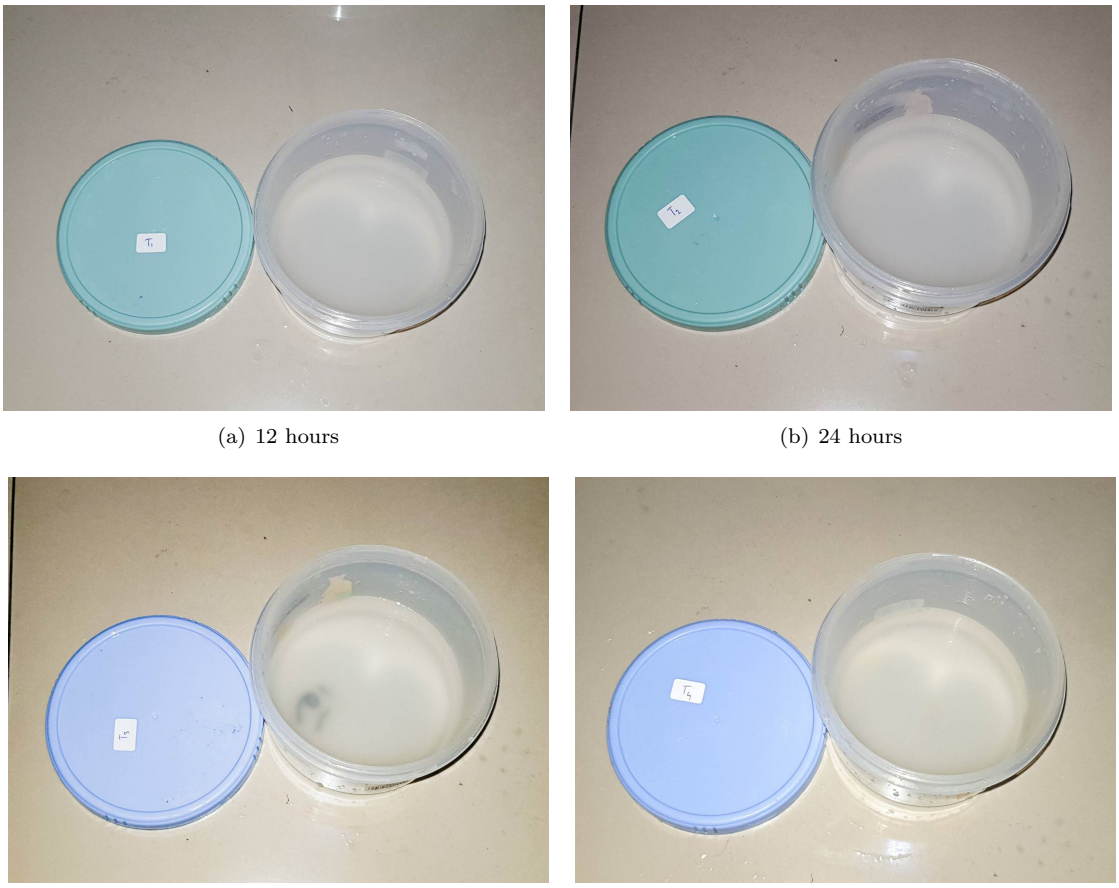
Sensory test results on green coconut water kefir showed that differences in fermentation time had significant effects ($P < 0.05$) on the level of sourness. As shown in Table 3, panelists could distinguish differences in the level of sourness of treatments. However, the level of sourness for the T2 (24 hours) and T4 (48 hours) treatments tended to be indistinguishable. The T3 (36 hours) treatment was known to have the highest sourness caused by the fermentation process. Fermentation by kefir grain produces lactic acid as the main metabolite. Acetic acid, glycerol and mannitol were also produced in low concentrations (Laureys & De Vuyst, 2014). The level of sourness should increase with increasing fermentation time. However, this did not occur in T4 where kefir grain cells entered the death phase due to an excessive fermentation process. Longer fermentation times can cause the accumulation of metabolites (lactic acid and carbon dioxide) which can then inhibit the growth of cells and result in a non-optimal fermentation process (Yuliana, 2012).

Soda sensation of green coconut water kefir

The soda sensation is the impression of numbing, burning or biting when consuming food products containing carbon dioxide (Kappes, Schmidt & Lee, 2007). Sensory tests on green coconut water kefir showed that the panelists could not distinguish the soda sensation between different treatments. The bursting of carbon dioxide bubbles is a metabolite result of sugar conversion by microorganisms (Wu et al., 2010). A limited sugar content in green coconut water could only produce a small amount of carbon dioxide through yeast fermentation thus the inability to distinguish between treatments.

Sour Aroma of Green Coconut Water Kefir

The differences in fermentation time had significant effects ($P < 0.05$) on the sour aroma of green coconut water kefir. The intensity of



(a) 12 hours (b) 24 hours

(c) 36 hours (d) 48 hours

Figure 1: Green coconut water kefir fermentation

sour aroma increased with fermentation time. This is shown in Table 3. The average value of sour aroma was the lowest in T4 (48 hours). Sour aroma increased due to the presence of volatile compounds during the fermentation process. Kefir grains produced aroma-forming compounds due to the presence of volatile compounds such as acetaldehyde, acetone, ethyl acetate, 2-butanone, diacetyl and ethanol (Cheng, 2010). The longer the fermentation time, the more volatile compounds that are produced, increasing the intensity of the sour aroma (Beshkova, Simova, Frengova, Simov & Dimitrov, 2003).

Viscosity of Green Coconut Water Kefir

Sensory tests showed that panelists could not distinguish the viscosity of the green coconut water kefir across the different treatments. This is also in accordance with the quantitative analysis of viscosity that showed there were no significant differences in viscosity with fermentation time. The viscosity of a solution tends to increase with the addition of ingredients such as sweeteners or fibers (Mattes & Rothacker, 2001). However, there was no addition of those ingredients in the manufacturing process of green coconut water kefir, thus viscosity was not affected. Furthermore, as green coconut water contains only a low amount of protein the resulting viscosity may

not change significantly as fermentation time increases. In a fermented beverage, protein denaturation could lead to texture thickening of a finished product (Novelina, Sayuti & Rahmadani, 2013).

Turbidity of Green Coconut Water Kefir

Sensory tests of green coconut water kefir showed that the differences in fermentation time had a significant effect ($P < 0.05$) on turbidity. As shown in Table 3, panelists were able to distinguish the turbidity of green coconut water kefir between T1 (12 hours) and the other treatments. However, T2 (24 hours), T3 (36 hours) and T4 (48 hours) tended to be indistinguishable. As shown in Figure 1(a), T1 (12 hours) produced a clearer solution than other treatments. While T2 (24 hours), T3 (36 hours) and T4 (48 hours), as presented in Figures 1(b)-1(d), were apparently similar. Panelists considered that T1 produced a lower turbidity intensity than the other treatments due to a shorter fermentation time. Under a short fermentation time, cells in the kefir grain were still in the adaptation phase, whilst in the other treatments the cells were already in the growth phase and thus increasing in number. This is in accordance with Parhusip and Kusuma (2003) who stated that the greater the number of microbes, the higher the turbidity of solutions.

4 Conclusions

This study showed that longer fermentation times were associated with less favorable physical and chemical characteristics in green coconut water kefir. The ideal fermentation time for producing green coconut water kefir was 12 hours, resulting in a pH of 4.6, viscosity of 0.09 cP, TDS of 3.8^o Brix, alcohol content of 1.16 %, water content of 97.14 %, protein content of 6.64 %, fat content of 1.17 % and a lower level of sourness that was considered more acceptable by the panelists.

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