

Development of mature coconut (*Cocos nucifera* L.) probiotic beverage: Physicochemical characteristics, microbial count, antioxidant activity, and sensory acceptance

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Abstract

Mature coconut (*Cocos nucifera* L.) water is a by-product of coconut milk and oil industries that can be transformed into value-added products as part of a sustainable and zero-waste effort. In the present work, mature coconut water was fermented using kefir grains to produce probiotic beverage, and the present work aimed to evaluate the physicochemical characteristics, microbial counts, antioxidant activities, and sensory acceptance of the developed mature coconut water kefir (MCWK) in comparison with mature coconut water (MCW) and water kefir (WK). Results showed significantly higher ($p < 0.05$) lactic acid and ethanol contents in MCWK relative to WK, which was attributed to the higher counts of lactic acid bacteria and yeasts. The fermentation was also apparent in MCWK, exhibiting significantly lower ($p < 0.05$) pH value at different fermentation days. Significantly ($p < 0.05$) highest total phenolic content (TPC) and antioxidant activities [DPPH radical-scavenging activity and ferric-reducing antioxidant power (FRAP)] were recorded by MCWK after three days of fermentation. Sensory acceptance test also demonstrated that fermentation of MWK with kefir grains improved the palatability and acceptance. MCWK fermented for three days resulted in significantly ($p < 0.05$) highest overall acceptance mean score ($n = 30$). In conclusion, the complex microbial consortia in kefir grains can be used to produce MCW probiotic beverage with functional properties.

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Introduction

Unlike young coconuts, mature coconuts are usually used to produce coconut milk and oil. Therefore, the mature coconut water is often discarded, particularly in large coconut milk industries, whereby only the coconut meat is used (Tan *et al.*, 2014; Kantachote *et al.*, 2017). Coconut water consists of water, sugar alcohols, lipids, amino acids, nitrogenous compounds, organic acids, and enzymes (Yong *et al.*, 2009); however, the composition can vary depending on factors such as cultivars, maturity stage, growing conditions, and nutrition (Burns *et al.*, 2020). Young coconut water is widely consumed as a soothing recreational beverage as well as sports rehydration beverage owing to its

electrolyte's composition (Kannangara *et al.*, 2018; Rodsamran and Sothornvit, 2018).

Mature coconuts are those that are aged over nine months (270 days) after pollination (Burns *et al.*, 2020), and generally, their water still contains substantial nutritional value aside from some metabolic changes occurring as the fruit matures. Reduction in total sugar and solids, as well as development of a slight sourish taste occur as the coconut fruit matures, cause the mature coconut water to lose its palatability (Chauhan *et al.*, 2014). As the coconut matures, the dominant sugar contained in the coconut water shifts from majority fructose (reducing sugar) to sucrose (non-reducing sugar) (Solangi and Iqbal, 2011). Furthermore, although the protein content in coconut water is relatively low, Tan *et al.*

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(2014) reported that the protein level increases significantly as the coconut matures. Past researchers have reported that the phytonutrient-rich coconut water delivers numerous nutritional benefits such as anti-obesity, rheumatism reliver, hepatoprotective, anaemia prevention, anti-hypercholesterolemia, and antioxidative properties (Chauhan *et al.*, 2014; Zulaikhah *et al.*, 2019; Segura-Badilla *et al.*, 2020; Kumar *et al.*, 2021). Mahayothee *et al.* (2015) reported that the total phenolic content (TPC) and antioxidative activities (DPPH and ABTS) of coconut water increased up to 190 days after pollination.

Fermenting beverages can be a two-pronged strategy which is to prolong the shelf life of product and add functionality to the product. Water kefir, also known as sweet or sugar kefir, is a form of fermented beverage raved for its health benefits, similar to its relative, the milk kefir. However, milk kefir may not be suitable for vegan and lactose-intolerant consumers (Fiorda *et al.*, 2017; Farag *et al.*, 2020). Therefore, water kefir is a great option for those who cannot consume dairy products but still want to reap the health benefits of kefir. Sucrose or brown sugar is added with kefir grains to allow fermentation instead of using common milk substrates. Fruit juices or the addition of dried fruits can also be used as a modification of the starting medium to produce water kefir with enhanced flavour profile and desirability (Gulitz *et al.*, 2011; Fiorda *et al.*, 2017; Farag *et al.*, 2020). Apart from the probiotic function, water kefir contains a multitude of microbial consortia and bioactive compounds with various health benefits (Tu *et al.*, 2019). Kefir grain diversified microbiota include various species of lactic acid bacteria (10^8 CFU/g), yeasts (10^6 - 10^7 CFU/g), and acetic acid bacteria (10^5 CFU/g), all connected to kefiran, a polysaccharide matrix (Prado *et al.*, 2015). Dwiloka *et al.* (2020) reported that fermentation time had significant effects on physicochemical (pH, total dissolve solid, water, and protein content) as well as sensory characteristics of green coconut water kefir. Their findings also showed that 12 hours of fermentation was the best for their kefir.

Current market trends of health-conscious consumers are moving toward the production of crafted functional products such as kefir (Wrage *et al.*, 2019). The fermentation process in kefir may enhance its nutritional benefits due to formation of secondary bioactive components. Therefore, in the present work, a mature coconut water-based probiotic drink was developed using water kefir grains, and

thereafter characterised for its microbial, physicochemical, and sensorial properties.

Materials and methods

Materials

Water kefir grains without medium were purchased from Bagogen Life Beverage, Kuala Lumpur, Malaysia. The kefir grains were activated in a 10% sugar solution (sugar dissolved in pre-boiled water) for 3 d prior to use. Mature coconut water (MCW) was supplied by Linaco Resources Sdn. Bhd. in frozen form, and kept at -18°C until further analyses. The MCW was then used for the preparation of mature coconut water kefir (MCWK). Sugar was purchased from a local supermarket. Chemicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 4,6-tris (2-pyridyl)-S-triazine (TPTZ), and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). De Man, Rogosa and Sharpe (MRS) agar, Maximum Recovery Diluent (MRD), and Potato Dextrose Agar (PDA) were purchased from ThermoFischer Scientific (Hampshire, UK). All other chemicals and reagents used were of analytical grade.

Preparation of mature coconut water kefir (MCWK) and water kefir (WK)

The preparation of kefir samples was adapted from Lestari *et al.* (2018). A glass jar was sterilised using hot boiling water, and allowed to cool at room temperature. Mature coconut water was subjected to pasteurisation at 75°C for 5 min, and cooled at room temperature before inoculation (Randazzo *et al.*, 2016). Water kefir grains were strained from the activating sugar solution, and mixed into mature coconut water at 5% (w/v) kefir grains in the sterilised glass jar. The mature coconut water kefir (MCWK) was fermented at 25°C for 6 d. Fresh sugar solution (10%) was prepared by dissolving sugar in pre-boiled water. The sugar solution was then mixed with kefir grains 5% (w/v) to produce water kefir (WK). WK was used to compare with the newly developed MCWK. WK and MCWK samples were collected at day 1 (MCWK1 and WK1), day 3 (MCWK3 and WK3), and day 6 (MCWK6 and WK6) of fermentation for analyses.

Determination of pH value and viscosity

pH was measured using an electronic pH meter (Mettler Toledo M210) following the method by

Laureys and De Vuyst (2014) but without the saline rinsing. Viscosity was measured using a rheometer (Anton Paar Physica MCR301) at a shear rate of 200 s⁻¹ with cone and plate geometry (Will *et al.*, 2008). Both analyses were performed in triplicates.

Determination of lactic acid formation

Lactic acid content was measured using high-performance liquid chromatography (Waters 2998 USA) with a photodiode array detector and Waters XBridge column (150 mm × 5 µm) at 60°C (Magalhães *et al.*, 2011). A total of 5 mM of sulphuric acid was used as an eluent at a flow rate of 0.5 mL/min, and the volume of the sample used was 20 µL. Standard curves were prepared using lactic acid (0.1 - 1.0%).

Determination of ethanol formation

Ethanol content was measured using Agilent 6890 (USA) gas chromatography (GC) with flame ionisation detector (FID) (Šertović *et al.*, 2019). Hydrogen was used as a carrier gas at a speed of 8 mL/min, while the injection temperature was adjusted to 210°C, and the detector temperature was 230°C. Ethanol solutions at concentrations 0.1 to 3.0% (v/v) were used as reference to identify the ethanol peaks on the chromatogram (Ebersole *et al.*, 2017).

Determination of total phenolic content

Total phenolic content was measured using Folin-Ciocalteu reagents as described by Aziz *et al.* (2018a). A total of 1 mL of the sample was mixed with 5 mL 10% Folin-Ciocalteu reagent and 4 mL 10% (w/v) of sodium carbonate. The mixture was stored in the dark for 90 min. Absorption at 760 nm was measured using Epoch™ Microplate spectrometer, BioTek® Instrument (USA). The standard curve was plotted using gallic acid at 0 - 100 ppm concentration. TPC was expressed as gallic acid equivalents (GAE) in milligrams per gram dry material.

In vitro antioxidant determination

Determination of DPPH radical scavenging activity

Radical scavenging activity was measured by mixing 1 mL sample with a methanolic solution of 2 mL 0.1 mM DPPH (Mokhtar *et al.*, 2021). The mixture was shaken and stored in the dark for 30 min, after which the absorbance of the solution was measured using a spectrophotometer at 517 nm. Standard curve was prepared by using standard

ascorbic acid at various concentrations (0 - 100 ppm). Antioxidant activity was expressed as ascorbic acid equivalent with antioxidant capacity in milligram units per gram of sample (mg AEAC/g).

Determination of ferric-reducing antioxidant power (FRAP)

Ferric-reducing antioxidant power (FRAP) was measured as described by Hashim *et al.* (2018). A total of 2 mL FRAP solution (300 mM acetate buffer, pH 3.6: 10 mM tripyridyltriazine solution (TPTZ): 20 mM ferric chloride solution (in 40 mM HCl) at 10: 1: 1 volume ratio) was mixed with 100 µL of the samples. After incubation at room temperature for 30 min, the absorbance was measured at 593 nm using the Epoch™ Microplate spectrometer, BioTek® Instrument (USA). The standard curve was plotted using the standard sulphate at various concentrations (0.1 - 1.0 mM), and the results were expressed as ferric sulphate equivalents in millimolar units per gram sample (mM FeSO₄/g).

Microbial growth assessment

Lactic acid bacteria colony was cultured on De Man, Rogosa and Sharpe (MRS) (Cetinkaya and Elal, 2012), and pour-plating method was used to enumerate them. Samples were homogenised with maximum recovery diluent (MRD) solution in the stomacher bag for 2 min and 10⁻⁷ serial dilutions were made. From the three highest dilutions, 1 mL of suspension was transferred onto an empty plate, and 15 - 20 mL of MRS agar was poured into the plate. Plates were placed at 30°C for 72 h before colony counting was carried out.

Acidified Potato Dextrose Agar (PDA) was used to culture yeasts (Cetinkaya and Elal, 2012), and spread-plating method was used to enumerate them. Around 15 - 20 mL of sterilised agar was poured into an empty plate, and stored in the refrigerator (4°C). The sample was homogenised with MRD solution in the stomacher bag for 2 min and 10⁻³ serial dilutions were prepared. The samples of the three highest dilutions were transferred to plates containing the agar. The plates were stored at 22°C for 5 d before colony counting was carried out.

Enumeration of Enterobacteriaceae, total coliforms, and *Escherichia coli* (*E. coli*) were carried out on the pasteurised mature coconut water and fermented coconut water kefir (Temeli *et al.*, 2006). For water kefir, the sugar solution before and after fermentation were also tested. Enterobacteriaceae

was enumerated using double layered pour-plate method in violet red bile glucose agar (VRBGA). Colonies formed were counted after aerobic incubation at 37°C for 24 h, and total coliform was enumerated using most probable number (MPN) method using lauryl sulphate tryptose broth as the media for presumptive test. Enumeration of *Escherichia coli* (*E. coli*) was carried out using MPN method by streaking a loopful of culture from any gas positive lauryl sulphate tryptose tubes onto eosin methylene blue agar (EMBA).

Sensory acceptance test

A total of 30 semi-trained panellists (10 males and 20 females; aged 20 to 40 years old) consisting of undergraduate and postgraduate students of the Department of Food Sciences were involved. A 7-point hedonic test was used to determine the degree of acceptability (Aziz *et al.*, 2018b). The comparison was carried out to evaluate if the fermentation of MCW into kefir could improve its acceptability. The panellists were randomly served four chilled (< 4°C) samples namely mature coconut water (MCW),

MCWK1, MCWK3, and MCWK6. Attributes evaluated were colour, aroma, sweetness, sourness, mouthfeel, fizziness, and overall acceptance.

Statistical analysis

The physicochemical measurements were all performed in triplicate, and results were expressed in mean \pm standard deviation. Results were then analysed using one way analysis of variance (ANOVA), and Pearson's correlation coefficients was used to demonstrate the correlation between physicochemical, functional, and microbiological responses using Minitab 19. Results were deemed significant when $p < 0.05$.

Results and discussion

Physicochemical analyses on coconut water and kefir samples

Figure 1 shows the pH for MCWK, WK, and MCW following one, three, and six days of fermentation.

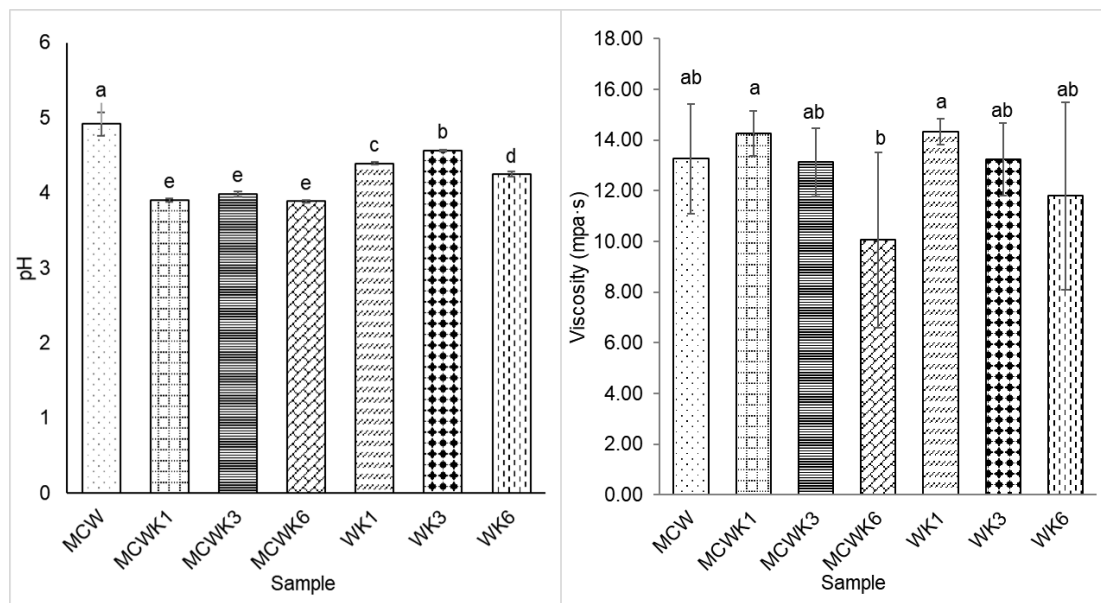


Figure 1. pH and viscosity values of mature coconut water kefir (MCWK) and water kefir (WK) following 1-, 3-, and 6-day fermentation, and mature coconut water (MCW). Different lowercase superscripts on the histogram bars indicate significant difference at $p < 0.05$.

Results showed that there were significant differences ($p < 0.05$) among the different types of samples. Both kefir types recorded significantly ($p < 0.05$) lower pH as compared to the unfermented MCW, thus indicating effectual fermentation was achievable by the kefir grains. All MCWK at different fermentation days were observed to have

significantly lower ($p < 0.05$) pH value as compared to MCW and WK. Although WK samples also underwent fermentation, MCWK naturally contained organic acids such as malic, citric, tartaric, and succinic acids (Tan and Easa, 2021). The lower pH in MCWK could also indicate the MCW's ability to support the growth of the fermentation culture, in this

case, the growth of lactic acid bacteria that might have reduced the pH value (Katsiari *et al.*, 2002). This corresponded to the significant ($p < 0.05$) negative Pearson correlation (r) shown in Table 1, thus indicating that the decrease of pH value correlated with the increase of lactic acid content as well as lactic acid bacteria (LAB) count. Lactic acid in water kefir contributes to the fresh sour taste (Laureys and

De Vuyst, 2014). The pH decreasing trend observed in the present work was similar with Dwiloka *et al.*'s (2020) report on green coconut water kefir showing significantly ($p < 0.05$) lower pH after only 24 hours of fermentation. Their findings also showed no significant difference of pH observed after 36 or even 48 hours of fermentation.

Table 1. Pearson correlation (r) of physicochemical, functional, and microbiological responses measured on MCW, MCWK, and WK.

	pH	Viscosity	Lactic acid	Ethanol	LAB count	Yeast count	DPPH	FRAP
Viscosity	0.212							
Lactic acid	-0.835*	-0.182						
Ethanol	-0.723*	-0.368	0.858*					
LAB count	-0.913*	-0.096	0.792*	0.697*				
Yeast count	-0.931*	-0.094	0.889*	0.772*	0.976*			
DPPH	0.077	0.018	0.387	0.310	-0.137	0.034		
FRAP	-0.127	-0.089	0.583*	0.497*	0.067	0.241	0.940*	
TPC	0.002	-0.039	0.491*	0.389	-0.094	0.090	0.949*	0.969*

* Significant at $p < 0.05$.

The viscosity of MCW, MCWK, and WK showed no significant difference ($p > 0.05$) between the types of samples (Figure 1), but there was significant difference ($p < 0.05$) between MCWK6 with MCWK1 and WK1. Although inconclusive, the overall viscosity trend of kefir samples was found to decrease with longer fermentation time. According to Manjunatha and Raju (2013), the viscosity started to decrease when total solid particles in coconut water decreased. Dissolved solids in the form of glucose, fructose, and sucrose will be used up during the fermentation due to microbial activities (Dwiloka *et al.*, 2020). In a water kefir study conducted by Irigoyen (2005), the viscosity was found to decrease from 425 to 188 mPa s as the period of storage increased from day 1 to day 28. Apart from ethanol, glycerol can also be produced from the alcoholic fermentation of yeasts in water kefir. The slight differences might have been due to the viscosity contributed by glycerol (Laureys and De Vuyst, 2014).

Lactic acid and ethanol contents for MCW, MCWK, and WK are shown in Table 2. All MCWK samples recorded significantly higher ($p < 0.05$) lactic acid content as compared to MCW and WK. The initial amount of sugar in coconut water might have

triggered the process of fermentation, hence producing more lactic acid as the main product. As earlier mentioned, pH and lactic acid content were found to be significantly ($p < 0.05$) correlated. Lactic acid is the main metabolite of the LAB species (Laureys and De Vuyst, 2014). Cheirsilp *et al.* (2018) described the production of lactic acid in kefir as a result of LAB growth, specifically *Lactobacillus kefiranofaciens*. The pH of all MCWK samples were around 4 or lower. The decrease in pH below 4 has been reported to inhibit or slow down further cell growth in the kefir (Cheirsilp *et al.*, 2018). This subsequently can be seen manifested in the similar microbial growth of all MCWK samples as will be discussed later.

In terms of ethanol content, all MCWK samples had significantly ($p < 0.05$) higher ethanol content as compared to MCW and WK. Yeast metabolism in the kefir grains used monosaccharides in coconut water to produce ethanol and carbon dioxide. Apart from lactic acid, other organic acids such as hexanoic, octanoic, and decanoic acid were involved in the production of ethanol as a result of fatty acid biosynthesis pathway in yeasts which contributed to the fruity and floral notes in the resulting kefir (Laureys and De Vuyst, 2014).

Table 2. Antioxidant analysis, lactic acid, and ethanol content of mature coconut water kefir (MCWK) and water kefir (WK) following 1-, 3-, and 6-day fermentation, and mature coconut water (MCW).

Assay	MCW	MCWK1	MCWK3	MCWK6	WK1	WK3	WK7
Lactic acid (%)	< 0.1 ^c	0.6212 ± 0.0112 ^b	0.6130 ± 0.0093 ^b	0.7105 ± 0.0163 ^a	0.0006 ± 0.0006 ^c	< 0.1 ^c	< 0.1 ^c
Ethanol (%)	< 0.1 ^b	1.0773 ± 0.5380 ^a	1.6246 ± 0.9508 ^a	1.6224 ± 0.5927 ^a	< 0.1 ^b	< 0.1 ^b	0.1929 ± 0.0637 ^b
Free radical scavenging activity	15.48 ± 1.9287 ^c	18.44 ± 0.8590 ^b	23.33 ± 1.098 ^a	19.86 ± 0.9150 ^b	9.52 ± 0.0714 ^e	11.62 ± 0.7346 ^d	11.16 ± 0.9946 ^{de}
DPPH (mg AEAC/g)	0.312 ± 0.0445 ^c	0.377 ± 0.0345 ^b	0.463 ± 0.0521 ^a	0.428 ± 0.0142 ^{ab}	0.028 ± 0.0216 ^e	0.090 ± 0.0107 ^d	0.067 ± 0.0234 ^{de}
Ferric-reducing antioxidant power (FRAP) (mM FeSO ₄ /g)	27.218 ± 1.172 ^c	28.636 ± 0.718 ^{bc}	43.885 ± 1.902 ^a	29.630 ± 0.780 ^b	1.765 ± 0.398 ^d	3.344 ± 1.115 ^d	1.852 ± 0.323 ^d

Values are mean ± SD of three replicates ($n = 3$). Means followed by different lowercase superscripts in a row are significantly different at $p < 0.05$.

In vitro antioxidant activity and total phenolic content of MCW, MCWK, and WK

It was found that MCWK3 exhibited significantly ($p < 0.05$) highest free radical scavenging activity (23.33 ± 1.098 mg AEAC/g) as compared to all other samples tested (Table 2). All MCWK samples had significantly ($p < 0.05$) higher DPPH scavenging activity as compared to the unfermented MCW. Similar trend was also exhibited by FRAP results, in which MCWK samples scored significantly ($p < 0.05$) higher FRAP activity as compared to MCW. This indicated that the fermentation process by the kefir grains had generated antioxidative bioactive compounds. Furthermore, probiotics microorganisms such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Streptococcus* spp. have been reported to scavenge reactive oxygen species, consequently contributing to antioxidant properties (Fiorda *et al.*, 2017).

In terms of TPC, there was a significant difference ($p < 0.05$) between MCW with MCWK3, MCK6, and all WK samples. The significantly ($p < 0.05$) highest TPC was observed in sample MCWK3 at 43.885 ± 1.902 mg GAE/g. Mahayothee *et al.* (2015) reported that the TPC of coconut water increased up to 190 days after pollination of the coconut fruits. Both DPPH and FRAP were found to be significantly correlated ($p < 0.05$) with TPC as shown in Table 1, thus suggesting that the antioxidant activity might have been contributed by phenolic substances (Razali *et al.*, 2019). Coconut water have been widely reported to possess antioxidative properties as well as phenolic compounds, specifically catechin, salicylic acid, and smaller amounts of caffeic, ferulic, and *p*-coumaric acids (Mahayothee *et al.*, 2015; Watawana *et al.*, 2016). Catechin have been widely reported to possess beneficial health properties such as anti-cancer, obesity control, anti-hypoglycaemic, anti-inflammatory, hepatoprotective, and neuroprotective effects (Isemura, 2019; Gutiérrez-Gamboa *et al.*, 2020). However, the concentrations of (+)-catechin and (-)-epicatechin in coconut water reported by Chang and Wu (2011) were 0.344 and 0.242 $\mu\text{g/mL}$, which are tremendously low in comparison to catechin-rich food such as green tea (> 1 mg/g) (Zhang *et al.*, 2014). Sabokbar and Khodaiyan's (2016) findings on pomegranate juice kefir also found that TPC significantly increased as the fermentation time was extended. This is due to the metabolic

activities of the microbial consortium in the kefir grains altering the bioactive compounds, such as the various phenolic components. The increase in TPC up to day 3 might also have been due to the release of some conjugated phenolics, either with sugar or proteins. The unbinding of these phenolic substances was suspected to correlate with the increase in the antioxidative properties as well (Saharan *et al.*, 2017). Furthermore, during fermentation, complex phenolic substances can undergo hydrolysis to a simpler and more potent form. Prolonged fermentation on the other hand has been reported to degrade the phenolic substances (Fernandez-Orozco *et al.*, 2008). However, further research could be carried out to elucidate the types of compounds generated during coconut water kefir fermentation. Foods with high antioxidant and phenolic substances have been commonly shown to enhance and support human well-being (Tu *et al.*, 2019), hence the developed MCWK in the present work was proved to have potential as a functional beverage.

Microbial growth evaluation of MCW, MCWK, and WK

The counts of LAB and yeasts for MCW, MCWK, and WK samples can be observed in Figure 2. No significant difference ($p > 0.05$) of LAB and yeast counts was detected between all MCWK regardless of fermentation days; however, all MCWK samples recorded significantly ($p < 0.05$) higher LAB and yeast counts as compared to both WK and MCW samples. This might have been due to the conducive composition of MCW as a medium for the growth of LAB. Laureys and De Vuyst (2014) reported that the most common species of bacteria found in WK were *L. casei*, *L. harbinensis*, *L. hilgardii*, *B. psychraerophilum*, *S. cerevisiae*, and *D. bruxellensis*. *L. casei* was found to be active at low pH around 3.0 (Estifanos, 2014). This result was in agreement with a previous finding, where the count of *L. plantarum* increased at the beginning of fermentation process, and decreased after 10 days of storage (Prado *et al.*, 2015). The LAB activity started to decrease with the decrease in pH value. Both mature coconut water and water kefir were absent of Enterobacteriaceae, total coliforms, and *E. coli* before and after the fermentation with kefir grains.

Sensory acceptance of MCW and MCWK samples

Sensory acceptance results for MCW and

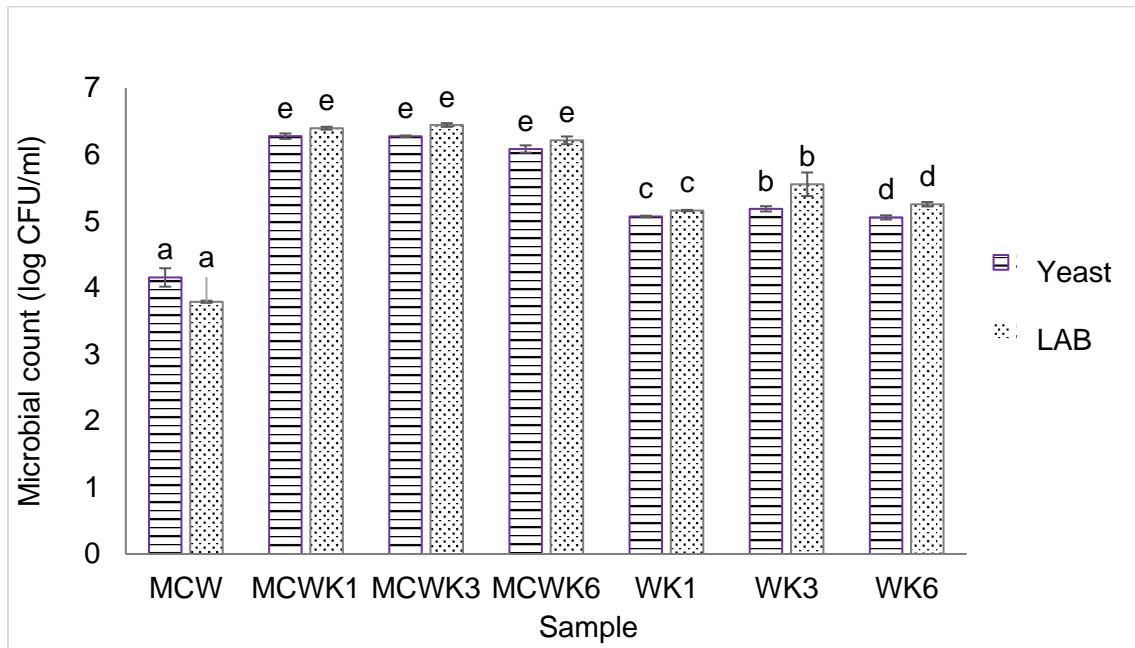


Figure 2. Yeast and lactic acid bacteria (LAB) counts of mature coconut water kefir (MCWK) and water kefir (WK) following 1-, 3-, and 6-day fermentation, and mature coconut water (MCW). Different lowercase superscripts on the histogram bars indicate significant difference at $p < 0.05$.

MCWK fermented for one, three, and six days are presented in Table 3. Results showed that colour, aroma, sweetness, and mouthfeel preference of the panellists had no significant difference ($p > 0.05$) for MCW, MCWK1, and MCWK3. However, the panellists preferred the sourness of MCW1 as compared to the unfermented MCW. Fizziness preference score was significantly higher for MCWK3 and MCWK6 as compared to MCW and MCWK1. Apart from lactic acid, carbon dioxide is also formed as the fermentation product that

contributes to the slight fizziness sensation in kefir beverages (Otlés and Cagindi, 2003; Arslan, 2015). Furthermore, MCW fermented up to three days recorded significantly ($p < 0.05$) highest score (5.92 ± 0.78) for overall acceptance. The symbiotic metabolic activity of the kefir grains microbiota improved the flavour, thus making the unwanted MCW more favourable (Arslan, 2015). However, prolonging the fermentation process for up to six days resulted in an acidic and pungent flavour that was unacceptable to the panellists.

Table 3. Sensory acceptance of mature coconut water (MCW) and mature coconut water kefir (MCWK) following 1-, 3-, and 6-day fermentation.

Attribute	MCW	MCWK1	MCWK3	MCWK6
Colour	5.58 ± 0.89^a	5.18 ± 1.22^{ab}	5.03 ± 1.12^{ab}	4.42 ± 1.56^b
Aroma	5.35 ± 1.23^a	5.21 ± 1.37^a	5.20 ± 0.93^{ab}	4.43 ± 1.01^b
Sweetness	5.16 ± 1.51^a	4.93 ± 1.51^a	3.98 ± 1.94^{ab}	3.52 ± 2.02^b
Sourness	4.14 ± 1.07^b	5.22 ± 1.06^a	3.32 ± 1.98^{bc}	2.90 ± 1.52^c
Mouthfeel	5.87 ± 0.78^a	5.86 ± 0.52^a	5.12 ± 1.30^a	4.35 ± 1.64^b
Fizziness	3.31 ± 1.37^b	4.16 ± 0.96^b	5.82 ± 0.96^a	5.47 ± 1.78^a
Overall acceptance	4.88 ± 1.04^b	5.15 ± 0.75^b	5.92 ± 0.78^a	4.05 ± 1.06^c

Values are mean \pm SD of 30 replicate ($n = 30$). Means followed by different lowercase superscripts in a row are significantly different at $p < 0.05$.

Conclusion

MCWK fermented up to three days showed high potential to be the next crafted probiotic and functional beverage as it showed significantly ($p < 0.05$) highest total phenolic content (TPC) and antioxidant activities [DPPH radical-scavenging activity and ferric-reducing antioxidant power (FRAP)]. The sensorial acceptance score of MCWK3 was also higher than MCW. Therefore, the utilisation of MCW will transform coconut oil and coconut milk production into a more sustainable and zero-waste industry.

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