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FULL PAPER

Storage stability assessment and quality performance of fermented mature coconut water beverage

Abdul Rahman, I., Mohd Lazim, M.I., Mohamad, S., *Soo Peng, K. and Mohd Asri, M.A.

Food Science and Technology Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia

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Abstract

Nutritional enhancement of mature coconut water was developed using mixed culture fermentation of *Lactobacillus acidophilus* and *Lactobacillus brevis*. The fermented mature coconut water (FMCW) was subsequently formulated to produce a palatable fermented beverage. For the FMCW to be considered safe for consumption, a storage study with a duration of 12 months was done. Prior to storage, the formulated FMCW was pasteurized at 90°C for 30 mins and allowed to cool to room temperature. The formulated FMCW was then stored at two different storage conditions of 4°C and approximately 24°C (refrigerator and room temperatures, respectively). Samplings for microbial growth by counting colony-forming unit (CFU/mL), Brix and pH values, and nutritional contents were done every three months by removing individual bottles of the formulated FMCW products for specific months to monitor the product quality quarterly. The results showed that the physical analyses and nutritional contents were maintained for the whole 12 months duration without any significant changes. In addition, samples at both storage temperatures did not show any microbial growth. The potential spoilage of the FMCW product by the common microbial spoilers was prevented probably by the presence of bio-preservatives in the form of high lactic acid or other antimicrobial compounds produced from the mixed culture fermentation. Therefore, the storage study concluded that the FMCW beverage was stable and remained safe for consumption for at least 12 months when kept at refrigerator and room temperatures.

1. Introduction

Coconut water (CW) is well-known not only for its unique and refreshing taste but also for its benefits in health and sports (Prades *et al.*, 2012). CW is also high in electrolytes such as potassium, calcium and magnesium (Campbell-Falck *et al.*, 2000). Unlike typical sports drinks, CW is naturally lower in sugar, making CW a healthy sports drink. CW was also shown to be consumed in large quantities during sports when compared with sports drinks and plain water (Saat *et al.*, 2002).

Nevertheless, fresh CW can only be found in the tropics, where coconuts are grown, whether natural or cultivated. Although CW is sterile in its shell, it can be easily spoiled by oxidation and contamination by microbes once the coconut shell has been cracked open (Prades *et al.*, 2012). In addition, the weight and size of coconut shells also make the storage and distribution of CW difficult. Therefore, several methods were developed to process CW to preserve its quality, making it lasts longer and ensuring its safety for consumption by reducing or eliminating the side effect from microbial spoilage upon its exposure to air. In addition, many CW beverages are bottled for easy distribution and improved stability.

Like many fruit juices, CW contains sugar, which promotes microbial growth and consequently turns CW rancid and unpalatable. The high temperature of the tropics makes the quality of exposed CW deteriorates even faster. Refrigeration is the most common practice to delay microbial growth and enzymatic degradation of the CW. However, the best approach used in CW processing for production is membrane processing due to the absence of heat to preserve all nutrition and the ease of handling the most common membrane processing technologies include microfiltration and reverse osmosis (Mohon Naik *et al.*, 2020). Conventional thermal heating is cheaper but may cause deterioration in quality and change in colour (Cunha *et al.*, 2020). Both filtration and

*Corresponding author.
Email: *karenkoh@mardi.gov.my*

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heating can effectively remove microbes and deactivate enzymes that contribute to the spoilage of CW. Chemical preservatives such as citric and acetic acids can also be used to limit microbial growth by reducing the pH of CW. Lastly, controlled microbial fermentation is also a viable processing method to preserve CW by producing natural bio-preservatives. However, this process can alter the taste significantly.

Despite the change in taste, there is an increasing number of health-conscious people who appreciate the distinct taste of fermented drinks and also for their health benefits. Fermentation turns ordinary food into functional food, which adds significant value. Fermented drinks such as vinegar can be added during cooking, establishing a unique taste while offering health benefits. Making vinegar is an ancient and cost-effective method to preserve fermented juice for an extended period of time. Vinegar is characterized as having an astringent taste with numerous health benefits, including weight loss, anti-tumour, anti-bacterial, anti-oxidant, and blood glucose control (Chen *et al.*, 2016).

Meanwhile, the coconut milk industry produces a huge amount of waste in the form of mature coconut water (MCW). Even though MCW has a slightly astringent and less palatable taste, certain bioactive compounds are higher than in the water from younger coconuts that are commonly used as CW beverages. For example, the Malayan Green Dwarf coconut variety contains the highest cytokinin in its mature stage (Lazim *et al.*, 2015). Another study also reported higher bioactive compounds which contributed to the anti-diabetic effect in mature CW from the West Coast Tall variety (Preetha *et al.*, 2013). Therefore, the opportunity to convert the waste of mature coconuts water into a functional CW beverage was carried out. Previous work showed that microbial fermentation using selected lactic acid bacteria (LAB) improved the overall quality of the bioactive compounds in MCW, including levodopa (L-DOPA), organic acids, GABA and amino acids profile (Rahman *et al.*, 2018). The fermented MCW was formulated to further improve its taste and palatability level. However, product storage stability studies are needed before the fermented product is deemed safe and edible after a long storage duration. Therefore, a year-long storage study was done to evaluate the product quality stability of the fermented MCW.

2. Materials and methods

2.1 Coconut water preparation

Mature coconuts (*Cocos nucifera* L.) of 6 to 11 weeks were harvested from the Malaysian Agricultural Research and Development Institute (MARDI) coconut plantation (Perak, Malaysia). The coconuts were disinfected with 70% (v/v) alcohol, and the CW was extracted in a sterile environment inside a laminar flow cabinet using autoclaved utensils to reduce risks of contamination. The MCW was stored at -20°C until further use.

2.2 Fermentation process

Starter cultures of *L. acidophilus* and *L. brevis* were prepared according to a method previously done with modifications (Kantachote *et al.*, 2017). The starter culture of each strain was prepared in MCW and incubated at 37°C using a static fermentation process for 24 hrs. Next, 1 % sugar was added into the MCW, and the mixture was pasteurized at 105°C for 10 mins and allowed to cool to room temperature. Then, the pasteurized mixture was inoculated with 3% (v/v) of the starter cultures (co-culture) and incubated at 37°C without any agitation (static fermentation) for 24 hrs. After 24 hrs, the MCW was centrifuged at 10,000 rpm for 10 mins to remove microbial cells. Then, the fermented MCW was formulated, bottled inside amber bottles, and pasteurized at 90°C for 30 mins before packaging.

2.3 Storage of fermented mature coconut water

The final FMCW product, which was prepared in one batch, was divided into two groups of storage temperatures: 4±1°C (refrigerator temperature) and 24±1°C (room temperature). These two groups were further divided into 5 subgroups, designated as 0, 3, 6, 9, and 12 months each to monitor the product quality at the interval of 3 months. Each sub-group was prepared in triplicates. All samples were stored at the designated temperatures, and the sampling was done by removing each triplicate at the end of the designated months (quarterly).

The final FMCW product was checked for any sign of microbial growth on nutrient agar plates by colony-forming unit (CFU/mL) estimation. The pH of samples was measured using a pH meter (Mettler Toledo, Switzerland), while Brix was measured using a hand-held refractometer (Fisher Scientific, USA). The unfermented MCW was used as a negative control. All analyses were done in triplicate. After 12 months, all samples (including the negative control MCW) were sent for microbiology testing (SGS, Malaysia).

2.4 Quantification of L-DOPA using UPLC

L-DOPA content was analysed using the Acquity ultra-performance liquid chromatography (UPLC) (Waters, USA) according to a method done previously with modifications (Danial *et al.*, 2016). A volume of 10

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µL of the sample was derivatized using 70 µL of AccQ-Tag™ borate buffer and 20 µL of AccQ-Tag™ Fluor agent. The mixture was heated to 55°C for 10 mins. Approximately 1 µL of the mixture was injected into the UPLC system. An AccQ-Tag™ Ultra column (2.1 mm × 100 mm, 1.7 µm) was used with a flow rate of 0.7 mL/min and a column temperature of 55°C. The absorbance was read at the wavelength of 260 nm. Mobile phase A was acetonitrile: formic acid: ammonium (10:6:84) and mobile phase B was acetonitrile: formic acid (98:2). The gradient strategy was performed as follows: 99.9% A (0.01% B) maintained from 0.00 to 0.54 mins, followed by a linear gradient of A from 99.9 to 90.9% at 0.54 to 5.74 mins, a linear gradient of A from 90.9 to 78.8% at 5.74 to 7.74 mins, a linear gradient of A from 78.8 to 40.4% from 7.74 to 8.50 mins, a constant flow rate of A at 40.4% for 0.30 mins, a linear gradient of A from 40.4 to 99.9% at 8.80 to 8.90 mins and finally, a constant flow of A at 99.9% for 2.10 mins. L-DOPA standard was used to set up an external calibration curve for quantification.

2.5 Quantification of lactic acid using HPLC

High-performance liquid chromatography equipped with Waters 2695 Alliance Separations Module and Waters 2996 photodiode array detector (Waters, USA) was used to quantify lactic acid. 10 µL of the sample were injected and separated using Synergi™ 4 µm Hydro -RP 80 A column (250×4.6 mm) with a flow rate of 0.6 mL/min and column temperature of 30°C. The mobile phase consisted of mobile phase A (KH_2PO_4) and mobile phase B (water) with a gradient elution program as follows: 100% A maintained from 0.00 to 30 mins, followed by a linear gradient of A from 100 to 0% at 30 to 31 mins, 0% of A remained from 31-45 mins before proceeding to a linear gradient of A from 0 to 100% at 45 to 46 mins and remained constant in flow rate of 100% A at 46-55 mins. The absorbance was read at the wavelength of 190 nm. The pure lactic acid standard was used to set up an external calibration curve for quantification. Both analyses were done in triplicates.

2.6 Statistical analysis

The data are expressed as means ± standard deviations (SD). Results were compared using one-way analysis of variance (ANOVA) using Tukey's post hoc test with 95% confidence using Minitab 19. All experiments and readings were done in triplicates.

3. Results and discussion

3.1 Microbiological study

One of the most important aspects to be considered in a storage study of a product is checking for the potential of microbial growth after long storage. CW can be easily contaminated with autochthonous microbes due to its sugar content, minerals, and amino acids (Walter *et al.*, 2014). Even though most microbes were killed during pasteurization, some microbes especially moulds can survive and spoil the product. Temperatures of between 30°C and 37°C are supportive for moulds to germinate and grow inside the CW. However, the very low pH of the end product could have prevented these moulds from growing. After 24 hrs of controlled fermentation by selected LAB strains, the pH of the MCW dropped from 5.74 to approximately 3.70 and maintained its stability for 12 months (Table 1). The drop in pH was due to the production of a large amount of lactic acid in the FMCW produced by the LAB.

Table 1 shows no sign of microbial contamination with zero CFU/mL on nutrient agar plates at both storage temperatures of 4°C and 24°C, implying that the quality of the FMCW product remained unspoiled for the 12 months storage duration. Further selective microbial growth tests were also done to detect any sign of the growth of yeasts and moulds, *Escherichia coli*, *Staphylococcus aureus*, and any type of microbial growth using total plate count (TPC). In general, no growth was detected for the FMCW product under these specific tests as summarized in Table 2. In contrast, the non-fermented MCW (negative control) failed the yeast and mould test, and TPC, showing signs of microbial growth after 12 months of storage. *E. coli* and *S. aureus* are common pathogens that come from unsanitary conditions. The pasteurization process was shown to successfully eliminate these pathogens in both samples. However, mould is known to be able to survive the pasteurization process and will repopulate once the condition is favourable.

On the other hand, the absence of mould in the FMCW product reinforced the assumption that the microbial growth was inhibited due to its low pH with an average pH of 3.79. Moulds can grow at low pH, but their growth was probably extremely slow especially at unfavourable conditions (Cutter 2002). At that level of acidity, most microbial growth is prevented, but some reports showed the growth of spore-forming bacteria and fungi at a temperature of 30°C (Silva and Evelyn, 2020). The presence of anti-microbial compounds, such as lactic acid could also be a reason that protected the FMCW product from spoilage by moulds. These findings concluded that the FMCW product is safe from microbial contamination. Meanwhile, the Brix value significantly dropped (P<0.05) when comparing the FMCW product to the unfermented MCW (negative control) due to sugar metabolism by the LAB during the 24 hrs fermentation. Other studies also reported sugar metabolism by LAB, which resulted in a reduction in Brix value and an

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Table 1. Analyses of microbial growth (CFU/mL), pH, Brix, lactic acid, and L-DOPA content over 12 months storage periods of FMCW product at two different storage temperatures.

	Months	Microbial growth (CFU/mL)	pH	Brix	Lactic acid (ppm)	L-DOPA (ppm)
MCW (control)	0	0	5.74±0.02[a]	8.3±0.1[a]	N.D.	21.50±0.03[a]
FMCW Product (4°C)	0	0	3.74±0.03[b]	6.7±0.1[b]	12,188.78±2.92[a]	120.50±0.04[b]
	3	0	3.76±0.03[b]	6.7±0.1[b]	12,244.52±5.41[a]	119.27±0.10[b]
	6	0	3.83±0.02[b]	6.8±0.1[b]	12,263.78±4.92[a]	123.95±0.07[b]
	9	0	3.82±0.02[b]	6.7±0.1[b]	12,267.36±4.23[a]	121.73±0.11[b]
	12	0	3.80±0.02[b]	6.8±0.1[b]	12,270.33±4.27[a]	121.12±0.08[b]
FMCW Product (24°C)	0	0	3.74±0.03[b]	6.7±0.1[b]	12,123.08±1.53[a]	121.52±0.03[b]
	3	0	3.73±0.03[b]	7.1±0.1[b]	12,247.10±3.73[a]	122.63±0.04[b]
	6	0	3.81±0.02[b]	7.0±0.2[b]	12,232.02±2.46[a]	123.98±0.05[b]
	9	0	3.83±0.03[b]	6.9±0.1[b]	12,245.77±3.25[a]	123.42±0.10[b]
	12	0	3.80±0.02[b]	7.0±0.1[b]	12,253.47±3.97[a]	122.12±0.07[b]

Values are presented as mean±SD in triplicates. Values with different superscripts within the same row are significantly different at 95% confidence level. FMCW: fermented mature coconut water; L-DOPA: levodopa, N.D.: not detected

Table 2. Microbiology test of MCW and FMCW product after 12 months of storage period

Sample	Total Plate Count (CFU/mL)	Yeast and Mould (CFU/mL)	*E. coli* (CFU/mL)	*S. aureus* (CFU/mL)
MCW	9.9×10^4	4.0×10^1	Not detected	Not detected
FMCW Product	Not detected	Not detected	Not detected	Not detected

MCW: mature coconut water; FMCW: fermented mature coconut water

increase in antioxidant (Wu *et al*., 2020) and phenolic acid levels (Han *et al*., 2021).

3.2 Nutritional contents

In our previous study, we identified the presence of L-DOPA, which was one of the bioactive metabolites produced by LAB. L-DOPA has therapeutic properties and was reported to have the potential to treat Parkinson's disease. In addition, L-DOPA has a neuroprotective property, which has been shown to delay age-related macular degeneration (Brilliant *et al.*, 2016). In this study, the stability of the L-DOPA level was evaluated in the FMCW product. In general, microbial contamination can spoil the FMCW product through the activity of microbial enzymes produced that can break down essential compounds initially generated *via* controlled fermentation, such as L-DOPA. Microbes have been shown to be able to metabolize L-DOPA, reducing its availability (Brüssow, 2020). Since no contamination occurred, no significant change in L-DOPA content was observed in the FMCW product after 24 hrs of fermentation and at the end of the 12 months storage study period as displayed in Table 1. Moreover, removing LAB from the FMCW product can help further maintain the stability of L-DOPA and the product in general.

Additionally, the high lactic acid content in the FMCW product, which was also constant throughout the storage study, did not affect the L-DOPA content. In addition, lactic acid was also reported to inhibit the growth of *Helicobacter pylori* in the stomach, which causes peptic ulcers (Midolo *et al.*, 1995). The reduction in pH of the intestine by lactic acid was also reported to regulate the growth of putrefactive bacteria, thereby reducing intestinal inflammation (de Vrese and Schrezenmeir, 2008). On the other hand, the presence of stable lactic acid in the FMCW product can act as a natural bio-preservative. Past studies reported anti-fungal compounds produced by LAB, including salicylic acid, peptides, and other anti-mycotic compounds (Garofalo *et al.*, 2012; Crowley *et al.*, 2013). Other findings also reported organic acids such as lactic and acetic acids as anti-fungal compounds (Quattrini *et al.*, 2019; Luz *et al.*, 2020). The presence of high lactic acid content on the FMCW product did not show any adverse effect on its organoleptic acceptability and was confirmed from our earlier public sensory evaluation study which showed a good response with a high scoring point. The product safety issue has been investigated using an acute toxicity study on a mice model with no harmful effect observed (data not shown). Cell-free supernatants of LABs were also shown to have high antifungal activity against moulds, including *Aspergillus niger*, *Fusarium oxysporum, Penicillium* sp. and *Rhizopus* sp. (Abouloifa *et al*., 2021).

4. Conclusion

A 12 months storage study was done to evaluate the safety and stability of the FMCW product stored at two different storage temperatures of 4°C and 24°C. The FMCW product did not show any sign of microbial

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growth and any significant changes in the value of pH, Brix, lactic acid, and L-DOPA levels at both storage temperatures. In comparison, the unfermented MCW product showed signs of microbial growth in TPC, and yeast and mould test with colony growth of 9.9×10^4 and 4.0×10^1 CFU/mL, respectively, after 12 months of storage. The presence of high lactic acid with low pH in the FMCW product was the main contributing factor, protecting it from microbial spoilage during the long storage period. The current study presented a feasible and economical way of preserving CW, which offers an alternative approach to preserve pure CW juice with additional health benefits of L-DOPA. Further studies on other LAB co-culture combinations and the resulting benefits in terms of health, sports, and taste can be explored in the future to maximize the utilization of MCW.

Conflict of interest

The authors declare no conflict of interest.

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