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ORIGINAL RESEARCH PAPER

USE OF THE LACTOCOCCUS LACTIS IO-1 FOR DEVELOPING A NOVEL FUNCTIONAL BEVERAGE FROM COCONUT WATER

OLAIDE OLAWUNMI AJIBOLA [1, 2]*, SAMUEL LIHAN[2], AHMAD HUSAINI[1], ROSMAWATI SAAT[1], FAZIA MOHAMAD SINANG[1], IDRIS ADEWALE AHMED[3], WAHAB A. ABIDEEN[4]

[1] Faculty of Resource Science & Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan.
[2]. Institute of Biodiversity and Environmental Conservation, University Malaysia Sarawak, 94300 Kota Samarahan,
[3] Center for Natural Products Research and Drug Discovery, University of Malaysia, 50603 Kuala Lumpur.
4 Department of Microbiology, Faculty of Basic and Applied Science, Osun State University, Osogbo, Nigeria.
**Corresponding author: olaideajibola@gmail.com*

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Abstract

The goal of this work was to add value to the coconut water (CW) by fermentation with the potential probiotic *Lactococcus lactis* subsp *lactis* IO-1 in order to produce fermented CW beverages. Unpasteurized coconut water (UPW) was fermented with *Lactococcus lactis* subsp *lactis* IO-1 for 48 h at 30°C, and the viable cell counts, total acidity, pH, degree of polymerization, antioxidant activity, antibacterial bioassay and lethality bioassay were studied at 24 and 48 h. We revealed that the fermentation process of UPW with probiotic *L. lactis* IO-1 increased the viable cell counts. The total phenolic compound exhibited a higher antioxidative ability in fermented UPW at 48 h (65.79µg/mL gallic acid equivalence). The fermented UPW exhibited the highest ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging abilities at 48 h (67.62 and 63.03%), The culture extracted from fermented UPW inhibited all the tested pathogenic foodborne such as *Listeria monocytogenes, Salmonella typhi, Staphylococcus aureus,* and *Escherichia coli*, although the degree of antagonistic varied between the pathogens. Furthermore, fermented UPW extract sample at 48 h, exhibited lower potent activity against the brine shrimp with LC_{50} values (7158.2 µg/mL). Comparatively, pasteurized coconut water (PCW90) fermented by *Lactococcus lactis* subsp *lactis* IO-1 produced a fermented beverage PCW90 with similar properties as the fermented UPW. Adding 0.4% (w/v) of coconut flavor and 20% pure honey (v/v) into the fermented CW gave the beverage a better taste. The obtained results showed that the CW product fermented by *Lactococcus lactis* subsp *lactis* IO-1 may be used as a novel functional beverage comprising both probiotics and electrolytes, which can serve as a good vehicle for developing a wider range of novel products.

Keywords: Antibacterial activity, antioxidant activity, brine shrimp lethality test, coconut water, cytotoxicity.

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Introduction

Coconut water (CW) is considered as tender coconut water (TCW) or matured coconut water (MCW), based on maturity, cultivation areas and subsequently, storage and post-harvesting processing (Zhang *et al*., 2018). TCW is commonly consumed as a sport beverage in the tropics (Giri *et al*., 2018), while MCW is mostly discharged because only coconut meats are utilized for different culinary purposes. Besides, CW is low in fat and calories, but rich in magnesium, calcium, enzymes, vitamin C and vitamin B and making it a good substitute for artificial sports beverages to replace the electrolytes after exercise (Giri *et al*., 2018). Edible coconut parts and various value-added products comprise a high content of flavonoid and phenolic compounds with antioxidant capacity (Arivalagan *et al*., 2018). Furthermore, (-)-epicatechin and (+)-catechin were reported in CW (Kantanchote *et al*., 2017). There are few current studies on fermentation, particularly fermentation with the probiotic strains (Prado *et al.,* 2015). In this study, more attention was given to the use of lactic acid bacteria (LAB) to develop a value-added CW as a probiotic beverage.

Functional beverages are described as alcoholic-free beverages fortified with an ingredient like herbs, minerals, vitamins, sugars, or additional raw vegetables or probiotics that give specific health qualities beyond those found in other general food sources (Kantanchote *et al*., 2017). Lactic acid bacteria have been widely accepted as safe (GRAS) and played a key role in fermented beverages and food production (Kantachote *et al.,* 2017). Consequently, lactic acid fermentation (LAF) is a natural method to improve the amount of the polyphenol compounds in feedstocks that are beneficial to consumer's health.

Lactococcus lactis subsp *lactis* IO-1 has been successfully utilized as a starting culture to obtained lactic acid from lactose and hydrolysis of citric acid and casein by fermentative pathway (Samaržija *et al*., 2001). The strain is regarded as safe by scientists because it has probiotic properties including the production of bacteriocins (Kato *et al*., 2012). The unique characteristic of this strain as compared to other LABs is its ability to ferment xylose to generate L-lactic acid (Kato *et al.,* 2012).

Aside from the maturity stage and cultivar types of fruits, bio-composition and bioactive components in fruit beverages are heavily influenced by post-harvesting processing (Talcott *et al*., 2003). In addition, the pasteurization process may increase antioxidant abilities due to the release of compounds that possess antioxidant activity from the cell matrix, while in some cases it may lead to the deterioration of antioxidant activity (Farnworth *et al*., 2001). However, several LAB strains are being recorded for their capabilities to produce antioxidant and antimicrobial bioactivities (Giri *et al.,* 2018) and can reduce the level of toxicity (Chelule *et al.,* 2010). For example, plants like *Houttuynia cordata* fermented with probiotic *Lactobacillus* spp. have been reported to be less toxic and safe for human consumption than non-fermented *H. cordata* (Chaiyasut *et al.,* 2018). Thus, in this work, the focus was on the development of functional CW using *L. lactis* IO-1, with the goal of enhancing the nutritional benefits of this beverage. Additionally,

alterations were analyzed in the degree of polymerization, total acidity, antioxidant ability, toxicity activity and antibacterial activity of the fermented CW.

Materials and methods

Chemicals

Chemical compounds such as DPPH (2,2-Diphenyl picrylhydrazyl), Folin-Ciocalteu phenol, Sodium chloride, Potassium chloride, Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Hydrochloric acid, Gallic acid, and Ascorbic acid reagents were purchased from Sigma Aldrich (St. Louis, MO, USA). Potassium ferricyanide, and Ferric chloride was purchased from Merck (KGaA, 64271 Darmstadt, Germany), Trichloroacetic acid, Sodium hydroxide, Sodium carbonate, Sodium phosphate monobasic, and Sodium carbonate were purchased from J.T Baker (Mallinckrodt Baker, Inc. Philipsburg, USA). Methanol solvent was purchased from Fisher Scientific (Pittsburgh, PA). Other chemical reagents were of analytical grades and were purchased from local suppliers.

Microorganisms and cultivation conditions

The microorganism used for fermentation was *Lactococcus lactis* IO-1. The strain was originated from the Japanese Microbe collection, JCM 7638. IO-1 strain stock cultivation was stored at – 40°C in 2 mL of vials comprising of 30% (v/v) glycerol. *L. lactis* IO-1 strain had previously been isolated by Chinachoti *et al*. (1998) and its potential probiotic properties *in vitro* were reported (Kato *et al*., 2012). Strain IO-1 (obtained from Biochemistry laboratory, Universiti Malaysia Sarawak,) was thrice sub-cultured to obtain an active cell culture for preparation of inoculum by inoculating it into the artificial broth (comprising 20 g glucose and 5 g yeast extract per L) and incubating at 37°C for 18 h. To prepare a starting culture, the cell was harvested by centrifugation at 7500 ×g for 12 min and washed once with 0.85% sodium chloride. Subsequently, the culture was diluted with sterile CW to obtain optical density OD_{570} = 0.39, using a UV-1601 spectrophotometer (Shimadzu Co., Kyoto, Japan) at a wavelength of 570 nm, of approx. 10^8 CFU/mL (based on a pre-established calibration curve of optical density against viable counts (CFU/mL) using standard plate count method), for use as a starter cell culture (Carvajal-Zarrabal *et al*., 2009).

The test microorganisms used for antibacterial activity were the well-known potential pathogenic foodborne bacteria as follows: *Listeria monocytogenes* ATCC® 33090™, *Escherichia coli* (Migula) Castellani and Chalmers ATCC® 25922™, *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC® 25923™ and *Salmonella typhimurium* ATCC® 14028™ (Microorganisms were collected from the stock of the Virology laboratory, Faculty of Resource Science Technology of University Malaysia Sarawak). The test organisms were maintained on nutrient agar slopes and kept in a refrigerator at 4°C. A 100 mL aliquots of nutrient broth were inoculated with the culture of test microorganisms using a loop and then incubated at 37°C for 18 h.

Sample preparation and fermentation

Harvested coconut fruits aged between seven and nine months were purchased from the farm market in Samarahan, Sarawak, Malaysia Borneo. The CW used in this research was obtained by perforating the fruit with a sterile stainless steel knife after the main epicarp has been washed and cleaned with running tap water. The water from 25 coconut fruits was collected and had an initial average of 4.78±0.16 pH, 15.42 ± 0.02 g/L reducing sugar, 41.12 ± 0.73 g/L total sugar and 6.7 ± 0.29 g/L total soluble solids. To prepare the CW medium, suspended solids in CW were pre-filtered with a 0.8 µm polysulfone filter (TISH scientific, USA) before filtering with 0.65 µm filter paper (TISH scientific, USA). It was then dispensed into sterilized transparent glass bottles, sealed immediately for unpasteurized samples (UPW) and then, pasteurized at 90°C for 5 min (PCW90) in a thermostatic water bath (Memmert, Schwabach, Germany) and then refrigerated at 4 until required for analyses (Ajibola *et al*., 2020). The pH of the two samples (UPW and PCW90) was adjusted to 6.7 with 6 M NaOH to achieve the optimum pH for the growth of *L. lactis* in the prepared medium. Both the UPW and PCW90 samples (800 mL) were inoculated with a 0.5% (v/v) pure culture of *L. lactis* ($10^{7.5}$ CFU/mL). The fermentation was conducted in triplicate at 30 °C for 48 h and samplings were done at every 24 h to check the required properties such as: viable cell counts, pH, total acidity. In addition, the degree of polymerization (DP) was also investigated to confirm the preliminary results; while viable cells were counted every week for 4 weeks at 4 °C.

Crude extracts were obtained from the fermented samples according to the method adapted from Mahayothee *et al*. (2016). The extracted fermented samples were used to evaluate various bioassays.

Microbiological analysis

The viable cell counts of *L. lactis* were estimated by standard plate count technique (Mueller Hinton agar medium, 48 h incubation at 30 °C) as reported by Nematollahi *et al*. (2016).

Analytical method

The change in pH was determined by pH meter (MW 102, USA). The total titrable acidity (%) was analyzed by titrating the samples with 0.1 N NaOH (Nematollahi *et al*., 2016).

The degrees of polymerization (DP) of the fermented samples were estimated according to the concentration of total sugar (TSC) and reducing sugar (RSC). The Anthrone test (Loewus *et al*., 1952) was employed to determine TS, whereas the dinitrosalicylic acid assay (Miller *et al*., 1957) was employed to read the RSC. The DP was estimated from the concentration of TSC divided by the concentration of RSC.

The antioxidant ability of the fermented samples was assessed by the following assays: Ferric reducing power activity (FRAP), 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical assay and total phenolic content employing the Folin-Ciocalteu

bioassay according to the assay adopted by Oyaizu *et al.* (1986) and Mahayothee *et al*. (2016).

The antibacterial bioassay of the fermented samples was examined by using the agar well diffusion bioassay as reported by Garcia *et al.* (2016). Prior to each test, the fermented samples (UPW/PCW90) were diluted in sterile water (10 mg/2mL) and then 50 µL of each aliquot of aqueous culture extracts were applied to newly inoculated plates.

The lethality bioassay of the fermented samples was examined using brine shrimp lethality bioassay according to the method of Otang *et al*. (2013). The eggs of brine shrimp (*Artemia salina*) nauplii were hatched in aerated seawater (SW) for 24 h at room temperature. The active larvae hatched were attracted to the direction of light, they were chosen and treated with both fermented samples extracts. Stock solutions (20 mg/2mL) of the each fermented sample extracts (UPW/PCW90) were prepared in Dimethyl sulfoxide (DMSO), diluted into different concentrations (1-500 µg/mL). A solution of 0.5 ml of DMSO and 4.5 mL of SW was used as negative control while 5 mg of thymol in 5.0 mL of SW was taken as a positive control. Ten brine shrimps were introduced into each triplicate vials containing 5 mL of solution and control and incubated; the effect of both fermented extracts at 24 h interval was monitored by examining the live larvae after 24 h of treatment. The death rate of the larvae showed cytotoxicity of the fermented test samples.

Sensory evaluation

Both fermented CW (UPW and PCW90) samples at 28 days of storage (4 °C) were sensory analysis determined by 10 trained panelists using 7-point hedonic scale test based on the following parameters; flavor, clearness, odor, color, and overall acceptance. The result of the sensory analysis showed no significant change in all parameters analyzed for UPW and PCW90 beverages produced by the IO-1 strain. Fermented UPW was further analyzed for its sensory analysis by supplementing with 20% honey (v/v) for formulation B and fermented UPW with the addition of 20% honey (v/v) and 0.5% (w/v) coconut flavor for formulation C (Apex Flavour Inc, Belcamp, USA). The score was presented on a seven-point hedonic scale varying from 1-being dislike, 7- like very much.

Statistical analysis

All tests in this study were conducted in triplicate; the data for all variables were tested using ANOVA. The mean $\overline{(x)}$ ± Standard error (S.E) were presented. Statistical significance was determined using Tukey's test and the p-value was < 0.05 was considered to be significant. The determination of the average death of the brine shrimp at different concentrations of the extracts and concentration that kills 50% (LC_{50}) of the *A. salina* nauplii were made using Microsoft Excel and probit analysis on statistical software SPSS.

Results and discussion

CW fermentation

Changes in viable cell count, total acidity, pH and DP in UPW or PCW90 fermented by *L. lactis* IO-1 are shown in table 1.

Table 1 Changes in physicochemical and microbiological parameters during fermentation of coconut water in UPW and PCW90 media with *Lactococcus lactis* subsp. *lactis* IO-1.

Parameter	Fermentation time (h)	UPW	PCW90
Viable cell count (log CFU/mL)	0	7.56±0.01[a]	7.52±0.01[a]
	24	9.23±0.02[b]	8.73±0.01[b]
	48	9.29±0.01[b]	8.85±0.02[c]
pH	0	6.80±0.01[a]	6.80±0.00[a]
	24	3.72±0.01[b]	4.21±0.01[b]
	48	3.28±0.01[c]	3.43±0.00[c]
Total acidity (%)	0	0.25±0.00 [a]	0.25±0.00 [a]
	24	0.88±0.04 [b]	0.80±0.02 [b]
	48	0.98±0.01 [c]	0.90±0.00 [c]
Degree of polymerization (DP)	0	2.76± 0.03 [a]	2.73±0.03 [a]
	24	4.32±0.03 [b]	4.60±0.04 [b]
	48	4.05±0.01 [c]	4.14±0.03 [c]

Results are presented as mean±S.E (n=3). Non-identical superscripts within the same column represent significant differences at $p<0.05$. UPW (UPW, initial pH 6.7, inoculums size 7.5 log CFU/mL). PCW90 (PCW90, initial pH 6.7, inoculums size 7.5 log CFU/mL)

Both fermentation processes (UPW and PCW90) had a dramatic increase of viable cell counts after 24 h of fermentation and no remarkable difference until 48 h for the UPW but a significant change for PCW90.This corresponded to a significant increase of the total acidity with a significant fall of pH at 24 h of fermentation. The viable cell count approximately increased with 1.73 log CFU/mL in the fermented UPW (9.29 log CFU/mL) while the increase was approximately 1.27 log CFU/mL in the fermented PCW90 (8.85 log CFU/mL). The final count of the IO-1 cells in both UPW and PCW was comparable to those previously reported in young CW and matured CW after 48h of fermentation with *L. acidophilus L10* and *L. casei L26* (Lee *et al*., 2013) and *L. plantarum* DW12 (Kantachote *et al*., 2017). In order to accept that the samples have potential probiotic, viable cells must have a minimum of 10^6-10^7 CFU/mL or g of product, based on a daily dose of 100 mL (Karimi *et al*., 2011). Therefore, both fermented CW in this study can be regarded as probiotic beverages since the strain IO-1 could survive well at 4 °C for 28 days. The results were consistent with those of Giri *et al*. (2018), who recorded that after 48 h fermentation of mature coconut beverage, probiotics (*L. casei* L4) reached approximately log 9 CFU/mL. Total acidity at 24 – 48 h of fermentation in both fermented samples (UPW and PCW90) was approximately 0.98 and 0.91% while

the pH was approximately 3.20 and 3.34, respectively. The DP values in both fermented samples significantly increased at 24 h and afterward slightly decreased. The various degrees of polymerization (DP3-DP8) oligodextran were studied for their abilities to support the probiotics growth and it was observed that the entire tested cell utilized majority of the DP4 and DP3 (≥ 80%), with the exception of *Lactobacillus rhamnosus*, *Lactobacillus helveticum* and *Bifidobacterium bifidum* 02 for D8, D7, and D4 (Kantachote *et al.,* 2017). With regard to both the fermented samples (UPW/PCW90) with DP values in the range of 4-5 might improve the growth of cell IO1 although prebiotics like FOS should be further investigated. Based on the result obtained in this study (Table 1), higher viable cell density, total acidity, and lower DP were observed in UPW compared to PCW90 during fermentation. This observation may be due to the micronutrients, such as vitamins, inorganic ions present in UPW (Prades *et al.,* 2012; Tan *et al.,* 2015), that supporting LAB during the 48 h of metabolic process.

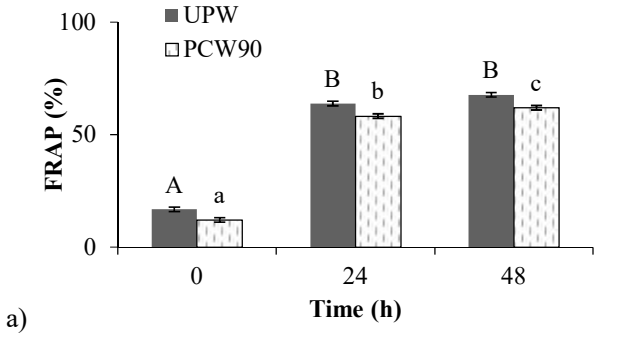
Antioxidant activity

The presence of potential antioxidants can help to prevent oxidative damages that occur in the human body and prevent lipid peroxidation in foods (Abountiolas and Nascimento, 2018). In this present study, in addition to the scavenging inhibitory activity of both FRAP and DPPH radical assays, the total phenolic compound (TPC) was also estimated. *L. lactis* IO-1 significantly increased the antioxidant ability of both fermented samples (UPW / PCW90) that were detected by different assays such as FRAP, DPPH, and total phenolic compounds as they revealed a remarkable increase at 24 h (Figure 1a and b, and Figure 2). The results indicated that the antioxidant potential of both fermented samples were produced by strain IO-1. The results are in accordance with Giri *et al.* (2018), who recorded that the radical scavenging activities (RSA) of CW fermented by *L. casei* L4 for 48 h were 58.4% for DPPH and 69.2% for ABTS. Both fermented samples (UPW and PCW90) produced by strain IO-1 had the antioxidant ability, as observed in their abilities, to scavenge the radical FRAP+ in a range of 61.98-67.62% (figure 1A). It was observed from Figure 1A that antioxidants in both fermented samples (UPW/PCW90) were found to produce similar patterns for their relative antioxidant abilities as the maximum ability was demonstrated at 48 h of batch fermentation.

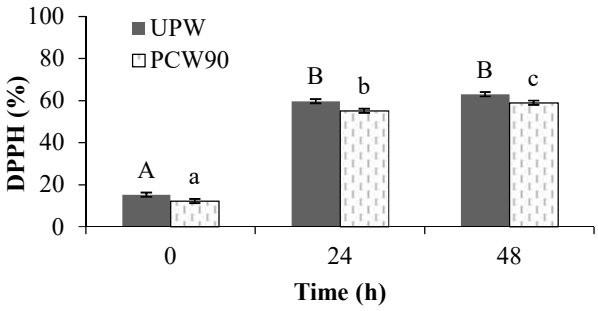
Figure 1B reveals that the antioxidant ability (UPW/PCW90) of the culture extracts, measured by DPPH assay was found to range between 58.99-63.03% with a similar trend for FRAP scavenging (figure 1a). Based on the DPPH method, both fermented samples can be classified for their antioxidant activities at the intermediate level like coffees (Ramadan-Hassanien, 2008).

However, the total phenolic amount of UPW culture extract exhibited a higher antioxidative ability (64.45-68.79 µg/mL GAE) than PCW90 (59.93-62.05 µg/mL GAE) (figure 2). Phenolic compounds, widely distributed in plants, are the most abundant antioxidants in the human diet (Giri *et al*., 2018). Some of them, including (+)(-) epicatechin and –catechin, were detected in coconut water (Chang and Wu, 2011). In this present investigation, phenolic content in CW should be low

as the detection for antioxidant ability at the starting of the fermentation was low (Figures 1 and 2). This supports the view that the free radical scavenging in both fermented extracts that occurred by probiotic strain IO-1 was due to other compounds rather than total phenolic contents (Figure 2). Between the studied samples, fermented UPW extract shows a higher amount of antioxidant activity and total phenolic content while lower content was observed in fermented PCW90 extract. The considerable difference between the results of antioxidant activity and phenolic content is due to inorganic acid, vitamins, L-arginine and ascorbic acid found in the UPW medium (Tan *et al.,* 2015). It was therefore presumed that, apart from the phytochemicals released from cell-matrix, the active natural phenolic compound in UPW media could also contribute to the TPC during the fermentation process. Although, it has been proven previously that CW contains micronutrients, L – arginine and ascorbic acid (Lima *et al*., 2015)



a)



b)

Figure 1. The radical scavenging activity of UPW and PCW90 samples (A) FRAP and (B) DPPH assay. Different alphabets above bars represent significant differences (p<0.05). Upper case for UPW and lowercase for PCW90.

Antibacterial activity

As indicated in table 2, no significant differences were detected between antimicrobial activities of both fermented samples (UPW/PCW90) against the growth of typical foodborne pathogenic bacteria.

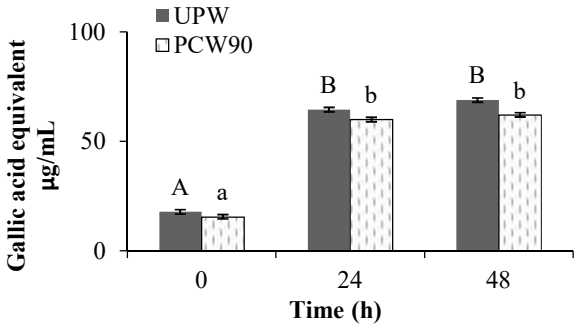


Figure 2. Total phenolic content (gallic acid equivalent: GAE) in fermented samples fermented with *L. lactis* IO1. Different alphabets (ABC for UPW; abc for PCW90) above bars represent the significant differences (p<0.05).

Table 2. Antibacterial bioactivity of the fermented samples.

Test microorganism	**UPW (zone inhibition, mm)**		**PCW90 (zone inhibition, mm)**	
	24 h	48 h	24 h	48 h
Listeria monocytogenes	7.20±0.23	8.30±0.11	7.10±0.15	8.20±0.60
Staphylococcus aureus	5.97±0.30	7.17±0.19	5.80±0.32	7.13±0.12
Salmonella typhi	4.60±0.57	5.47±0.15	4.52±0.14	5.43±0.18
Escherichia coli	3.50±0.03	4.10±0.02	3.49±0.04	4.06±0.12

No antibacterial bioactivity was reported at the starting of the batch fermentation process (t=0).

The result indicated that both UPW and PCW90 fermented samples inhibited the growth of all tested microbes and the inhibition were in the order of *L. monocytogenes*> *S. aureus*> *S. typhi*> *E. coli.* It was ascertained that there was a slight difference in the inhibitory effects of both samples against the clinically Gram-positive bacterial strain test. However, the clinically Gram-negative bacterial strain, *S. typhi*, was more sensitive to UPW/PCW90 culture extracts compared with strains tested. Also, the results showed that *E. coli* revealed a higher resistance. The efficacy of the antimicrobial activity against all tested pathogenic foodborne microbes in this study was comparable to that of the fermented CW beverage, which was incubated for 72 h using *L. plantarum* DW12 as a starter culture (Kantachote *et al*., 2017). The antibacterial activity of fermented food-beverage was mainly associated with the acidity acid of the beverages as no inhibition against the growth of all target microorganisms at the starting of fermentation (Table 2). The major compound in the fermented food-beverage extract from either the UPW or PCW90 was organic acid as the total acidity was approximately 0.90%. However, other polyphenol constituents present in the beverage extract may be involved in the antimicrobial action.

Toxicity against brine shrimp

The brine shrimp lethality test (BSLT) is among the preliminary toxicity tests based on the ability to kill laboratory-cultured brine shrimp. BSLT is a reliable, fast and cheap bioassay for testing the bioactivity of the fermented beverage extracts. The determination of the lethal concentration of 50% (LC_{50}) was based on the average death of brine shrimp (*Artemia salina*) after 24 h of using probits analysis technique (Table 3).

Table 3. Average death of *A. salina* nauplii at different concentrations of fermented samples at 24 h interval.

Fermented samples	Average death of *A. salina* nauplii Concentration (µg/mL)							LC_{50} (µg/mL)
	Time (h)	1	10	25	50	100	500	
UPW	0	0.33±0.6	1.00±1.0	1.33±0.6	1.67±1.2	2.33±1.5	3.00±0.0	4800.5
	24	0.00±0.0	0.67±0.6	1.00±0.0	1.30±1.2	2.33±0.6	2.33±0.6	6567.5
	48	0.00±0.0	7.33±0.5	1.33±1.1	1.67±1.2	2.33±0.6	2.33±0.6	7158.2
PCW90	0	0.33±0.6	0.67±0.6	1.33±1.5	2.00±1.0	2.33±2.1	2.67±1.2	5712.2
	24	0.30±0.6	0.67±0.6	0.33±0.6	1.33±1.2	2.00±1.0	2.33±2.1	6879.8
	48	0.00±0.0	0.33±0.6	0.67±1.2	1.33±1.5	1.33±0.6	2.00±1.0	8070.4
+Control		5.00±0.8	7.33±0.5	10.0±0.6	10.0±0.0	10.0±0.0	10.0±0.0	10.58
-Control		0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0

Fermented samples with the LC_{50} values higher than 1000 µg/mL are considered non-toxic while fermented extracts with LC_{50} values lower than 1000 µg/mL are considered significantly bioactive and toxic according to Meyer's toxicity index (Meyer *et al*., 1982). The result of both fermented samples (UPW/PCW90) at 24 h interval showed a concentration dependent increment in the mortality rate of the brine shrimp nauplii. The fractions of both fermented samples extract were lower potent against the brine shrimp activity with LC_{50} values ranging from 4800.5 to 6879.8 respectively (Table 3). Rakić *et al*. (2007) suggest that reduction of toxicity during processing is influenced by the cell matrix in the degradation of hydrolysable tannins, a larger polyphenolics compound, which results in the formation of smaller degradation products, such as gallic acid, that maintains antioxidant activity. These findings were in accordance with Quiao-Won *et al*. (2016), which study the kombucha fermentation cytotoxicity from various substrates. Furthermore, both fermented beverage extracts (at 24 h interval) showed significantly lower mortality compared to a positive control (thymol). This toxicity reduction is, therefore, one of the important technological characteristics used to select a lactic acid strain starter. In addition, it can be suggested that both fermented samples suited to brine shrimp. This could be due to the absorption and metabolism of phenolic compounds by the fermenting strain, which were influenced by 1) degree of glycosylation/ acylation, 2) fundamental structure, 3) conjugation with other phenolics, 4) molecular size, 5) degree of polymerization

and 6) solubility (Bravo, 1998). Furthermore, coconut water may contain some phenolic compounds in the stable forms of glycosides, which may be deglycoslated by probiotic LAB, which may enhance its non-cytotoxicity (Bravo, 1998).

Sensory determination

Using 10 survey subjects, we tested the acceptability of fermented UPW (formulation A), fermented UPW supplemented with 20% honey (formulation B) and fermented UPW supplemented with 20% honey and 0.4% (w/v) coconut flavor (formulation C) (Table 4). It was observed that there were no significant changes in the appearance or color of the samples. However, the addition of 20% coconut flavor and honey (formulation C) significantly improved the sensory evaluation based on flavor, odor and the overall acceptance of the beverage, showing that formulation C was widely acceptable to most participated individuals in this study. These results could be due to the reduced acidity and improved aroma and sweetness of this beverage after adding coconut flavor and honey. Our findings are in agreement with those recorded by Giri *et al*. (2007), who revealed that the addition of coconut flavor and 15 % honey enhanced the acceptance of CW fermented with *L. casei* L4. Furthermore, Kantachote *et al.* (2017) supplemented fermented mature CW with 20% honey, which enhanced the acceptance of the studied beverage. Alternatively, the fermented beverage may be supplemented with other sweetener based on desired products such as saccharin (Angelov *et al*., 2006), that is good for diabetic patients, and fruit ingredients to enhance their appeal to the consumer.

Table 4. Sensory determination of CW fermented by *L. lactis* IO-1 and its modified formulation.

Parameters	Control A	Recipe B	Recipe C
Flavor	3.75 ± 0.16^{a}	4.65 ± 0.17^{b}	5.30 ± 0.15^{c}
Appearance	3.85 ± 0.13^{a}	3.95 ± 0.14^{a}	4.05 ± 0.15^{a}
Odor	3.70 ± 0.19^{a}	3.85 ± 0.13^{a}	4.65 ± 0.11^{b}
Color	4.40 ± 0.18^{a}	5.00 ± 0.16^{b}	5.05 ± 0.17^{b}
Overall acceptance	3.95 ± 0.14^{a}	4.45 ± 0.11^{b}	5.00 ± 0.15^{c}

Formulation A: (Fermented CW), Formulation B: (Fermented CW + 20% honey), Formulation C: (Formulation B + coconut flavor). Non-identical superscript alphabets with in the same row represent the significant difference, $p<0.05$.

Conclusions

It can be concluded that *Lactococcus lactis* IO-1 strain showed a capability for utilizing both CW samples. Following the 48 h fermentation process with *L. lactis* IO-1, we determined that fermented beverages contain antioxidant, antibacterial and non-cytotoxic activity. Furthermore, even after 4 weeks of refrigerated storage (4°C), both fermented beverage samples retained the desired *L. lactis IO-1 levels*, which are in accordance with daily-recommended probiotic dose. The fermented CW supplemented with 20% pure honey and 0.4% (w/v) coconut flavor was revealed to have the highest acceptance rate by the panelists. Further studies are

needed for the production of inexpensive *L. lactis* IO-1 fermented functional beverages with several health benefits for vegetarians, lactose intolerant people, and those who are allergic to milk products.

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