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CELL VIABILITY, PHYSICOCHEMICAL AND SENSORY CHARACTERISTICS OF PROBIOTIC COCONUT JUICE DURING COLD STORAGE

OLAIDE OLAWUNMI AJIBOLA[1,2], SAMUEL LIHAN[2], AHMAD HUSSAINI[1], CIRILO NOLASCO HIPOLITO[3], OCTAVIO CARVAJAL ZARRABAL[4], SHAHRUL RAZID SARBINI[5] AND SHARIFAH AMINAH SYED MOHAMAD[6]*

[1]Faculty of Resouce Science & Technology, Universiti Malaysia Sarawak, Kota Samarahan, 94300, Malaysia. [2]Institute of Biodiversity and Enviornmental Conservation, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia. [3]Insituto of Biotechnology, Universidad Del Papaloapan, Circuito Central 200, Col. Parque Industrial, CP, 68301, Tuxtepec, Oaxacs, 68000, Mexico. [4]Institute of Forensic Medicine, University of Veracruz, SS Juan Pablo II s/n, 94294 Boca del Río, Ver., México. [5]Faculty of Agriculture, Science and Technology, Universiti Putra Malaysia Bintulu Campus, 97008, Jalan Nyabau, Malaysia. [6]School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.

**Corresponding author: sharifah459@uitm.edu.my*
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Abstract: Probiotic fermentation has gained considerable attention in the pharmaceutical and food industries. Milk-based products are not in demand among vegetarians, people with allergies to certain proteins, and lactose-intolerant consumers. Therefore, there is a genuine interest in the production of crop juice-based probiotic beverages with probiotic potentials. The objective of this study is focused on the development and storage of probiotic coconut juice using lactic acid bacteria (LAB) as a starter. The viability of the probiotic, its physicochemical properties, and antibacterial potentials of the stored probiotic coconut juice employing LAB (*Lactobacillus casei* ATCC 393, *Lactobacillus plantarum* ATCC20174, *Lactobacillus rhamnosus* ATCC 7469, and *Lactococcus lactis* IO-1) as a single starter culture were studied. Sensory tests were also performed on the samples. There was an increase in total acidity production and a decrease in pH and brix levels, as well as phenolic, antioxidant and tannin contents during the refrigerated condition. At weeks three and four, coconut juice inoculated with *L. lactis* IO-1 samples had the highest total acidity (1.32%). However, the level of phenolic compound, antioxidant, and tannin contents showed a slight decrease during storage. The probiotic strains were viable throughout the refrigerated condition. *L. lactis* IO-1 showed greater viability compared with other strains (8.426 log CFU/mL). There were no significant differences between all the samples in terms of taste, aroma, colour and appearance. It could be inferred from this study that high acidity and the presence of inhibitor phenolic compounds in the probiotic coconut juice have no negative impact on the viability of the probiotics and antibacterial potential of the samples throughout storage. Hence, probiotic fermentation could provide an alternative outlet for coconut juice utilization, and could be used to produce novel probiotic beverages for consumers, especially in terms of sports nutrition.

Keywords: Coconut juice, probiotic, antioxidant, sensory test.

Introduction

The term "probiotics" means "for life", but in terms of health or beneficial microbes, probiotics can be defined as "viable or inviable microbial strains (ruptured or intact; spore or vegetative) that are potentially healthful to the host" (Zendeboodi *et al.*, 2020). According to the definition, probiotics can be divided into three classes; true probiotic (viable and active strains), pseudoprobiotic (viable and inactive strains, in vegetative or spore form), as well as ghost probiotic (non-viable or dead strains, in ruptured form). However, each of these classes is divided into two groups based on their site of impact or action (in vivo or in vitro). For example, a probiotic cell that produces a healthy metabolite or bioactive compound in a diet matrix is considered a "true probiotic external". But when this viable and active probiotic is transformed into the system to perform its specific function in

a target organ, it is considered a "true probiotic internal". If the former probiotic becomes inactive (but still viable) in a diet, the probiotic is known as "pseudoprobiotic vegetative external". Another example is a ruptured probiotic cell in a diet, which is referred to as "ghost probiotic ruptured external". But when the cell fragments of this probiotic are consumed in a diet or as a supplement for health benefits, it is referred to as "ghost probiotic ruptured internal". The use of dairy products to deliver probiotics has brought health benefits to the host by balancing the microbial population in the body system (Tomar, 2019; Ryan *et al.*, 2020). The low viability and functionality of probiotic cells are one of the critical problems associated with probiotic food products due to their sensitivity to the adverse environment, such as potent enzymes of the gut and the low pH of food products (Tamang *et al.*, 2016). Standardised probiotic products should have at least 10^6 CFU/mL active and viable cells during consumption time (Terpou *et al.*, 2019).

In recent years, there has been a growing number of studies on the development of functional beverages that confer nutritional value on consumers' gastrointestinal health (Nazir *et al.*, 2019). The development of functional foods has been associated with the reduction of some diseases related to mental wellbeing (Zhu *et al.*, 2020). Functional foods are processed products with enhanced or added benefits over and above their essential micro- and macronutrients (Mantzourani *et al.,* 2018). In addition, functional beverages contain a significant amount of certain bioactive components, such as symbiotic, probiotic, and prebiotic components (Nazhand *et al.*, 2020).

Dairy fermented products are the most common means to deliver probiotic bacteria (da Cruz Rodrigues *et al.*, 2019; Roobab *et al.*, 2020). However, these products may cause some inconveniences for those with cholesterol problems and those with lactose intolerance (da Silva *et al.*, 2019). Therefore, there is a need to develop a novel and acceptable product that can be consumed by all, regardless of sex, age, and culture.

Zhu *et al.* (2020) reported the probiotic actions of apple, orange, juices using *Lactobacillus sanfranciscensis* strains.

The production of a probiotic coconut water juice by lactic acid bacteria (LAB) has been reported by Giri *et al.* (2018). He concluded that coconut water could be considered as a suitable matrix for the growth of probiotic bacteria and functional juice production.

The coconut (*Cocos nucifera* L.) is among the important agricultural products in tropical countries due to the pleasing fragrance and sweet smell of various parts of the crop (Ajibola *et al.*, 2018). The juice of the coconut is commonly consumed fresh or processed into coconut beverage (Naik *et al.*, 2020). Fresh coconut water is a popular beverage in hot and humid climates and is also the main ingredient of coconut wine (Ajibola *et al.,* 2020b). It is a very good source of ascorbic, tannin, cysteine and phenolic compounds (Zhang *et al.,* 2018). These bioactive compounds have been reported to possess antibacterial properties against various food pathogens, exhibit antioxidant and anti-inflammatory properties, as well as antithrombotic effects (Ajibola *et al.*, 2020a; X. Zhang, *et al.*, 2020). In addition, the availability of rich mineral content makes it an important medium for probiotic beverage production. Edible coconut fruit parts and various value-added products contain a high concentration of polyphenol constituents with antioxidant abilities (Ajibola *et al*., 2020a). In addition, coconut water has been documented to contain (+) catechin and (-) epicatechin (Ajibola *et al.,* 2020b). However, coconut water is low in calories and fat, but rich in ascorbic acid, vitamin and L-arginine, making it a good substitute for artificial sports beverages to replace electrolytes following an exercise (Ajibola, 2020b).

Nowadays, there are many different kinds of probiotic-based cereal and milk products. The interest in probiotic-based vegetable products derived from fruits and leafy vegetables has also increased due to a growing number of vegetarian consumers, as well as cholesterol-conscious and lactose-intolerant customers (Ajibola *et al.*,

2018; Chaturvedi & Chakraborty, 2021). Plant materials, such as fruit and leafy vegetables, are a good source of vitamin A, protein, mineral concentration, carbohydrate, fibre, polyphenol, and antioxidants, which have a positive impact on some important organs in the body system (Santos *et al.,* 2017). Therefore, agricultural raw materials, such as leafy vegetables and fruits, which lack certain milk allergens, could be utilised (Nguyen *et al.,* 2019; Ajibola, 2020). The ability to obtain coconut water to serve as a novel carrier of probiotic lactic acid (LA) bacteria and the use of *Lactococcus lactis* IO-1 to develop a novel functional beverage with coconut water has been reported (Ajibola *et al.,* 2020b).

The objective of this study is focused on the production and storage of probiotic coconut juice using LAB as a starter, and the determination of the physicochemical parameters of the products (total soluble solid [°Brix], viability, antibacterial activity, phenolic content, tannin content, titratable acidity, and pH).

Materials and Methods

Coconut Juice

Newly harvested coconuts between seven to eight months old were procured from the Kota Samarahan market in Sarawak, Malaysia. Coconut juice was collected by perforating the fruits with a sterile knife after the epicarp was cleaned, rinsed with distilled water, and then air-dried. The coconut water had an initial average pH of 4.78±0.16, reducing sugar of 15.42 ± 0.02 g/L, total sugar of 41.12 ± 0.73 g/L, and total soluble solids of 6.7 ± 0.29 g/L. To prepare the coconut juice medium, suspended solids in the juice were pre-filtered with 0.8 µm polysulfone filters (TISH scientific, USA) and then with 0.65 µm filter papers (TISH scientific, USA). It was then dispensed into sterilised transparent glass bottles and sealed immediately. The initial brix level was 6.7 while the pH was adjusted to 6.8 using 6 N NaOH.

Probiotic Strains Collection and Inocula Cultivation

Commercial probiotic strains *Lactobacillus casei* ATTC 393, *Lactobacillus plantarum* ATCC20174, and *Lactobacillus rhamnosus* ATCC 7469 were collected from DSM Co. The cultures were prepared separately in the sterile de Man Rogosa Sharpe (MRS) medium (Sigma-Aldrich Chemicals company, St. Louis, USA) for 48 hours at 30°C. The *Lactococcus lactis* IO-1 obtained from Universiti Malaysia Sarawak was prepared in a sterile artificial broth (comprising 20 g glucose and 5 g yeast extract per l) and incubated at 37°C for 18 hours. The strains were harvested by centrifugation at 7500 g for 12 minutes and washed once with 0.85% sodium chloride. Each culture was diluted with sterile coconut juice to obtain optical density (OD) of the strain suspension using a UV-1601 spectrophotometer at 575 nm. The OD of the strains suspension was compared with the OD of 0.5 Mc Farland containing 10^7 CFU/mL (based on a preliminary study using standard plate count method) for use as a starter cell culture.

Fermentation of the Probiotic Coconut Juice Samples

The fermentation of coconut juice samples was carried out in triplicates. A total of 800 mL of each CJ samples were inoculated with 1% (v/v) pre-cell of respective probiotic starter culture (0.5 McFarland standard containing 10^7 CFU/ml mL). The inoculated samples with the probiotic strain (*L. casei* ATTC 393, *L. plantarum* ATCC201, *L. rhamnosus* ATCC 7469, and *Lactococcus lactis* IO-1) were labeled as Coscasei (coconut juice inoculated with *L. casei*), Cosplanta (coconut juice inoculated with *L. plantarum*), Cosrhamn (coconut juice inoculated with *L. rhamnosus*), Coslact1 (coconut juice inoculated with *L. lactis* IO-1) and control (coconut juice without inoculated strain). Each inoculated coconut juice sample was incubated for 48 h at 30°C for the fermentation process, and refrigerated at 4°C for four weeks. The viability of the cell, along with the changes in chemical properties, including brix level, titrable acidity, pH, total tannin

content, total phenolic content and antibacterial activity, antioxidant ability (DPPH), were determined during cold storage (four weeks, 4°C). Crude extracts were obtained from the probiotic coconut juice samples according to the method adapted from Mahayothee *et al.* (2016). The extracted samples were used to evaluate various bioassays.

Microbiological Analyses

The viable cell counts of the probiotics were estimated using the standard plate count technique (MRS agar medium, 48 hours incubation at 30°C) as reported by Ajibola *et al.* (2020b).

Extraction

The extraction of the phenolic compounds from the samples was performed according to the method described by Ajibola *et al.* (2020b) with some modifications. The samples were thawed and mixed with methanol (1:5 v/v), and the mixture was shaken at room temperature (27°C) for three hour using an orbital shaker ((New Brunswick Scientific, Edison, New Jersey, USA) at 90 rpm. The mixture was filtered with 0.8 µm polysulfone filters (TISH scientific, USA). The filter sample extract was heated at 40°C using a rotary evaporator (R-114, Buchi, Switzerland). All extractions were carried out in triplicates.

Chemical Analysis

The total acidity was determined by titrating the samples with 0.1 N NaOH (Nematollahi *et al.,* 2016). A change in pH and brix level was determined using a pH meter and refractometer (ATAGO, Yushima, Japan).

Total Phenolic Content Determination

The change in total phenolic content was determined using Ciocalteu's method described by Mahayothee *et al.* (2016). The methanolic extract samples (0.2 mL) was mixed with 10% v/v Folin–Ciocalteu's reagent (1 mL) and allowed to react for three minutes. Sodium carbonate (7.5% w/v, 0.8 mL) was added to the mixture and allowed to react at room temperature for two hours. The absorbance at 765 nm was measured using a spectrophotometer. Gallic acid was used for standard calibration and the total phenolic content was expressed in GAE µg/mL.

Total Tannin Content Determination

The total tannin content was determined using the Folin-ciocaltueu method described by Makkar *et al.* (1993). Extract samples (0.1 mL) were made up to 0.5 mL in volume with distilled water. The Folin-Ciocalteu reagent (0.25 mL) was added to the mixture followed by 1.25 mL of a sodium carbonate solution. The vortexed mixture was recorded at 725 nm using a spectrophotometer. The tannin acid was used for standard calibration and the total tannin content was expressed in TAE µg/mL.

DPPH Assay

The change in DPPH assay was performed according to the method described by Mahayothee *et al.* (2016). Diluted extract samples (0.1 mL) were mixed with 6×10^{-5} M DPPH in methanol (3.9 mL). The mixture was kept at room temperature in the dark for two hours. Absorbance was measured at 515 nm and the DPPH radical scavenging activity was calculated as:

DPPH free radical scavenging activity (%)
$= \left(\frac{A_0 - A_1}{A_0}\right) \times 100$ ---------- Equation 1.0

where, A_0 = the absorbance of the control (without sample) at 517 nm
A_1 = the absorbance of the samples at 517 nm

Antibacterial Activity of the Probiotic Samples Against Foodborne Pathogenic Bacteria

The antibacterial activity of the probioticated substrate against selected pathogenic strains (*Listeria innocua* Seeliger ATCC® 33090™, *Escherichia coli* (Migula) Castellani and Chalmers ATCC® 25922™, *Staphylococcus aureus* subsp. aureus Rosenbach ATCC® 25923™ and *Salmonella typhimurium* ATCC® 14028™) was examined using the agar

diffusion bioassay. The test strains containing 2.310^7 CFU/mL were seeded on a sterile molten agar. Following solidification, wells were bored on seeded agar plates, and the probioticated juice samples were introduced into the wells. The plates were first incubated at 4°C for one hour to enable the test samples to diffuse in the agar, and they were then incubated at 30°C for 24 hours. Following incubation, the diameter of the clear zone was measured in the center of the well.

Sensory Evaluation

Each coded probiotic coconut juice sample was served to ten trained participants. Participants were asked to assess the taste, colour, appearance, and aroma of each coded probiotic sample using a hedonic scale of 1 to 7, where one (1) corresponds with "dislike" and seven (7) corresponds with "like very much" (Adebayo & Akeji, 2016). Triplicate evaluations were made per sample. The obtained outcomes were subjected to analysis of variance using one-way ANOVA, and the difference between means was separated using Tukey's post-hoc test.

Statistical Analysis

All experiments were conducted in triplicates. Chemical and sensory properties were subjected to one-way ANOVA (IBM SPSS statistical software 21.0). The values of the mean () ± standard error (S.E) were presented. The statistical significance was determined using Tukey's test and the *p*-value of <0.05 was considered to be significant. The determination of the antibacterial test (mean) and viability of probiotic of samples ± S.E) were done using the SPSS software.

Results and Discussion

pH of the Stored Probiotic Samples

Table 1 shows the pH changes in the probiotic coconut juice samples during four weeks of cold storage. There was a remarkable difference in the pH values during cold storage. High pH values were recorded in the fourth week following cold storage for all samples. This may be due to the conversion of other organic acids to lactic acid (LA) metabolised by the probiotic LAB (Ajibola, 2020) or due to autolysis of the bacterial body and release of peptides into the juice (Mortazavian *et al.,* 2011). There were reductions in pH following the first week of refrigerated storage (4°C). During refrigerated storage, the Coslact1 juice sample exhibited the lowest pH value (3.30) at week three. This may be due to the metabolic processes occurring during probiotic production and cold storage.

The decrease in pH values of the probiotic juice samples during the fermentation and refrigerated storage periods shows a positive quality of the juice samples. Our results are in agreement with the findings reported by Adebayo and Akpeji (2016), in which the reduction in pH is of major importance to the nutritional quality of the final product. The changes in pH values

Table 1: Weekly pH analysis of the probiotic coconut juice samples (weeks)

Juice Sample Code	pH					
	Time of Cold Storage (week)					
	1	2	3	4	Mean	S.E
Coslact1	3.30^{b}	3.28^{a}	3.25^{a}	3.40^{ab}	3.30^{ab}	0.023
Coscasei	3.36^{b}	3.29^{a}	3.27^{a}	3.57^{ab}	3.37^{b}	0.042
Cosplanta	3.39^{b}	3.23^{a}	3.26^{a}	3.61^{ab}	3.37^{b}	0.050
Cosrhamn	3.41^{b}	3.30^{a}	3.24^{a}	3.62^{ab}	3.39^{b}	0.048
C control	6.80^{a}	6.78^{a}	6.72^{a}	6.73^{a}	6.77^{a}	0.017

Means with different superscript letters across the row are significantly different from each other at $p<0.05$. KEY: Coslact1 - coconut juice with *Lactococcus lactis* IO-1, CosCasei - coconut juice with *L. casei*, CosPlanta - coconut juice with *L. plantarum,* Cosrhamn - coconut juice with *L. rhamnosus*, C control - coconut juice without probiotic.

and the concomitant increase in total acidity composition during fermentation, as well as the cold condition of the samples, may be attributable to the metabolic bioactivity of the probiotic bacteria.

Total Soluble Solid of the Stored Probiotic Samples

Table 2 presents the total soluble solid (°Brix) changes in the probiotic coconut juice samples during four weeks of cold storage. Evidently, during cold storage, all the samples exhibited a significant difference ($p<0.05$) in Brix value. The obtained results revealed that the lowest Brix value was achieved in all the probiotic coconut juice samples in the fourth week.

The decrease in Brix value may be due to the utilisation of sugar, as well as other metabolic activities, by the probiotic bacteria during the fermentation process and refrigerated storage of the probiotic coconut juice samples. This result is in agreement with the findings by Adebayo and Akpeji (2016), who reported a similar decreasing Brix value in pineapple juice with *Lactobacillus rhmnosus* and *Pediococcus pentosaceus*.

Total Acidity Production in the Stored Probiotic Samples

The total acidity production in the samples during four weeks of refrigerated storage is shown in Table 3. The result revealed that in the first week of the cold storage, the total acidity production ranged from 0.25% to 0.93%, with Coslact1 having the highest total acidity percentage. In the second week (4°C), the total acidity production ranged from 0.25% to 1.17 %, with the Coslact1 juice sample having the highest total acidity percentage. Following the third and fourth weeks of cold storage, the

Table 2: Weekly total soluble solid (°Brix) analysis of the probiotic coconut juice samples

Juice Sample Code	Total Soluble Solid (°Brix)					
	Time of Cold Storage (week)					
	1	2	3	4	Mean	S.E
Coslact1	6.21^{a}	5.69^{ab}	5.31^{bc}	5.10^{c}	5.59^{c}	0.121
Coscasei	6.15^{a}	5.78^{ab}	5.56^{bc}	5.20^{c}	5.67^{b}	0.100
Cosplanta	6.36^{a}	5.84^{ab}	5.48^{ab}	5.25^{b}	5.73^{ab}	0.140
Cosrhamn	6.32^{a}	5.82^{ab}	5.58^{ab}	5.38^{b}	5.78^{ab}	0.132
C control	6.51^{a}	6.49^{a}	6.47^{a}	6.45^{a}	6.48^{a}	0.001

Means with different superscript alphabets across the row are significantly different from each other at $p<0.05$. KEY: Coslact1 - coconut juice with *Lactococcus lactis* IO-1, CosCasei - coconut juice with *L. casei*, CosPlanta - coconut juice with *L. plantarum,* Cosrhamn - coconut juice with *L. rhamnosus,* Control - coconut juice without probiotic.

Table 3: Weekly total acidity (%) of the probiotic coconut juice samples (weeks)

Juice Sample Code	Total Acidity (%)					
	Time of Cold Storage (week)					
	1	2	3	4	Mean	S.E
Coslact1	0.93^{a}	1.17^{b}	1.21^{bc}	1.32^{c}	1.15^{a}	0.023
Coscasei	0.86^{a}	1.09^{b}	1.19^{bc}	1.28^{c}	1.10^{b}	0.044
Cosplanta	0.83^{a}	1.04^{b}	1.18^{bc}	1.27^{c}	1.08^{b}	0.047
Cosrhamn	0.81^{a}	1.03^{b}	1.14^{bc}	1.25^{c}	1.06^{b}	0.046
C control	0.25^{a}	0.25^{a}	0.24^{a}	0.23^{a}	0.24^{a}	0.001

Means with different superscript alphabets across the row are significantly different from each other at $p<0.05$. KEY: Coslact1 - coconut juice with *Lactococcus lactis* IO-1, CosCasei- coconut juice with *L. casei*, CosPlanta- coconut juice with *L. plantarum,* Cosrhamn- coconut juice with *L. rhamnosus,* Control - coconut juice without probiotic.

total acidity content was found to be in the range of 0.24% to 1.21% and 0.23% to 1.32%, respectively. There were significant differences in the total acidity production during the cold storage period.

The increase in total acidity could be due to the metabolic processes occurring during probiotic production and cold storage. This corresponds to an increase in the total acidity with a remarkable drop of pH during storage. The remarkable increase in total acidity will inhibit the growth of the food spoilage microbes (Giri *et al.*, 2018). This is in line with the study by Kantachote *et al.* (2017). In addition, Adebayo and Akpeji (2016) stated that the changes in pH and total acidity level are a result of the production of organic acid by probiotic bacteria.

Phenolic Content, Antioxidant Ability, and Tannin Content of the Stored Probiotic Samples

Tables 4, 5, and 6 indicate phenolic, antioxidant ability, and tannin content of the probiotic coconut juice samples during four weeks of cold storage. The tested parameters were found to have decreased significantly in all probiotic coconut juice samples, but this reduction was less evident during the refrigerated period (p<0.05). The control sample, without added probiotic, exhibited the lowest change. In contrast, samples with probiotic strains exhibited the highest change in all three parameters. The increase in the phenolic content and antioxidant abilities during the fermentation process may be a result of other compounds rather than pthe henolic compounds by the probiotic LAB in the probiotic coconut juice samples (Kantachote *et al.,* 2017). In addition, it might also be due to the presence of a superoxide dismutase enzyme that catalyses the hydrogen peroxide and oxygen dismutation of the superoxide anion (Ajibola *et al.*, 2020b).

However, the decrease in the phenolic and tannin contents, as well as antioxidant abilities, may be a result of the slight activity of probiotic LAB in cold temperature conditions and also the presence of dissolved oxygen in the probiotic coconut juice samples, which resulted in oxidation of the phenolic compounds. The sample containing *L. lactis* showed the highest oxidation as a result of the growth of LAB during the refrigerated condition. It has been revealed that in the absence of oxygen and light, the amount of phenolic bioactive compounds did not change significantly throughout the cold storage condition (Patthamakanokporn *et al.,* 2008). Nematollahi *et al.* (2006) revealed that the antioxidant potential of food beverages from fermented cherry juice supplemented with *L. casei, L. plantarum,* and *L. rhamnosus* significantly decreased following 28 days of cold storage (4°C), which is in line with the outcomes of the present investigation.

Table 4: Weekly phenolic content (GAE µg/mL) analysis of the probiotic coconut juice samples

Juice Sample Code	Phenol (gallic acid equivalent [GAE] GAE µg/mL)					
	Time of Cold Storage (week)					
	1	2	3	4	Mean	S.E
Coslact1	72.10^{a}	60.46^{ab}	56.83^{bc}	50.64^{c}	60.00^{ab}	2.750
Coscasei	71.47^{a}	64.43^{ab}	61.33^{bc}	56.70^{c}	63.48^{ab}	2.030
Cosplanta	71.27^{a}	62.80^{ab}	59.58^{bc}	53.73^{c}	61.85^{ab}	2.200
Cosrhamn	71.67^{a}	62.11^{ab}	59.48^{bc}	52.70^{c}	60.01^{bc}	2.230
C control	16.36^{a}	16.19^{a}	16.03^{a}	15.95^{ab}	16.13^{a}	0.120

Means indicated with different superscript alphabets across the rows are significantly different from each other at p<0.05. KEY: Coslact1 - Coconut juice with *Lactococcus lactis* IO-1, CosCasei - coconut juice with *L. casei*, CosPlanta - coconut juice with *L. plantarum,* Cosrhamn - coconut juice with *L. rhamnosus,* Control - coconut juice without probiotic.

Table 5: Weekly tannin content (TAE µg/mL) analysis of the probiotic coconut juice samples

Juice Sample Code	Tannin (Tannic Acid Equivalent [TAE] TAE µg/mL)					
	Time of Cold Storage (week)					
	1	2	3	4	Mean	S.E
Coslact1	11.53^{a}	10.23^{ab}	9.50^{bc}	8.29^{c}	9.89^{bc}	0.400
Coscasei	11.43^{a}	10.79^{ab}	10.11^{ab}	9.18^{bc}	10.38^{ab}	0.330
Cosplanta	11.47^{a}	10.41^{ab}	10.06^{b}	9.50^{bc}	10.36^{ab}	0.280
Cosrhamn	11.58^{a}	10.53^{ab}	10.04^{bc}	9.00^{c}	10.29^{ab}	2.230
C control	12.47^{a}	12.33^{a}	12.18^{a}	12.16^{a}	12.29^{a}	0.110

Means indicated with different superscript alphabets across the rows significantly different from each other at $p<0.05$. KEY: Coslact1- coconut juice with *Lactococcus lactis* IO-1, CosCasei- coconut juice with *L. casei*, CosPlanta- coconut juice with *L. plantarum,* Cosrhamn- coconut juice with *L. rhamnosus,* Control- coconut juice without probiotic.

Table 6: Weekly antioxidant capabilities (% to scavenge the radical DPPH) of the probiotic coconut juice samples

Juice Sample Code	Antioxidant ability (% Radical Scavenging activity of DPPH)					
	Time of Cold Storage (week)					
	1	2	3	4	Mean	S.E
Coslact1	65.43^{a}	59.99^{b}	55.95^{bc}	51.40^{c}	58.19^{ab}	1.110
Coscasei	63.54^{a}	57.64^{ab}	51.47^{b}	49.30^{bc}	55.49^{ab}	1.810
Cosplanta	61.46^{a}	53.51^{ab}	51.99^{b}	46.86^{bc}	53.46^{ab}	2.050
Cosrhamn	61.71^{a}	55.38^{ab}	53.48^{b}	49.00^{bc}	54.89^{ab}	1.650
C control	11.44^{a}	10.36^{a}	10.17^{ab}	9.88^{ab}	10.46^{a}	0.150

Means indicated with different superscript alphabets across the rows are significantly different from each other at $p<0.05$. KEY: Coslact1 - coconut juice with *Lactococcus lactis* IO-1, CosCasei - coconut juice with *L. casei*, CosPlanta - coconut juice with *L. plantarum,* Cosrhamn - coconut juice with *L. rhamnosus,* control - coconut juice without probiotic.

Viability of Probiotic Bacteria in the Stored Samples

The change in viable cell counts (log CFU/mL) of probiotic strains in the probiotic juice samples during cold storage is presented in Figure 1. There was a slight reduction in viability during the cold storage period. The highest viable cell count (8.33 log CFU/mL) was observed in Coslact1, followed by CosCasei and CosPlanta, and the least viable cell count was observed in Cosrhamn after four weeks of cold storage. After the first week of cold storage, the lowest reduction rate was observed in Coslact1. The probiotic strains used in this investigation were viable throughout the refrigerated condition. After the fourth week of refrigerated storage (4°C), the probiotic coconut juice produced by *L. lactis* IO-1 had the lowest reduction rate of viable cell count.

The slight reduction in viable cell counts during cold storage from the first week to the fourth week is likely due to the reduction in the sugar levels in the product samples as a result of metabolic activities of the probiotic culture, and the accumulation of organic acid by some metabolites during the storage condition (Zhang *et al.*, 2018). Adebayo and Akpeji (2016) demonstrated that the remarkable increase in total acidity is likely due to the fermentation processes, which can decrease the viability and survivability of the strains. Nematollahi *et al.* (2016) equally demonstrated that the reduction rate of the probiotic strains might be remarkably influenced by the fermentation period, storage period, pH, and type of probiotic bacteria, as well as the oxygen level in the sample product.

In order to accept that the samples have potential probiotics, viable cells must have a

minimum of 10^6 CFU/mL or g of product based on a daily dose of 100 ml (Ajibola, 2020). Therefore, all fermented coconut juice in this study can be regarded as probiotic juice since all the strains could survive well at 4°C for 28 days. This agrees with the report by Ajibola (2020b), who said probiotic coconut juice could be considered as a probiotic beverage without any nutrient supplementation.

Antibacterial Activities of the Probiotic Samples against Foodborne Pathogenic Bacteria

The antibacterial activities of the probiotic samples against foodborne pathogenic bacteria were presented in Table 7. The highest antimicrobial activity was found to be against *L. monocytogenes,* with a zone of inhibition of 9.23 mm. The Cosrhamn samples had the least antimicrobial activity against *S. aureus*, with a zone inhibition of 2.43 mm.

The ability of the probiotic coconut juice to inhibit foodborne pathogenic bacteria could be due to the production of bioactive substances, such as organic acid, lactic acid, diacetyl, hydrogen peroxide, and secondary metabolites (Gaanappriya *et al.,* 2013). This outcome agrees with the study by Adebayo and Akpeji (2016), who observed that the antibacterial compounds

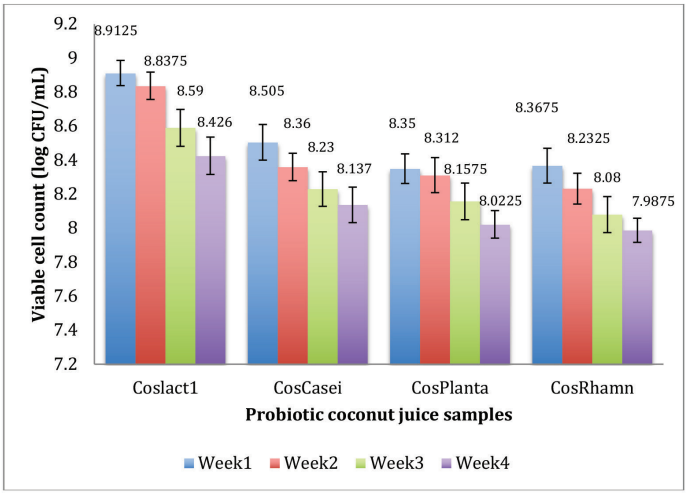

Figure 1: *Viability of probiotic bacteria in the stored samples.* KEY: Coslact1 - coconut juice with *Lactococcus lactis* IO-1, CosCasei - coconut juice with *L. casei*, CosPlanta - coconut juice with *L. plantarum,* Cosrhamn - coconut juice with *L. rhamnosus,* control - coconut juice without probiotic. Note: Control has no viable cell count throughout the cold storage

Table 7: The antibacterial activities of the probiotic coconut juice samples against some foodborne pathogenic microbes

	Inhibition (mm)			
	L. monocytogenes	***S. aureus***	***S. typhi***	***E. coli***
Coslact1	9.23	7.24	4.94	4.21
CosCasei	5.90	3.90	3.70	3.29
CosPlanta	8.40	6.99	6.28	4.16
Cosrhamn	7.09	2.43	3.43	8.13
Control	ND	ND	ND	ND

KEY: Coslact1 - coconut juice with *Lactococcus lactis* IO-1, CosCasei - coconut juice with *L. casei*, CosPlanta - coconut juice with *L. plantarum,* Cosrhamn - coconut juice with *L. rhamnosus,* Control - coconut juice without probiotic

produced by probiotic bacteria have inhibitory activities against certain foodborne pathogens, as reported in this study.

Sensory Test of the Stored Probiotic Samples

Table 8 indicates a sensory test of the probiotic juice samples during four weeks of refrigerated storage (4°C). The results obtained from the sensory test showed no significant difference in appearance, aroma, colour and taste of the probiotic juice samples during refrigerated storage.

The results obtained from the seven-scale hedonic analysis show that the probiotic juice samples had an excellent organoleptic quality and the probiotic bacteria did not have an impact on the appearance, aroma, and taste of the product following four weeks of refrigerated storage.

The preferred sample was Coslact1 throughout the four weeks of cold storage. This may be due to the effect of the starter culture on the sample. This outcome agrees with the study by Adebayo and Akpeji (2016), who revealed the impact of probiotic culture on the sensorial attributes of the probiotic beverage that is a fermented pineapple product. Nematollahi *et al.* (2016) reported that there was no off-flavour in cornelian cherry juice containing probiotic strains when compared with the control sample (without probiotic).

For the food sector, the outcomes are important because they report remarkable results that can be obtained using sensory test. Furthermore, sensory tests can provide the most important attributes for consumer acceptance, guiding the sector, and allowing the sector to focus on them when developing the product. Finally, sensory tests could be used to test consumer acceptance of beverage products, mainly in the early process of product development, enabling the utilisation of a lower number of individuals.

Non-dairy products are generally good carrier of LAB for vegetarians, people with allergies to certain proteins, and lactose-intolerant consumers (Horáčková *et al.*, 2018). But it is necessary to evaluate them, when the aim is to characterise bacterial strains. Besides estimating microbial viability, it is important to verify the influence of these strains on the chemical characteristics of the beverage during storage. It was verified that the strains had an influence the chemical analysis of the samples during cold storage. However, this did not negatively affect the product as the seven-scale hedonic test indicated that most participants attributed scores of 3-4 for all attributed tested. It can be observed that microbial fermentation may be used as a suitable technology in formulating health promoting foods.

Table 8: Sensory test (taste, aroma, colour, and appearance) of the probiotic samples during cold storage

Juice Code Sample	Taste				Aroma				Color				Appearance			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Coslact1	3.0^{a}	3.0^{a}	3.0^{b}	3.0^{b}	3.0^{a}	3.0^{a}	3.0^{b}	3.0^{b}	3.0^{a}	3.0^{b}	3.0^{b}	3.0^{b}	3.0^{a}	3.0^{a}	3.0^{b}	3.0^{b}
Coscasei	3.0^{a}	3.0^{a}	3.0^{b}	4.0^{c}	3.0^{a}	3.0^{a}	3.0^{b}	3.0^{b}	3.0^{a}	3.0^{b}	4.0^{b}	3.0^{b}	3.0a	3.0^{a}	3.0^{b}	4.0^{b}
Cosplanta	3.0^{a}	3.0^{a}	3.0^{b}	3.0^{c}	3.0^{a}	3.0^{a}	3.0^{b}	3.0^{b}	3.0^{a}	3.0^{b}	4.0^{b}	3.0^{b}	3.0a	3.0^{a}	3.0^{b}	4.0^{b}
Cosrhamn	3.0^{a}	3.0^{a}	3.0^{b}	3.0^{c}	3.0^{a}	3.0^{a}	3.0^{b}	3.0^{b}	3.0^{a}	3.0^{b}	4.0^{b}	3.0^{b}	3.0a	3.0^{a}	3.0^{b}	4.0^{b}
P control	3.0^{a}	3.0^{a}	6.0^{a}	7.0^{a}	3.0^{a}	3.0^{a}	6.0^{a}	7.0^{a}	3.0^{a}	3.0^{b}	6.0^{a}	7.0^{a}	3.0a	3.0^{a}	7.0^{a}	7.0^{a}
SE	0.0	0.0	0.25	0.28	0.0	0.0	0.25	0.26	0.0	0.0	0.23	0.24	0.0	0.0	0.26	0.27

Scoring points: 7=dislike extremely, 6= dislike strongly, 5=dislike, 4=like moderately, 3= like, 2=like strongly, 1=like extremely. KEY: Coslact1 - coconut juice with *Lactococcus lactis* IO-1, CosCasei - coconut juice with *L. casei*, CosPlanta - coconut juice with *L. plantarum,* Cosrhamn - coconut juice with *L. rhamnosus,* Control - coconut juice without probiotic.

Conclusion

In this study, lactic acid bacteria (*Lactobacillus casei* ATTC 393, *Lactobacillus plantarum* ATCC20174, *Lactobacillus rhamnosus* ATCC 7469, and *Lactococcus lactis* IO-1) as a single starter culture were employed to investigate the viability of the probiotic and physicochemical properties of stored probiotic coconut juice. Sensory tests of the samples were also performed. The study showed that coconut juice was very favourable to the probiotic strains and they were able to survive under high acidity and low temperature (4°C) conditions for four weeks. The changes in pH, total soluble solids and total acidity, as well as phenol, tannin and antioxidants contents during cold storage were similar between the four cultures. The stored samples had an inhibitory effect against the selected foodborne pathogen. No remarkable changes were revealed in the sensory test. Therefore, the four mentioned strains were realised as good potential probiotics for utilisation in food matrices commonly consumed, such as fruit beverages. However, further research may be conducted regarding the effect of probiotic coconut beverages on postprandial glycemia in healthy individuals.

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