

Research article

***Lactobacillus plantarum* and *Lactobacillus helveticus* fermented tender coconut water probiotic beverage: A comparison study**Rakshitha N. R.^{1#}, Mayookha V. P.^{1,3#}, Dasari Swaroopa Rani², Mohan A. Dhale², Suresh Kumar G.¹¹Department of Biochemistry, ²Department of Microbiology and Fermentation Technology, Central Food Technological Research Institute, Mysore, 570020, Karnataka, India³Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India

(Received: September 2022

Revised: November 2022

Accepted: December 2022)

Corresponding author: **G. Suresh Kumar**. Email: sureshg@cftri.res.in; sureshg1977@yahoo.co.in

These authors contributed equally to this work

ABSTRACT

Introduction and Aim: Coconut water is reflected as an elixir to the body. It has become a popular beverage among different age groups because of its deliciousness and high nutritional value. The growth, fermentation efficiency, and bioactive content generation by the probiotic bacteria *Lactobacillus plantarum* and *Lactobacillus helveticus* in coconut water were evaluated in this study to develop a new non-dairy probiotic beverage. The classification of species was based on its biochemical features, morphology, physiology, as well as its probiotic properties.

Materials and Methods: Batch fermentations were conducted for the aseptically collected coconut water for two days at a constant temperature of 37°C with *Lactobacillus plantarum* and *Lactobacillus helveticus*. The fermented samples were microbiologically and chemically analysed for checking the presence of bioactive components and the physicochemical properties.

Results: After a 48-hour fermentation with *Lactobacillus plantarum* and *Lactobacillus helveticus*, tender coconut water showed a significant increase in the bio-actives including cyanocobalamin and riboflavin content. The antibacterial activity shown by the final fermented product indicated its potential for acting against foodborne pathogens.

Conclusion: The findings revealed that following fermentation with *Lactobacillus helveticus* and *Lactobacillus plantarum*, the species were able to improve the nutritional content of tender coconut water. Between these two *Lactobacillus* strains, *L. plantarum* fermented tender coconut water exhibited higher nutritional values compared to the latter.

Keywords: Tender coconut water; fermentation; probiotic beverage; *Lactobacillus* bacteria.

INTRODUCTION

Coconuts (*Cocos nucifera* L.) come in a variety of categories and are widely planted in tropical and subtropical locations, particularly in Southeast Asian countries. Coconut water, one of the edible portions of the coconut, is widely used in processing and is becoming incredibly popular because of its exquisite taste and valuable minerals. Unfortunately, during dehusking for the production of coconut oil or coconut milk, a substantial volume of coconut water is generally discarded (up to 200,000 tonnes) (1).

Coconut water is a liquid endosperm, which begins to form in modest amounts in the third month of maturity and peaks in eight months before gradually diminishing as the nut matures. It is a clear, colourless

liquid with a pH range of 4.2 to 6.0 that is sweet, naturally flavoured, and just mildly acidic. Coconut water includes modest amounts of the carbohydrates such as glucose, fructose, sucrose, and sorbitol, as well as the essential amino acids; lysine, histidine, tyrosine, tryptophan and organic acids including tartaric, citric, and malic acids (2). There are many different ratios of carbohydrates, with a total composition of up to 8% (w/v). Coconut water also includes majority of the nutrients, which are growth stimulating elements necessary for plant and microbial cell growth. Not only is coconut water consumed widely worldwide as a delightful beverage, but it also has numerous medicinal benefits. Exploiting such a unique medium for human wellbeing is difficult, since the tender coconut water is wasted or environmental pollution is avoided. Fermentation with specific culture one can save or utilize wasted coconut water

for the production of health beneficial functional beverages (3).

Consumers may experience some impacts if they drink Coconut Water (CW) on a regular basis. Chang and Wu (4) investigated CW for the first time and discovered (+)-catechin and (-)-epicatechin. Catechins are having antimicrobial, antioxidant, and anticancer activities (5). Fresh coconut water is high in kinetin, which is a cytokinin plant growth hormone that can help human skin cells to delay the onset of ageing symptoms (6). Coconut water contains a variety of antimicrobial peptides having its own set of characteristics and methods of action. Antimicrobial peptides from CW with action against human pathogenic bacteria were described by Mandal (7). Several studies have proved the numerous health advantages of different coconut components in various ways. Deb Mandal and Mandal (8) analysed a number of studies on coconut water properties and its effects, including the electrolyte nature as well as antioxidant, antibacterial, anticancer, antidiabetic, antiatherosclerotic, cardio-protective, anti-thrombotic, hypolipidemic, anti-cholecystitis, anti-viral, anti-fungal, anti- protozoal, immunestimulatory, hepatoprotective, and hormone-like properties. Despite the fact that coconut has numerous benefits, there are very few studies on the technological development of fermented beverages based on CW have been published (9). Because CW is prone to oxidative, enzymatic, and microbiological deterioration, a suitable preservation method is required to market it as a ready-to-drink beverage. Fermented foods are gaining appeal and acceptability due to their functional properties, according to De Dea Lindner (10). Lactic acid bacteria are used in food products all over the world, and their useful features have been scientifically established. Some LAB strains have been found to include antioxidant, antimicrobial, cholesterol- lowering, immune- modulating, chaperone- like, and opioid/opioid antagonist peptides (11). New products, especially non-dairy beverages based on fruit and grains, have been introduced. For example, Probiotic beverages constitute a prospective market. However, there are obstacles to this sector's growth, like proper processing and storage procedures required to ensure the existence of these microbes.

Vitamin B12 (cobalamin) insufficiency has previously been linked to haematological and neurological problems, as well as myocardial infarction, because humans cannot synthesise it and must rely on animal-derived foods such as meat, eggs, and milk. As a result, vegetarians are more likely to suffer vitamin B12 insufficiency. There are very few bacterial and archaeal species, like *L. plantarum* LZ95 and *L. reuteri* CRL1098, can synthesise substantial amounts of vitamin B12 (12). With the goal of increasing the

health advantages of this beverage, we concentrated on the development of a functional beverage from CW utilising *Lactobacillus plantarum* and *Lactobacillus helveticus*.

MATERIALS AND METHODS

Bacterial cultures

The two probiotic strains, *L. plantarum* and *L. helveticus* were procured from the Department of Microbiology and Fermentation Technology, CFTRI, Mysuru. The selected strains were propagated separately in sterile MRS broth, incubated for 24 hrs at 37°C and then kept at 4°C for later use.

Optimization studies

For the optimization of growth temperature, the two bacterial strains were grown at 5 different temperatures such as 30, 35, 40, 45 & 50°C and the growth was analysed for 30 hrs, at every 6 hrs of interval. Spectrometric readings of optical density at 540nm were recorded for the bacterial biomass analysis. Optimum pH was also noted using a pH meter for every 6 hrs of interval till 30 hrs. Furthermore, pour plates were carried out with the culture for 12, 24 and 30 hrs. Culture grown at 35°C showed better biomass of the bacterial strains.

Freeze drying of the bacterial cultures

The bacterial cells were cultured in bulk using MRS broth incubated at 37°C for 24 hrs in a normal Erlenmeyer flask. Centrifugation of incubated cultures was carried out at 4000 rpm for 15 minutes and the pellets containing bacterial cells were collected. The cryoprotectant (5% sucrose solution) was added to the bacterial cells as they act as preservative to retain the cells viability. Freeze drying is a common technique for the production of dried powders of probiotic bacterial cultures. In this process, bacterial cells were exposed to freezing temperature and dehydrated to restore the viability and probiotic potential.

Preparation of the inoculum for fermentation

Tender coconut water (27 ml) that was sterile (membrane filtered to 0.2 µm) was inoculated with 3 ml of bacterial culture suspension and incubated at 37°C for 18–24 hours. This was employed as the initial inoculum. A workable culture of 10⁻⁸ CFU/ml was then obtained by serially diluting an 18–24-hour fresh bacterial culture. The appropriate tube was used to pour 1 ml of sample onto MRS agar plates, which were then incubated at 37°C for 48 hours. The appropriate number of colonies for counting was thought to be between 30 and 150.

Calculation of viable spore count was done by the following formula:

Viable spore count = Number of colonies per plate × Final dilution factor.

Fermentation of tender coconut water

Fresh tender coconuts (nearly 8 months old) were purchased from a local market in Mysore, Karnataka, India. The water from green tender coconuts was collected by perforating it with a sterile knife. Total solid content (⁰Brix) and pH of the water was 5.2 and 6.5 respectively.

Tender coconut water (TCW) was first fermented using 100 ml of sample in sterile Erlenmeyer flasks that held 250 ml. LAB cultures were used to inoculate the flasks containing TCW. Two days of batch fermentations were conducted in duplicate at a constant temperature of 37 °C. After incubation for two days, samples were taken aseptically by gently whirling the conical flasks, and they were then exposed to microbiological and biochemical analysis. In a similar manner, fermentation was carried out in large quantities and used to create the product.

Proximate compositional analysis of fresh and fermented tender coconut water

The samples were subjected to proximate analysis by AOAC method (13). Reducing sugar (DNS), ash (AAS method), TSS (refractometer) and acidity (titrimetric method). moisture, proteins (Kjeldahl apparatus) and viscosity (viscometer) were analysed.

Determination of pH, total acidity, and cell viability

The pH was measured using a digital pH metre from Sigma-Aldrich in the US. 10 g of each sample were combined with 90 mL of distilled water and then titrated with 0.1 N NaOH using 0.1 percent (w/v) phenolphthalein in 95 percent ethanol as an indicator to measure the total titrable acidity. Samples collected at 0 hours were utilised as a control. On MRS agar plates, viable numbers of LAB were found for the fermented products. On duplicate MRS agar plates, 1 mL of each sample was disseminated after being diluted with 0.1 percent (w/v) bacteriological peptone (Himedia). Viable LAB counts were tested once per week for 4 weeks after storage at 40 °C and after 2 days of storage at room temperature. The viability was assessed using the colony-forming units (CFU)/mL, and the cell concentration was determined as the log of the CFU/mL.

Analysis of various bio-actives in TCW, PSFTCW and HSFTCW

The analysis of various bio-actives presented in TCW, PSFTCW & HSFTCW were conducted using HPLC Systems with C18 column, with various mobile solvent system mentioned below.

Cyanocobalamin: was measured by reverse phase HPLC analysis utilizing the Shimadzu LC-10A, a device equipped with a Photodiode array detector (PDA) and fitted phenomenex C18 column (25 cm x 4.6 mm, 5 mm i.d.), detector set at 361 nm; mobile phase- Distilled water (A) and acetonitrile (B) (70:30), flow rate 1 mL/min.

Riboflavin: By combining an HPLC system (model LC-10 AVP Shimadzu Corp., Tokyo, Japan) with a C18 column of water superisorb 3 µm ODS2, the content was calculated. Used with λEx 445 nm and λEm 550 nm is a fluorescence detector. Riboflavin is employed as the standard for identification and quantification in an isocratic assay using a mobile phase made up of water, methanol, and acetic acid (68:32:0.1 v/v), with a flow rate of 1 mL/min.

Total Phenolic Content: Using the Folin-Ciocalteu colorimetric technique the total phenolic content was examined. To make a calibration curve, gallic acid (0–8.2 mg/mL) was utilized as the standard. Per millilitre of material, the results were expressed as micrograms of gallic acid equivalents (GAEs).

Antibacterial activity of fermented TCW

Using the agar-diffusion method, the antibacterial activity of the culture supernatant was assessed. To acquire cell-free supernatants, culture supernatants were filtered over 0.45-µm size Millipore filter. As target organism the following foodborne pathogens were investigated: ATCC 9341, *Micrococcus luteus*, *B*; *E. coli* EFR02 Each strain was cultivated aerobically overnight at 37 °C in brain heart infusion (BHI; Sigma-Aldrich) broth before being centrifuged (4500g, 15 min, 4 °C), washed, and re-suspended in sterile saline solution. 50 mL of each cell-free supernatant was poured into wells containing BHI agar, and a 1-mL aliquot of each bacterial suspension (with final viable counts of about 8 log CFUs/mL) was disseminated onto BHI soft agar plates (5-mm diameter and 5-mm depth; drilled using a sterile glass cannulas). The diameter (mm) of the growth-inhibition zones surrounding each well was determined after the plates had been incubated aerobically at 37°C for 48 hours. Uninoculated CW served as the positive control, and MRS broth served as the negative control.

SEM Analysis

Before being fixed with 2.5 g/100 ml glutaraldehyde, the fermented tender coconut water products were gently washed in a 50 mM phosphate buffer solution (pH 7.3). In a series of exposures, the specimen was dehydrated with ethanol concentrations ranging from 30 to 100 percent. After drying, the samples were distributed over a gold-coated, double-sided conductive adhesive tape that had been glued to a metallic stub and was then examined for surface morphology using a scanning electron microscope (LEO 435VP, UK) connected to a video copy processor (Mitsubishi, Japan) at a voltage of 20 kV. A 35 mm camera (Ricoh, Japan) was used to take the pictures, and it was optically connected through fibre optics to the monitor.

Statistical analysis

The data were analysed using one-way analysis of variance, and Tukey's test was performed to evaluate differences across treatments. The data are displayed as mean values \pm with standard deviations where $p < 0.05$ was considered statistically significant.

RESULTS

Lactobacillus plantarum and *Lactobacillus helveticus*, both of which have been reported to have potential probiotic properties, were shown to be effective in the production of a fermented CW beverage in this study. After a 48-hour fermentation utilising *Lactobacillus plantarum* and *Lactobacillus helveticus*, we discovered that the fermented beverage contains essential minerals, vitamin B12, antioxidants, and antibacterial activity.

Proximate composition and bioactive analysis

The proximate composition of the coconut water is similar to the work carried out by Walter (14) and Tan

(15). The results showed a significant difference between the fresh tender coconut water and fermented tender coconut water in the composition of reducing sugar, ash, TSS and acidity. Slight differences were observed in other parameters like moisture, proteins and viscosity as presented in Table 1. The appearance of the tender coconut water before and after fermentation is presented in Fig. 1. In the current study the pH reduced up to 4.7 at 40°C and 30 hrs of incubation time, whereas normal broth had reduction of pH into 6.2. Total soluble solids in tender coconut water and probiotic fermented coconut water were determined to be 5.2, 6.4 (PS), and 6.3°(HS) Brix, respectively. Samples of coconut water were found to have a titratable acidity of 0.18% and 0.53%, respectively. The inclusion of ascorbic acid gave tender coconut water a titratable acidity value of 0.18%. The titratable acidity value after fermentation was 0.53% lactic acid.

Mineral composition in the tender coconut water and fermented product was found to have slight differences (Table 1). Among the fermented coconut water samples, PS fermented product has a little higher mineral composition compared to HS. Reducing sugar of both raw and fermented tender coconut water were measured by the DNS method. Reducing sugar content was found to decline significantly in the fermented product, when compared to control (raw) as in Fig. 2(A). The change in pH during fermentation indicated that most of the carbohydrates may be utilized by the bacteria and converted into short chain fatty acids, which may have resulted in the reduction of the pH (Fig. 2 B). It was observed that cyanocobalamin increased two times after fermentation. It was also visible that the content was more in the PS fermented product than the HS one, at room temperature and 30 hrs. of incubation (Fig. 2C). However, the production of riboflavin was found to be more in the PSFCW fermented product than in the HSFCW (Fig. 2D). There was a decrease in the phenolic content of the fermented product (Fig. 2 E).

Table 1: Proximate composition of fresh tender coconut water (TCW) and fermented tender coconut water (FCW)

Sl no.	Proximate analysis	Fresh tender coconut water	Fermented tender coconut water PS	Fermented tender coconut water HS
1.	Moisture content (%)	96.64 \pm 5.0 ^a	98.04 \pm 7.2 ^a	97.09 \pm 5.60 ^a
2	Ash content (%)	0.40 \pm 0.01 ^a	0.60 \pm 0.01 ^b	0.59 \pm 0.02 ^b
3	Proteins (g/100 ml)	0.76 \pm 0.03 ^a	0.81 \pm 0.02 ^a	0.80 \pm 0.02 ^a
4	Reducing sugars(g/100 ml)	0.59 \pm 0.02 ^a	0.42 \pm 0.04 ^b	0.40 \pm 0.02 ^b
5	TSS(Brix)	5.20 \pm 0.40 ^a	6.40 \pm 0.10 ^b	6.30 \pm 0.20 ^b
6	Titration acidity	0.18 \pm 0.02 ^a	0.53 \pm 0.05 ^b	0.50 \pm 0.07 ^b
7	Viscosity	10.08 \pm 0.20 ^b	11.35 \pm 0.6 ^a	11.02 \pm 0.8 ^a
8	Mineral composition			
a	Calcium	28.0	32.10	30.60

b	Iron	0.21	00.26	00.22
c	Magnesium	10.45	10.49	10.48
d	Sodium	35.01	38.10	36.90
e	Potassium	120.0	127.1	124.9
f	Zinc	00.14	00.18	00.16
g	Copper	00.07	00.09	0.072
h	Manganese	00.31	00.36	00.31

^{a & .b} Different alphabets represented in the row indicate significant at $P < 0.05$ level

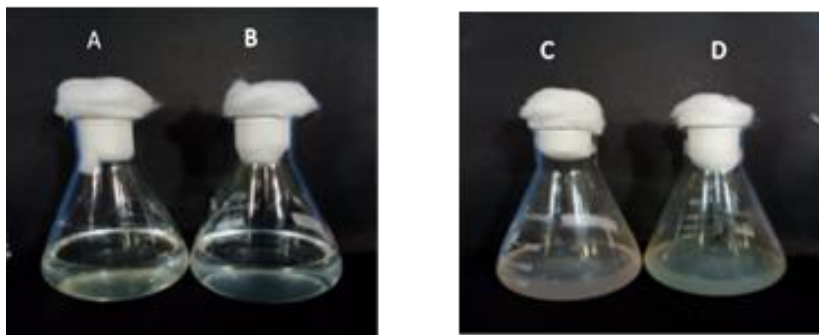


Fig. 1: Fresh tender coconut water (A&B), fermented coconut water with bacterial strains of PS(C) and HS (D).

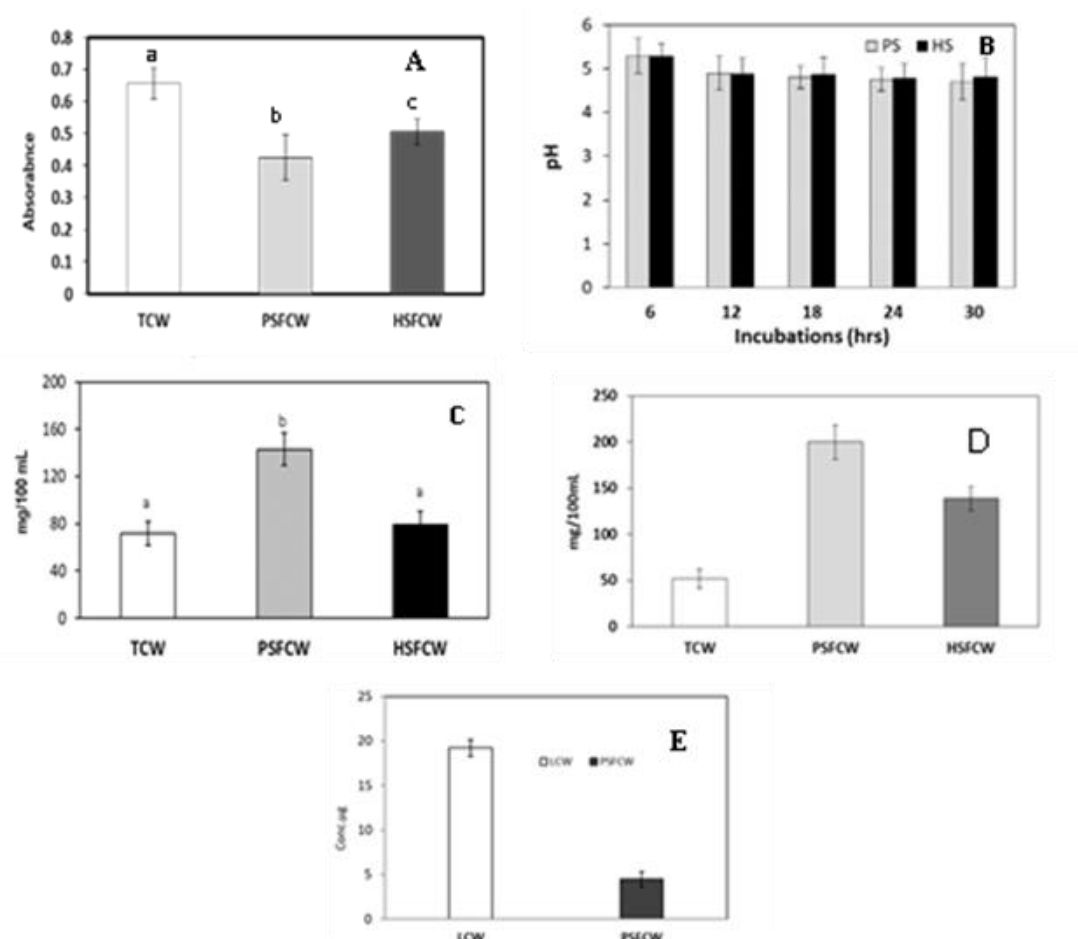


Fig.2: Reducing sugar content (A), Media pH (B), Content of Cyanocobalamin (C), Content of Riboflavin (D), Phenolic content (E) in control and after fermentation. ^{a & .b} Different alphabets represented in the bar graph indicate significant at $P < 0.05$ levels.

Antimicrobial activities

Fig. 3A trial has been made to study the antimicrobial activities using *Micrococcus luteus* ATCC 9341, B; *Escherichia coli* EFR02 which are food borne

pathogens. The fermented sample showed a better inhibition zone with *E. coli* strain when compared to the unfermented sample. Positive control showed a clear inhibition. Freezing dried samples does not have

much effect. However, all the test samples had a very good zone of inhibition.

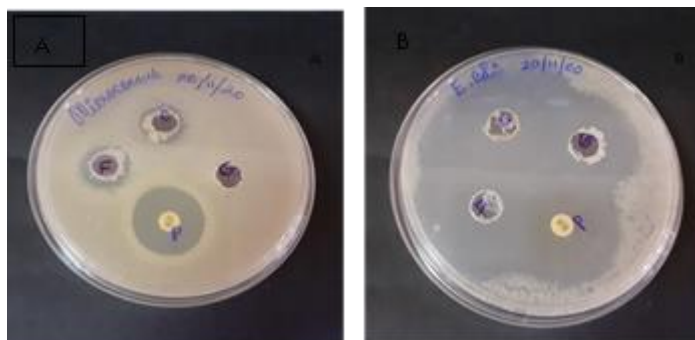


Fig. 3: Antimicrobial activities of control and fermented samples using plate method showing the growth-inhibition zones' diameter (mm) of *M. luteus* ATCC 9341(A) and *E. coli* EFR02(B).

Abbreviations: F- Fermented Tender Coconut Water, C-Control, P-Positive control (Tetracycline).

Strain/Antibiotics	Inhibition zone (mm)*	
	<i>M. luteus</i> ATCC 9341	<i>E. coli</i> EFR02
Control	14	44
Fermented TCW	16	42
Freeze Dried Fermented TCW	-	40
Positive Control (Tetracycline)	24	36

Surface morphology through SEM analysis

With the help of a scanning electron microscope, the colony morphology of the *Lactobacillus plantarum* and *Lactobacillus helveticus*, on the surface of

fermented coconut water were observed. The surface morphology of the solids separated from the sterilized medium after inoculation of *L. plantarum* and *L. helveticus*, was observed by using SEM, as shown in Fig.4 (A–D).

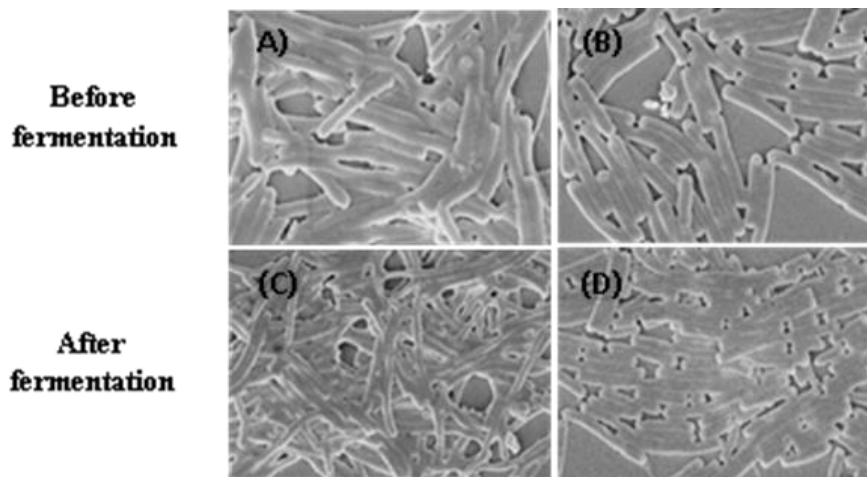


Fig. 4: Scanning electron micrographic (SEM) images of before and after fermentation of *Lactobacillus plantarum* (A & C), *Lactobacillus helveticus* (B & D)

DISCUSSION

In the present investigation, fermentation of coconut water was analysed with focussing on two different probiotic strains, i.e. *Lactobacillus plantarum* and *Lactobacillus helveticus*. To make sure that it was the only raw material that controlled the probiotic bacteria's growth and metabolism, tender coconut water was used as the only fermentation medium in this study, without any additions. Using a possible probiotic strain as a starter culture can provide a

fermented product with distinct and consistent properties as well as potential health benefits, as shown by the improved growth of *Lactobacillus plantarum* and *Lactobacillus helveticus* in tender coconut water. Tender coconut water that has been fermented offers the advantages of plant-based foods with the extra benefit of probiotic-rich live microorganisms.

The finding simply states that the rise in viable cell count correlated with the fall in pH and amount of

sugars taken during fermentation. The total soluble solids content of tender coconut water was 5.2 °Brix, indicating that the majority of the solids were soluble solids like sugars. The total soluble solids content of the fermented probiotic coconut water increased as a result of the increase in viable cell counts after fermentation. Soluble solids are another indicator of how sweet CW is. The sugars fructose, glucose, and sucrose are known to contribute to the sweet flavour of CW (16). The primary by-product of the conversion of carbs caused by the use of coconut water's sugars is lactic acid. The most common strains reported for the generation of lactic acid are *Lactobacillus plantarum* and *Lactobacillus helveticus*. Thus the observed increase in the acidity is due to the production of lactic acid during fermentation.

The exopolysaccharide production by *Lactobacillus plantarum* and *Lactobacillus helveticus* resulted in an increase in the soluble solid content, which led to a substantial increase in the viscosity of fermented coconut water to 11.35 and 11.02, respectively (17). The viscosity of tender coconut water is governed by the strength of hydrogen bonds and intermolecular spacing, both of which are significantly influenced by concentration and temperature. These forces between molecules and water-solute interactions, that is, interactions between sugars and acids, determine the viscosity of tender coconut water. Increased soluble solid content leads to more hydrated molecules and hydrogen bonding with the hydroxyl groups of the solute, which improves fluid resistance and increases liquid viscosity. Soluble solids in tender coconut water were mostly owing to sugar content, whereas live cells and exopolysaccharide played a significant impact in viscosity values in fermented coconut water (18).

The pH values obtained points out the low acidic nature of the coconut water (14). Fermentation and multiplication are carried out by the bacteria using nutrients and substrate. More lactic acid is produced as a result of active metabolism, which lowers the pH and increases the acidity of the final product (19).

The increase in the mineral contents between fermented and fresh TCW indicates that these minerals were released from chelated complex compounds through fermentation (20). Additionally, fermentation enhances the bioavailability of certain minerals (21). Inorganic substances known as minerals are necessary for the control of metabolic and structural processes. As previously documented, both fermented and unfermented CW included significant mineral components such calcium, magnesium, potassium, and sodium (22,23). Numerous enzymes involved in energy metabolism and protein synthesis require magnesium as a cofactor (24). According to this research, fermented coconut water will likely have similar hydration and nutritional benefits to fresh

coconut water. Drinks made specifically for rehydration should be able to replace lost water volume and electrolytes, especially sodium, lost through sweat (25). According to Mitchell *et al.*, (26), a rehydration beverage should have a sodium level of 20-30 mmol/litre to restore lost fluid and hasten absorption. Additionally, Saat *et al.*, (25) found that the majority of people are likely to experience more effective hydration from beverages containing about 50 mmol/litre of sodium. In addition to other nutrients like calcium, potassium, and magnesium, the fermented coconut water had substantially higher sodium levels than the other coconut waters. The reduction observed after fermentation in both the samples indicates the utilization of sugars by LAB.

Vitamin B12, also known as cobalamin, is important for cellular metabolism and DNA synthesis. This vitamin, which can only be produced by microorganisms, is primarily found in animal products. Both LAB and the strain that produces riboflavin are frequently used in the dairy industry and added to fermented foods. A vitamin-producing LAB's obvious technical benefit is that fortification occurs in real time. Due to their benefit of in situ fortification, LAB are a viable solution for bio prospecting microorganisms that can serve as vitamin suppliers to human hosts (27). It has been discovered that both processing methods and the microorganisms used for fermentation have an impact on its composition. Riboflavin belongs to the vitamin B family, which is necessary for curing various ailments including skin health. When compared to unfermented milk, certain yoghurt starter cultures lower the content of riboflavin (vitamin B2), while others dramatically increase it (28). The reduction in phenolic content could be due to utilization of phenolics by the bacterial culture after depletion of media nutrients. Proteolytic enzymes hydrolyze phenolic complexes into simple soluble free phenols that are chemically more potent and quickly absorbed during fermentation. Furthermore, bacteria may metabolise simpler soluble free phenols, increasing bacterial proliferation (29). The majority of antioxidants consumed by humans come from phenolic compounds, which are widely distributed in plants. A few of them have been found in CW, including (C)-catechin and (+)-epicatechin (4).

The antimicrobial activity of bacterial culture supernatants against a variety of foodborne diseases. The results showed that all of the pathogens (*Micrococcus luteus* ATCC 9341 and *Escherichia coli* EFR02) studied were hindered in their growth, indicating the potential of the fermented tender coconut water for inhibiting the major foodborne diseases.

In the SEM analysis, the bacteria strains appeared as regular rods of about 1–1.5 µm long, with some shorter

as well as elongated shapes as explained by Kang (30). The increase in the number of rod structures are visible in the fermented TCW, compared to Fresh TCW.

CONCLUSION

The present investigation showed that probiotic *Lactobacillus plantarum* and *Lactobacillus helveticus* grew well in tender coconut water. The study's findings suggest that coconut water might be fermented into a probiotic beverage for its nutrition and enormous health advantages. Among these two strains, *L. plantarum* showed better results in major biochemical parameters as well as in nutritional quality. Advance studies are needed for developing an economical *L. plantarum* -fermented functional beverage.

ACKNOWLEDGEMENT

The authors wish to gratefully acknowledge the Director, CSIR-CFTRI and Mr. Jayaprakashan S G (Sr. Technician (2), Department of Food Engineering, CSIR-CFTRI) and Girish Kempanna Ghiwari (Sr. Technical Officer (1), Department of Food Engineering, CSIR-CFTRI) for their constant support. This work was carried out under the project GAP-561 funded by Coconut Development Board (CDB), Kochi, India.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

1. Unagul, P., Assantachai, C., Phadungruengluij, S., Suphantharika, M., Tanticharoen, M., Verduyn, C. Coconut water as a medium additive for the production of docosahexaenoic acid (C22: 6 n3) by *Schizochytrium mangrovei* Sk-02. *Bioresource Technology*. 2007 Jan 1;98(2):281-287.
2. Yong, J.W., Ge, L., Ng, Y.F., Tan, S. N. The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. *Molecules*. 2009 Dec;14(12):5144-5164.
3. Camargo Prado, F., De Dea Lindner, J., Inaba, J., Thomaz-Soccol, V., Kaur Brar, S., Soccol, C.R., Development and evaluation of a fermented coconut water beverage with potential health benefits. *Journal of Functional Foods*. 2015; 12: 489-497.
4. Chang, C., and Wu, R. Quantification of (C)-catechin and (+)-epicatechin in coconut water by LC-MS. *Food Chem*. 2011; 126: 710-717.
5. Zaveri, N., T. Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. *Life sciences*. 2006 Mar 27;78(18):2073- 20780.
6. Ge, L., Yong, J. W., Goh, N. K., Chia, L. S., Tan, S. N., Ong, E. S. Identification of kinetin and kinetin riboside in coconut (*Cocos nucifera* L.) water using a combined approach of liquid chromatography–tandem mass spectrometry, high performance liquid chromatography and capillary electrophoresis. *Journal of Chromatography B*. 2005 Dec 27;829(1-2):26-34.
7. Mandal, S., M., Dey, S., Mandal, M., Sarkar, S., Maria-Neto, S., Franco, O. L. Identification and structural insights of three

- novel antimicrobial peptides isolated from green coconut water. *Peptides*. 2009 Apr 1;30(4):633-637.
8. Deb Mandal, M., Mandal, S. Coconut (*Cocos nucifera* L.: Arecaceae): in health promotion and disease prevention. *Asian Pacific Journal of Tropical Medicine*. 2011 Mar 1;4(3):241-247.
9. Ferrari Pereira, F.L. I., De Dea Lindner, J., Thomaz-Soccol, V., Parada, J. L., Soccol, C. R. Development of an innovative nutraceutical fermented beverage from herbal mate (*Ilex paraguariensis* A.St.-Hil.) extract. *International Journal of Molecular Sciences* (2012). 13, 788-800.
10. De Dea Lindner, J., Penna, A. L. B., Demiate, I. M., Yamaguishi, C. T., Prado, M. R. M., & Parada, J. L. Fermented Foods and Human Health Benefits of Fermented Functional Foods. In Carlos Ricardo Soccol, A. Pandey, & C. Larroche (Eds.), *Fermentation processes engineering in the food industry* (263–297). Boca Raton: Taylor & Francis Inc. (2013).
11. Pessione, E., Cirrincione, S. Bioactive molecules released in food by lactic acid bacteria: encrypted peptides and biogenic amines. *Frontiers in microbiology*. 2016 Jun 9;7:876.
12. Li, P., Gu, Q., Yang, L., Yu, Y., Wang, Y. Characterization of extracellular vitamin B12 producing *Lactobacillus plantarum* strains and assessment of the probiotic potentials. *Food chemistry*. 2017 Nov 1;234:494-501.
13. AOAC., 1990. Official Methods of Analysis of the Association of Chemists. Analysis of the Association of Chemists, Washington, DC. 223-225, 992-995.
14. Walter, E. H., Kabuki, D.Y., Esper, L. M., Sant'Ana, A., S., Kuaye, A.Y. Modelling the growth of *Listeria monocytogenes* in fresh green coconut (*Cocos nucifera* L.) water. *Food Microbiology*. 2009 Sep 1;26(6):653-657.
15. Tan, T. C., Cheng, L. H., Bhat, R., Rusul, G., Easa, A. M. Composition, physicochemical properties and thermal inactivation kinetics of polyphenol oxidase and peroxidase from coconut (*Cocos nucifera*) water obtained from immature, mature and overly-mature coconut. *Food Chemistry*. 2014 Jan 1;142:121-128.
16. Campbell-Falck, D., Thomas, T., Falck, T. M., Tutuo, N., Clem, K. The intravenous use of coconut water. *The American journal of emergency medicine*. 2000 Jan 1;18(1):108-111.
17. Kodali, V. P., Sen, R. Antioxidant and free radical scavenging activities of an exopolysaccharide from a probiotic bacterium. *Biotechnology Journal: Healthcare Nutrition Technology*. 2008 Feb;3(2):245-251.
18. Gangwar, A. S., Bhardwaj, A., Sharma, V. Fermentation of tender coconut water by probiotic bacteria *Bacillus coagulans*. *International Journal of Food Studies*. 2018 Apr 13;7(1): 10.7455/ijfs/7.1.2018.a9.
19. Praia, A. B., Júnior, G. C. A. C., Guimarães, A. G. S., Rodrigues, F. L., Ferreira, N. R. Coconut Water-Based Probiotic Drink Proposal: Evaluation of Microbiological Stability and Lactic Acid Estimation. *J Food Sci Nutr*. 2020; 6: 062.
20. Obadina, A.O., Akinola, O.J., Shittu, T.A., Bakare, H.A., Effect of Natural Fermentation on the Chemical and Nutritional Composition of Fermented Soymilk Nono. *Nigerian Food Journal*. 2013;31: 91-97.
21. Sripriya, G., Antony, U., Chandra, T. S. Changes in carbohydrate, free amino acids, organic acids, phytate and HCl extractability of minerals during germination and fermentation of finger millet (*Eleusine coracana*). *Food chemistry*. 1997 Apr 1;58(4):345-350.
22. Lee, P. R., Boo, C., X., Liu, S. Q. Fermentation of coconut water by probiotic strains *Lactobacillus acidophilus* L10 and *Lactobacillus casei* L26. *Annals of microbiology*. 2013 Dec;63(4):1441-1450.
23. Kantachote, D., Ratanaburee, A., Hayisama-ae, W., Sukhoom, A., Nunkaew, T. The use of potential probiotic *Lactobacillus plantarum* DW12 for producing a novel functional beverage from mature coconut water. *Journal of Functional Foods*. 2017 May 1;32:401-408.

24. Berdanier, C. D., Berdanier, L. A. Advanced nutrition: macronutrients, micronutrients, and metabolism. CRC press; 2021 Jul 6.
25. Saat, M., Singh, R., Sirisinghe, R. G., Nawawi, M. Rehydration after exercise with fresh young coconut water, carbohydrate-electrolyte beverage and plain water. Journal of physiological anthropology and applied human science. 2002;21(2):93-104.
26. Mitchell, J. B., Grandjean, P.W., Pizza, F.X., Starling, R. D., Holtz, R.W. The effect of volume ingested on rehydration and gastric emptying following exercise-induced dehydration. Medicine and science in sports and exercise. 1994 Sep 1;26(9):1135-1143.
27. Burgess, C. M., Smid, E. J., van, Sinderen, D. Bacterial vitamin B2, B11 and B12 overproduction: an overview. International journal of food microbiology. 2009 Jul 31;133(1-2):1-7.
28. Zhang, Z. P., Ma, J., He, Y. Y., Lu, J., Ren, D.F. Antioxidant and hypoglycemic effects of Diospyros lotus fruit fermented with *Microbacterium flavum* and *Lactobacillus plantarum*. Journal of Bioscience and Bioengineering. 2018 Jun 1;125(6):682-687.
29. LeBlanc, J. G., Laino, J. E., del Valle, M. J., Vannini, V. V., van Sinderen, D., Taranto, M. P., et al., B-Group vitamin production by lactic acid bacteria—current knowledge and potential applications. Journal of Applied Microbiology. 2011 Dec;111(6):1297-1309.
30. Kang, C. H., Han, S. H., Kim, J. S., Kim, Y., Jeong, Y., Park, H. M., et al., Inhibition of nitric oxide production, oxidative stress prevention, and probiotic activity of lactic acid bacteria isolated from the human vagina and fermented food. Microorganisms. 2019 Apr;7(4):109.