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ORIGINAL ARTICLE

Fermentation of coconut water by probiotic strains *Lactobacillus acidophilus* L10 and *Lactobacillus casei* L26

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Abstract Coconut water is becoming an increasingly popular beverage and sports drink in tropical countries due to its high mineral content. Probiotic fermentation of coconut water would provide consumers with a novel probiotic beverage which can provide both hydration and probiotic benefits. The aim of this study was to assess the growth, survival and fermentation performance of two probiotic bacteria in coconut water. *Lactobacillus acidophilus* L10 and *L. casei* L26 grew well in coconut water and showed similar growth patterns. The viable cell count of the two probiotic cultures reached approximately 10^8 CFU/ml after 2 days fermentation at 37 °C and maintained approximately10^7–10^8 CFU/ml after 26 days at 4 °C. Changes in total soluble solids (°Brix), pH, sugars, organic acids and minerals were similar between the two probiotic cultures, except for fructose, glucose, copper, phosphorus and lactic, acetic and malic acids. There were significant variations between the two cultures in their ability to produce and consume these compounds. *L. acidophilus* produced higher amounts of 2-heptanone, 2-nonanone, benzaldehyde, 2-heptanol, 2-nonanol, δ-octalactone and δ-dodecalactone, whereas *L. casei* produced higher amounts of acetic acid, diacetyl, acetoin, δ-decalactone, 3-methyl-3-buten-1-*ol*, linalool, 1-octanol, *p*-tolualdehyde and ethyl 2-hydroxypropanoate. There was no substantial change in mineral content. These results suggest the feasibility of fermenting coconut water into a probiotic beverage, especially for sports nutrition, with the dual benefits of electrolytes and probiotics.

Keywords Coconut water · Lactic acid bacteria · Minerals · Probiotic

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Introduction

Coconut water, the aqueous portion of the coconut endosperm, is a common by-product of the coconut industry. On average, up to 200,000 tons of coconut water is wasted each year in Thailand, and the amount is increasing yearly due to the increasing demand for coconut milk as an ingredient in many processed food products (Unagul et al. 2007). Interestingly, the perception and utilisation of coconut water has evolved over the years owing to its unique chemical composition of sugars, vitamins, minerals, amino acids, enzymes and phytohormones that play different functional roles in the human system (Yong et al. 2009). One example is the consumption of coconut water as a refreshing and hydrating beverage due to its rich mineral content, such as sodium, potassium, magnesium and calcium, which can replenish the electrolytes of the human body excreted through perspiration (Saat et al. 2002). Studies have shown that coconut water has hydrating and exercise performance effects that are comparable to those of carbohydrate–electrolyte sports drinks (Saat et al. 2002; Idárraga and Aragón-Vargas 2010; Kalman et al. 2012).

Probiotics are living microorganisms which confer a health benefit on the host when administered in adequate amounts (FAO/WHO 2001). Nowadays, probiotic bacteria are increasingly incorporated into food products due to the associated health benefits, which include aiding digestion, modulating the immune system, suppressing infections and even potentially reducing cancer risk (Shah 2007). In general, species and strains of *Lactobacillus* and *Bifidobacterium* are used in most of the probiotic applications (Parvez et al. 2006). In the food industry, the majority of the probiotic foods and beverages are dairy based as dairy products are good matrices for the delivery of probiotics to humans (Sarrela et al. 2000). In the last decade, there has been a growing interest in the utilisation of non-dairy ingredients (e.g. soymilk, meats, fruit,

vegetables, cereals and soja) as substrates to deliver the physiological benefits of probiotics to a wider group of consumers (Rivera-Espinoza and Gallardo-Navarro 2010), partially explained by a number of drawbacks related to dairy products, such as their lactose (causing lactose intolerance) and cholesterol content. The survival and persistence of some probiotic strains of *Bifidobacterium* and *Lactobacillus* have been examined in some non-dairy matrices (Rivera-Espinoza and Gallardo-Navarro 2010), and the results of these studies have demonstrated possibilities for producing probiotic products from other matrices, such as coconut water. Current knowledge on the fermentation of coconut water is rather limited (Shivakumar and Vijayendra 2006; Unagul et al. 2007; Kuswardani et al. 2011; Seesuriyachan et al. 2011), especially fermentation with probiotic bacteria. However, Dharmasena (2012) recently developed a novel non-dairy probiotic beverage with a mixture of oatmeal and coconut water using probiotic *Lactobacillus plantarum* Lp 115-400B.

Given the increasing interest in natural drinks for sports nutrition, it would be of value to evaluate probiotic fermentation of coconut water with the aim of producing a novel probiotic beverage which can provide both hydration and probiotic benefits to all individuals, but especially to athletes and recreationally active fitness enthusiasts. The aim of this study was to assess the growth, survival and fermentation performance of two probiotic bacteria in coconut water. Two probiotic strains from *Lactobacillus* genus, namely, *L. acidophilus* L10 and *L. casei* L26 were chosen for this study. These two probiotic strains are the most commercially available and investigated probiotic strains, with proven beneficial impacts on health in animal and human trials, such as improved protection against intestinal pathogens (e.g. *Escherichia coli* 0111 and *Listeria monocytogenes*) and enhancement of the immune system (Pidcock et al. 2002; Crittenden et al. 2005; Paturi et al. 2008; Baarlen et al. 2011).

Materials and methods

Bacterial strains and cultivation

Freeze-dried probiotic *L. acidophilus* L10 and *L. casei* L26 were obtained as gifts from DSM Food Specialties (Heerlen, The Netherlands). The cultures were propagated separately in sterile de Man Rogosa Sharpe (MRS) broth (Oxoid, Cambridge, UK) (De Man et al. 1960) for up to 48 h at 37 °C aerobically and then stored at −80 °C until use.

Preparation of coconut water and fermentation

Fresh coconut water was obtained from young coconuts imported from Thailand (*Cocos nucifera* L.) and purchased from a supermarket in Singapore. The initial total soluble solids content (°Brix) was 6.60 % and the pH was 6.78. The coconut water was prefiltered through a 0.65-μm prefilter (Sartorius, Goettingen, Germany) before being aseptically filtered through a 0.45-μm polyethersulfone filter membrane (Sartorius). Triplicate fermentations with each probiotic strain were carried out in 400 ml of coconut water in sterile 500-ml conical flasks, which were inoculated with 1 % (v/v) pre-culture of the respective probiotic strain (pregrown in the sterile coconut water at 37 °C for 48 h until the cell count reached approx. 10^8 CFU/ml). The batch fermentations were carried out for 2 days at 37 °C statically. Samples were taken aseptically after swirling the conical flasks gently for homogenisation on days 0, 1 and 2, and subjected to microbiological and chemical analyses.

Effects of cold storage on cell viability, pH and °Brix of probiotic coconut water

After 2 days of fermentation at 37 °C, the remaining fermented samples (300 ml) were stored at 4 °C for 26 more days. Samples were taken at weekly intervals, and the viability of the probiotic cultures, pH and °Brix of the probiotic coconut water were determined.

Analytical determinations

The pH and °Brix were measured using a pH meter (Metrohm, Herisau, Switzerland) and a refractometer (ATAGO, Yushima, Japan), respectively. Viable (cultivable) cell counts were estimated by plating the appropriate 0.1 % peptone dilution on MRS agar. The plates were incubated at 37 °C for 48 h aerobically.

Sugars and organic acids were analysed by high-performance liquid chromatography based on the method described by Lee et al. (2012). The identification and quantification of sugars and organic acids were carried out by using retention time and standard curves of the corresponding standards (Sigma-Aldrich, St. Louis, MO). The mineral concentrations were analysed by dual-view Optima 5300 DV inductively coupled plasma-optical emission spectrometer (ICP-OES) (Perkin-Elmer Life Sciences, Boston, MA). The operation conditions employed for the ICP-OES were similar to those described in Marin et al. (2011). Concentrations of minerals were determined by using the linear regression equation of the corresponding analytical standards (High-Purity Standards, Charleston, SC). All samples were analysed in triplicate.

Analysis of volatile compounds was carried out using headspace solid-phase microextraction sampling combined with gas chromatography-mass spectrometer and flame ionisation detector (FID) according to the method described in Lee et al. (2012). The identification of volatile compounds

was achieved by matching the mass spectra against the Wiley 275 and NIST 8.0 mass spectral databases and further confirmed with linear retention indices (LRI) of standard compounds and literature references. The volatile compounds were compared by FID peak area, which reflects the relative amount of each volatile compound (Lee et al. 2012).

Statistical analysis

All experimental data were subjected to one-way analysis of variance and Scheffe's test using SPSS ver. 17.0 (SPSS Corp, Chicago, IL). Means and standard deviations were calculated based on data obtained from triplicate fermentations, and significant differences were evaluated at the 95 % confidence level.

Results

Growth profile and fermentation behaviour of probiotic bacteria in coconut water

The two probiotic cultures showed similar characteristics in terms of growth, pH changes and total soluble solids (°Brix) (Fig. 1). During the fermentation, the two cultures multiplied continuously, reaching the late log phase on day 2, with *L. casei* showing higher growth at 3.58×10^8 CFU/ml, while *L. acidophilus* grew less well, with a population of 1.41×10^8 CFU/ml (Fig. 1a). The cell count on day 2 varied significantly between the two cultures at $p<0.05$ (data not shown). The pH value decreased significantly from pH 6.78 to around pH 3.6 after 2 days of fermentation, while °Brix level decreased slightly from 6.60 % to around 6.22–6.38 % (Fig. 1b, c). After storage at 4 °C for 26 days, *L. acidophilus* and *L. casei* growth remained relatively constant or decreased slightly and achieved considerably high viable cell counts of 5.04×10^7 and 1.80×10^8 CFU/ml, respectively, on day 28 (Fig. 1a). Similarly, the pH and the °Brix value remained relatively constant or decreased slightly during the 26 days of storage at 4 °C (Fig. 1b and c).

Fructose, glucose and sucrose were the sugars detected in the coconut water. There was no significant change to the overall sugar composition of the coconut water before and after fermentation (Fig. 2a). However, the sugar contents of the coconut water inoculated with the two cultures decreased significantly after 2 days of fermentation ($p<0.05$), except for sucrose (Fig. 2a). *L. acidophilus* consumed more glucose than *L. casei*, while *L. casei* consumed more fructose than *L. acidophilus*. The sucrose consumption was similar between the two cultures (Fig. 2a). Nevertheless, the coconut water fermented by both cultures still had high concentrations of residual sugars (Fig. 2a), which would enable retention of sweetness.

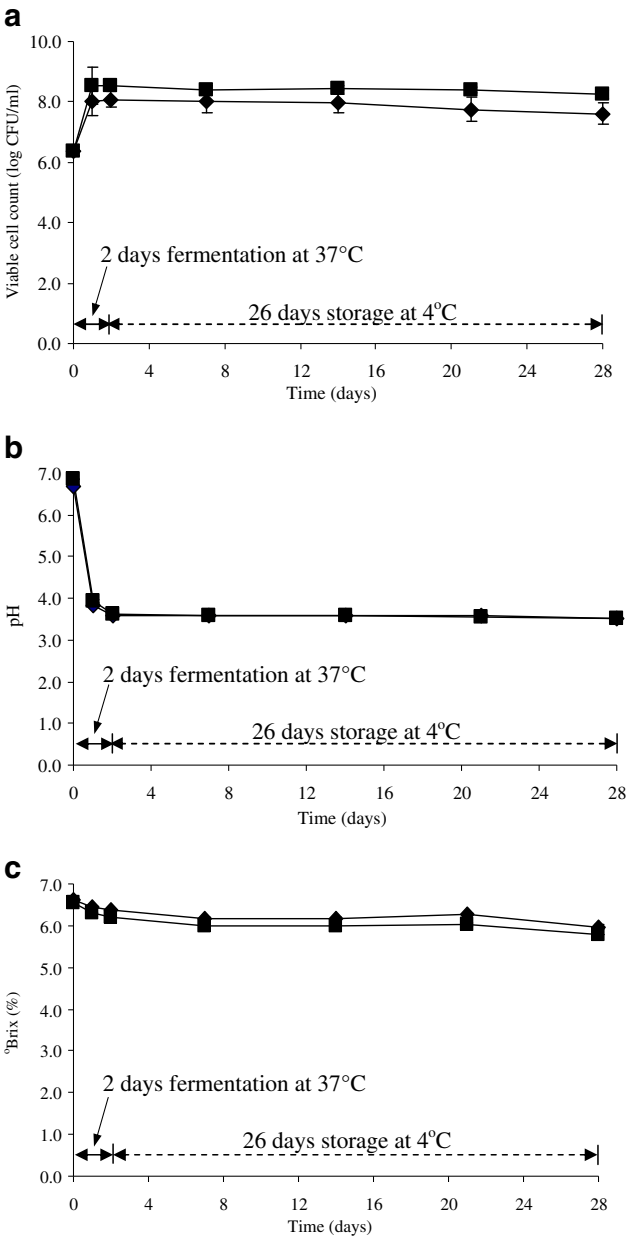

Fig. 1 Evolution of probiotic bacteria (**a**), pH (**b**) and total soluble solids content (°Brix; **c**) during the fermentation and storage of coconut water. *Filled diamond Lactobacillus acidophilus* L10, *Filled square L. casei* L26

Fermentation of the coconut water resulted in significant and different changes in the organic acid composition (Fig. 2b). Lactic acid was the dominant organic acid formed after fermentation, with *L. casei* producing a higher amount of lactic acid (9.87 g/l). Acetic acid was also produced by both cultures, with *L. acidophilus* producing a lower amount (0.37 g/l) (Fig. 2b). The changes in other organic acids were similar between the two cultures, except for malic acid which was utilised by *L. casei* but it remained unchanged after fermentation by *L. acidophilus* (Fig. 2b). Tartaric acid remained almost unchanged after fermentation by the two cultures (Fig. 2b). In

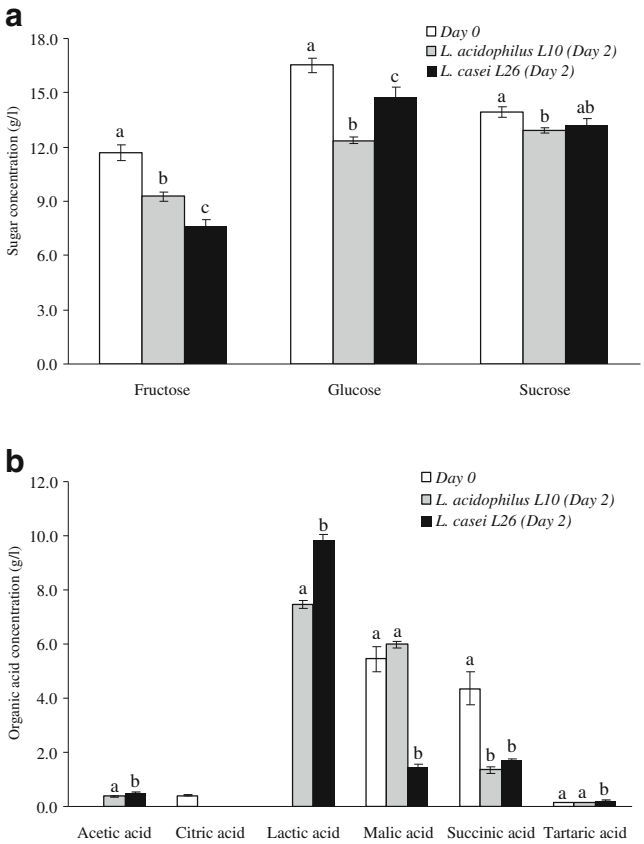

Fig. 2 Concentrations of sugars (**a**) and organic acids (**b**) of coconut water (day 2) fermented with *L. acidophilus* L10 and *L. casei* L26. The data are presented as the mean±standard deviation (n=3). *Different lowercase letters above bars* for each compound indicate a significant difference at $p<0.05$

contrast, the content of citric and succinic acids decreased substantially after fermentation by the two cultures, with citric acid being completely metabolised by day 2.

The composition and concentration of minerals in coconut water before and after fermentation are shown in Table 1. Sodium, potassium, magnesium, phosphorus and calcium were the major minerals detected in both fresh and fermented coconut water.

There was no significant alteration to the mineral composition of coconut water after fermentation by the two cultures (Table 1). Most of the minerals remained relatively unchanged, except for boron, copper, magnesium and phosphorus, the concentrations of which either increased or decreased slightly after fermentation (Table 1). The two cultures released comparable amounts of boron and magnesium, while *L. casei* showed a faster utilisation of copper and phosphorus than *L. acidophilus*, with residual concentrations of 0.45 and 166.43 mg/l at day 2, respectively (Table 1).

Volatile composition of coconut water before and after fermentation

A wide range of volatile compounds, including fatty acids, alcohols, aldehydes, esters, ketones, lactones and volatile phenol, were identified in both the fresh and fermented coconut water (Table 2). There was a variation among volatiles in terms of changes due to fermentation, and some differences between the two cultures were significant (Table 2).

Alcohols were the most copious volatiles in the fermented coconut water with a total relative peak area (RPA) of 37.61–42.47 % (Table 2). Most of the alcohols were produced after fermentation, except for ethanol, 1-butanol and isoamyl alcohol, which either remained unchanged or were utilised (Table 2). *L. acidophilus* had most of the production and utilisation of alcohols comparable to *L. casei* (Table 2). *L. casei* produced more 3-methyl-3-buten-1-*ol*, linalool and 1-octanol, while the formation of 2-heptanol and 2-nonanol was greater by *L. acidophilus* (Table 2). In particular, the concentration of 2-nonanol was 9.49-fold higher in the coconut water fermented by *L. acidophilus* than that fermented by *L. casei*.

Table 1 Concentrations of mineral in coconut water fermented with *Lactobacillus acidophilus* L10 and *L. casei* L26 on day 2

Mineral content (mg/l)	Day 0	Day 2	
		L. acidophilus L10	*L. casei* L26
Boron	0.31±0.00 a	0.32±0.01 b	0.32±0.01 b
Calcium	127.93±7.13 a	138.00±0.76 a	133.80±1.45 a
Copper	0.57±0.01 a	0.47±0.00 b	0.45±0.01 c
Iron	<0.10 a	<0.10 a	<0.10 a
Magnesium	200.17±2.65 a	205.00±0.61 b	204.00±0.96 ab
Manganese	0.65±0.06 a	0.75±0.02 a	0.67±0.03 a
Phosphorus	181.17±1.10 a	171.73±1.38 b	166.43±1.97 c
Potassium	2212±45.04 a	2206±52.73 a	2188±31.90 a
Selenium	0.24±0.01 a	0.23±0.01 a	0.23±0.01 a
Sodium	2805±63.66 a	2847±78.35 a	2748±36.53 a
Zinc	0.22±0.01 a	0.23±0.00 a	0.23±0.00 a

Values followed by the same lowercase letters are not significantly different at the 95 % confidence level

Table 2 Major volatile compounds (GC FID peak area × 10^6) and relative peak area in coconut water fermented by *L. acidophilus* L10 and *L. casei* L26 after 2 days of fermentation

Volatile compounds	LRI		Day 0		*L. acidophilus* L10		*L. casei* L26	
	FFAP[a]	Reference[b]	Peak area	RPA (%)	Peak area	RPA (%)	Peak area	RPA (%)
Acids								
Acetic acid	1427	1461[c]	10.30±1.89 a	3.49	25.80±2.25 b	5.33	20.30±1.08 c	5.81
Butyric acid	1594	1630[c]	3.41±0.35 ab	1.16	3.54±0.03 a	0.73	2.86±0.16 b	0.82
Pentanoic acid	1631	1698[d]	0.44±0.11 ab	0.15	0.24±0.04 a	0.05	0.55±0.14 b	0.16
(*E*)-2-Butenoic acid	1663	-	1.20±0.21 a	0.41	0.00±0.00 b	0.00	0.00±0.00 b	0.00
Hexanoic acid	1805	1847[c]	26.10±3.01 a	8.84	25.00±1.62 a	5.16	23.70±0.34 a	6.78
2-Ethylhexanoic acid	1908	-	0.73±0.15 a	0.25	0.63±0.05 a	0.13	0.55±0.05 a	0.16
Heptanoic acid	1918	1900[d]	0.91±0.03 a	0.31	0.71±0.01 b	0.15	0.74±0.10 b	0.21
Octanoic acid	2020	2061[c]	87.90±18.90 a	29.78	111±5.55 a	22.91	87.40±6.51 a	25.01
Nonanoic acid	2136	2169[c]	3.45±0.70 a	1.17	3.22±0.36 a	0.66	3.35±0.47 a	0.96
Decanoic acid	2243	2275[c]	9.97±2.12 a	3.38	10.90±0.59 a	2.25	0.00±0.00 b	0.00
Subtotal			144.41	48.92	181.04	37.37	139.45	39.90
Alcohols								
Ethanol	950	954[c]	127±4.97 a	43.03	139±20.80 a	28.69	123±19.40 a	35.20
1-Butanol	1139	1142[d]	1.88±0.24 a	0.64	0.89±0.16 b	0.18	0.00±0.00 c	0.00
Isoamyl alcohol	1201	1223[c]	4.53±0.82 a	1.53	3.01±0.36 a	0.62	3.16±0.49 a	0.90
3-Methyl-3-buten-1-*ol*	1244	-	0.00±0.00 a	0.00	1.57±0.05 b	0.32	4.80±0.58 c	1.37
2-Heptanol	1298	-	0.00±0.00 a	0.00	3.55±0.29 b	0.73	0.00±0.00 a	0.00
1-Hexanol	1331	1370[e]	2.92±0.12 a	0.99	8.39±1.38 b	1.73	6.70±2.06 b	1.92
2-Ethylhexanol	1466	1513[e]	0.00±0.00 a	0.00	1.43±0.19 b	0.30	1.26±0.18 b	0.36
2-Nonanol	1494	-	0.00±0.00 a	0.00	20.50±0.44 b	4.23	2.16±0.37 c	0.62
Linalool	1513	1544[d]	0.00±0.00 a	0.00	0.00±0.00 a	0.00	0.35±0.04 b	0.10
1-Octanol	1544	1586[e]	0.00±0.00 a	0.00	3.84±0.32 b	0.79	6.99±0.12 c	2.00
Subtotal			136.33	46.19	182.18	37.61	148.42	42.47
Aldehydes								
Octanal	1256	1291[d]	0.00±0.00 a	0.00	1.24±0.09 b	0.26	1.13±0.24 b	0.32
Benzaldehyde	1537	1538[c]	4.39±0.22 a	1.49	6.29±1.32 b	1.30	0.00±0.00 c	0.00
p-Tolualdehyde	1680	1666[c]	1.15±0.16 a	0.39	3.12±0.28 b	0.64	9.40±0.42 c	2.69
Ethylbenzaldehyde	1869	-	1.08±0.20 a	0.37	1.34±0.05 a	0.28	1.31±0.17 a	0.37
Subtotal			6.62	2.24	11.99	2.47	11.84	3.39
Esters								
Ethyl acetate	931	907[c]	0.00±0.00 a	0.00	3.89±0.49 b	0.80	3.92±0.03 b	1.12
Ethyl hexanoate	1189	1217[c]	0.00±0.00 a	0.00	0.53±0.07 b	0.11	0.65±0.09 b	0.19
Ethyl 2-hydroxypropanoate	1325	-	0.00±0.00 a	0.00	3.32±0.66 b	0.69	7.12±0.73 c	2.04
Ethyl octanoate	1387	1428[c]	0.44±0.09 a	0.15	0.67±0.03 b	0.14	0.74±0.07 b	0.21
Subtotal			0.44	0.15	8.41	1.74	12.43	3.56
Ketones and lactones								
2,3-Butanedione	979	959[e]	0.00±0.00 a	0.00	15.60±1.86 b	3.22	15.80±2.40 b	4.52
2,3-Heptanedione	1123	-	0.00±0.00 a	0.00	1.75±0.03 b	0.36	0.00±0.00 a	0.00
2-Heptanone	1157	-	0.00±0.00 a	0.00	40.70±0.61 b	8.40	4.51±0.40 c	1.29
4-Methyl-2-heptanone	1178	-	1.12±0.11 a	0.38	1.02±0.05 a	0.21	0.93±0.09 a	0.27
3-Hydroxy-2-butanone	1289	1308[c]	0.00±0.00 a	0.00	0.53±0.09 a	0.11	3.21±0.44 b	0.92
2-Nonanone	1353	-	0.00±0.00 a	0.00	27.00±1.78 b	5.57	2.18±0.06 a	0.62
2-Undecanone	1570	-	0.00±0.00 a	0.00	0.62±0.05 b	0.13	0.00±0.00 a	0.00
β-Damascenone	1790	1831[c]	0.32±0.00 a	0.11	0.60±0.14 b	0.12	0.37±0.02 a	0.11
δ-Octalactone	1985	2038[e]	0.38±0.06 a	0.13	3.57±0.44 b	0.74	1.65±0.29 c	0.47

Table 2 (continued)

Volatile compounds	LRI		Day 0		*L. acidophilus* L10		*L. casei* L26	
	FFAP[a]	Reference[b]	Peak area	RPA (%)	Peak area	RPA (%)	Peak area	RPA (%)
δ-Decalactone	2208	2208[e]	0.53±0.08 a	0.18	0.00±0.00 b	0.00	3.54±0.31 c	1.01
δ-Dodecalactone	2438	-	0.00±0.00 a	0.00	3.55±0.47 b	0.73	0.00±0.00 a	0.00
Subtotal			2.35	0.80	94.94	19.60	32.19	9.21
Phenols								
2,4-Di-*tert*-butylphenol	2277	2314[c]	5.02±0.50 a	1.70	5.89±0.37 a	1.22	5.15±0.37 a	1.47
Total			295.17		484.45		349.48	

GC, Gas chromatography; FID, flame ionisation detector; LRI, Linear retention indices; RPA, relative peak area

Values followed by the same lowercase letters are not significantly different at the 95 % confidence level

[a] Experimentally determined linear retention index on the DB-FFAP column, relative to C5–C40 hydrocarbons

[b] Literature reference for LRI value

[c] LRI values reported in Lee et al. (2012)

[d] LRI values reported in Goodner (2008)

[e] LRI values reported in Prades et al. (2012)

Ketones and lactones were the next highest diverse group of volatile compounds in the fermented coconut water (Table 2). Among the ketones and lactones, 4-methyl-2-heptanone and β-damascenone remained unchanged in the coconut water fermented by both cultures and by *L. casei*, respectively, while other ketones and lactones increased significantly after fermentation. 2,3-Heptanedione, 2-undecanone and δ-dodecalactone were not detected in both fresh and *L. casei*-fermented coconut water. *L. acidophilus* produced higher amounts of ketones and lactones than *L. casei*, except for 2,3-butanedione (diacetyl), 3-hydroxy-2-butanone (acetoin) and δ-decalactone, the levels of which were higher in the coconut water fermented by *L. casei* (Table 2). There was a drastic decrease in the level of δ-decalactone in the coconut water fermented by *L. acidophilus*, resulting in this lactone being absent in the fermented coconut water on day 2 (Table 2).

Among the volatile fatty acids, acetic acid was the only volatile acid produced by the two cultures, with *L. casei* producing a higher amount of acetic acid (5.81 % RPA) (Table 2), which is in line with the trend in organic acid levels shown in Fig. 2b. Other fatty acids remained relatively unchanged after fermentation, except for the (*E*)-2-butenoic, heptanoic and decanoic acids which were metabolised by either one of the cultures or both (Table 2). Other volatiles, including aldehydes, esters and volatile phenols, were also detected in the fermented coconut water (Table 2). Most of these were produced after fermentation except for ethylbenzaldehyde, benzaldehyde and 2,4-di-*tert*-butylphenol (Table 2). Both cultures displayed similar production capabilities for most of these volatiles, with the exception of ethyl 2-hydroxypropanoate and *p*-tolualdehyde (Table 2). *L. casei* was a better producer of ethyl 2-hydroxypropanoate and *p*-tolualdehyde than *L. acidophilus* (2.14- and 3.01-fold higher, respectively) (Table 2).

Discussion

In this study, probiotic fermentation of coconut water was examined with a focus on the viability and fermentation performance of *L. acidophilus* and *L. casei*. To date, only a limited number of studies have been conducted on the fermentation of coconut water. Among these, Shivakumar and Vijayendra (2006), Unagul et al. (2007) and Seesuriyachan et al. (2011) highlighted the possibility of exopolysaccharide or docosahexaenoic acid production using coconut water, while Kuswardani et al. (2011) demonstrated the growth and survival of *L. bulgaricus* and *Streptococcus thermophilus* in a coconut water-based medium. Similarly, Dharmasena (2012) highlighted the growth and survival of *L. plantarum* in a fermented oatmeal–coconut water matrix at successive levels during refrigerated storage. All of these studies revealed the possibility of microbial growth in coconut water. In our study, we used coconut water as the sole fermentation medium, without any additive relative to those used in the previous studies, to ensure that the coconut water was the only raw material that regulated the growth and metabolism of the probiotic bacteria.

In probiotic products, it is important to have a significant number of active and viable probiotic bacteria present (at least 10^6–10^7 CFU/g or ml of product) in order to exert beneficial effects (Liu and Tsao 2010). There are several factors that affect the viability of probiotic cultures in probiotic food products, such as the strains used, culture

condition, pH of the fermentation medium, final acidity of the product, and the concentration of organic acids (lactic and acetic acids) (Shah and Jelen 1990; Yoon et al. 2004). In our study, both probiotic cultures survived well in the fermented and highly acidic (low pH) coconut water at 4 °C for up to 26 days. Nevertheless, the viable cell population of *L. casei* in the fermented coconut water was 3.6-fold higher than that of *L. acidophilus* after 26 days of storage at 4 °C (Fig. 1a), indicating that the former is a better potential candidate for the production of a probiotic coconut water beverage. Given the extensive survival of the probiotic cultures in fermented coconut water, studies can now be conducted to evaluate the shelf-life of the resultant product.

Both *L. casei* and *L. acidophilus* are homofermentative and therefore produced lactic acid as the major organic acid, together with a small amount of acetic acid, during the fermentation of coconut water (Fig. 2b). Interestingly, there were still substantial amounts of residual sugars after 2 days of fermentation despite the significant lactic acid production and the growth of the two probiotic cultures (Figs. 1, 2). This result is in agreement to those reported by Mousavi et al. (2011) who found high amounts of residual sugars in pomegranate juice even after 3 days of fermentation by *L. paracasei* and *L. acidophilus*. Similarly, the different rate of fructose and glucose consumption by the two probiotic cultures corresponded to those reported by Mousavi et al. (2011), where the metabolism of carbohydrates by *Lactobacillus* varied from strain to strain and depended on the substrate and even on the fermentation time. Interestingly, there was a slight reduction in sucrose content after fermentation by both *L. casei* and *L. acidophilus* (Fig. 2a), possibly due to acid hydrolysis of sucrose and/or the capability of the two probiotic cultures to utilise sucrose. This finding is in agreement with that of Donkor (2007) who observed that *L. casei* L26 and *L. acidophilus* L10 could utilise sucrose, the main disaccharide in soymilk, as an energy source for the growth and production of acids. The decrease in succinate concentration (Fig. 2b) suggests that the two lactobacilli strains studied could have metabolised succinate through the oxidative TCA cycle, rather than via the reductive TCA cycle reported for other lactobacilli that would lead to succinate production (Dudley and Steele 2005). Possible explanations for this metabolism of succinate by *L. casei* and *L. acidophilus* strains is the availability of oxygen during the early stages of fermentation and that some lactobacilli, such as *L. casei*, lack one or more enzymatic activities for succinate metabolism via the reductive TCA pathway (Dudley and Steele 2005). This is a surprising result and merits further research.

Other than sugar metabolism, the production of lactic acid can also be derived from the metabolism of polyols (mannitol, sorbitol), organic acids (malate, citrate) or amino acids (serine, alanine) (Liu 2003). Most lactic acid bacteria are able to decarboxylate malate to lactate and CO_2 by the malolactic enzyme (commonly known as malolactic fermentation), while *L. casei* may degrade malate in a different manner: malate→pyruvate+CO_2→lactate (Landete et al. 2010). The results of our study show a significant reduction in malic acid after the fermentation by *L. casei*, whereas in agreement with current literature, the *L. acidophilus* strain did not degrade malic acid (Fig. 2b).

The ability of *L. casei* to utilise citrate has previously been reported (Hickey et al. 1983; Díaz-Muñiz et al. 2006), and this capability would provide a mechanism for lactate and acetate production from citrate by citrate lyase via the following pathway: citrate→acetate+oxaloacetate→pyruvate→lactate (Díaz-Muñiz et al. 2006). In addition, diacetyl and acetoin can also be produced by *L. casei* from pyruvate via a series of enzymatic and non-enzymatic reactions (Hickey et al. 1983; Díaz-Muñiz et al. 2006). On the other hand, little has been reported on the metabolism of citrate by *L. acidophilus*. Both *L. casei* and *L. acidophilus* also possess pyruvate oxidase and acetate kinase, which are responsible for the production of acetate from pyruvate and acetyl phosphate, respectively (Hickey et al. 1983). The results of our study show that both probiotic cultures degraded citrate and produced substantial amounts of lactate, acetate, diacetyl and acetoin after fermentation (Fig. 2b; Table 2). The higher formation of these compounds in the *L. casei*-fermented coconut water could be due to either metabolic differences between the two strains or the higher cell count reached by *L. casei* (Fig. 1a). The production of acetic acid by the two cultures (Fig. 2b), especially the fermentation by *L. casei*, may be expected to impart acidic, vinegar and pungent flavours (Bartowsky and Pretorius 2009). Similarly, the formation of diacetyl and acetoin by the two cultures (Table 2) can contribute creamy, buttery, sweet and pungent notes in the fermented coconut water near their flavour threshold of 0.2 and 150 mg/l, respectively (Bartowsky and Pretorius 2009). However, sensory analysis is required to evaluate the relative contribution of these volatiles to the organoleptic characteristics of the fermented coconut water.

In addition, non-volatile compounds can undergo transformation into numerous volatile compounds, such as alcohols, aldehydes, ketones and esters (Table 2). The production of these volatiles is associated with the metabolism of sugars, pyruvate, citrate, fatty acids or amino acids, giving rise to both positive attributes and defects, depending on the absolute and relative levels (McSweeney and Sousa 2000; Liu 2003; Coolbear et al. 2011). *L. acidophilus* was a higher producer of 2-heptanone, 2-nonanone, benzaldehyde, 2-heptanol, 2-nonanol, δ-octalactone and δ-dodecalactone, while *L. casei* was a better producer of δ-decalactone, 3-methyl-3-buten-1-*ol*, linalool, 1-octanol, *p*-tolualdehyde and ethyl 2-hydroxypropanoate (Table 2). The volatile

production capabilities of *L. casei* observed in our study are in agreement with those reported by Martínez-Cuesta et al. (2001) and Sidira et al. (2010), both of whom found the production of several volatile compounds by *L. casei* in other food products, such as cheese and sausages. This is mainly attributed to the high esterase and peptidase activities of *L. casei* (Dako et al. 1995) and its broad system of enzymes responsible for hydrolyzing activity towards a number of peptides and the conversion of amino acids (Martínez-Cuesta et al. 2001). Little has been reported on the mechanism of lactone and methylketone production by *L. acidophilus* and *L. casei*, but the formation of these compounds is well-known in eukaryotic microbes, such as *Geotrichum candidum and Penicillium roqueforti* (Molimard and Spinnler 1996; McSweeney and Sousa 2000). Methyl ketones are formed by the β-oxidation and decarboxylation of fatty acids, while lactones are produced by intramolecular esterification of corresponding hydroxyacids (McSweeney and Sousa 2000). It is not known whether similar mechanisms operate in prokaryotic lactobacilli.

The volatile production capabilities of *L. acidophilus*, on the other hand, have not been extensively studied in fermented products. Nevertheless, the production of benzaldehyde and alcohols by *L. acidophilus* could have resulted from the degradation of amino acids, such as phenylalanine (Groot and de Bont 1999; McSweeney and Sousa 2000; Marco et al. 2006). It remains to be ascertained whether these statistical differences translate into sensory differences. Formal sensory analysis should be carried out in future studies to determine the overall aroma profile of the fermented coconut water in the presence of a wide range of volatile compounds, as shown in Table 2. In contrast to production, there were some volatile compounds that decreased after fermentation by *L. acidophilus*, such as (*E*)-2-butenoic acid, heptanoic acid, 1-butanol and δ-decalactone (Table 2). The reduction of these acids and 1-butanol could be due to their metabolism or their utilisation as precursors for the formation of other volatiles. However, the decline in δ-decalactone level could be related its hydrophobic characteristic, which has a high volatility in a medium with high water content (Adhikari et al. 2006).

To date, the mineral content in probiotic products is still not an area of interest to the food industry or researchers, as most focus is placed on the viability of the probiotic bacteria (Yoon et al. 2004; Liu and Tsao 2010; Rivera-Espinoza and Gallardo-Navarro 2010). In this study, we examined the mineral content of the fermented coconut water in order to evaluate the effects of the fermentation on the mineral content originally present in the coconut water. It is the mineral content that would make coconut water a suitable alternative to carbohydrate–electrolyte sports drinks (Kalman et al. 2012). We found that there were no major changes in the concentration and composition of most minerals in the coconut water after fermentation, except for slight reductions or increments in the concentration of a few minerals (Table 1). This result indicates that the nutritional benefits and hydrating effects of fermented coconut water can be expected to be comparable to those of the fresh coconut water. Generally, drinks formulated specifically for rehydration should be able to replace the volume of water lost and replenish the electrolytes, especially sodium, excreted through sweat (Saat et al. 2002). Mitchell et al. (1994) revealed that a sodium content of 20–30 mmol/l is recommended for a rehydration beverage to provide fluid replacement and to stimulate absorption. Moreover, Saat et al. (2002) pointed out that drinks containing approximately 50 mmol/l of sodium are likely to provide more effective hydration for most people. The fermented coconut water contained a significantly higher amount of sodium than those previously mentioned and contained other minerals, such as calcium, potassium and magnesium (Table 1).

Other than the mineral content, the ingestion of carbohydrates during rapid rehydration is also advisable as it aids the rate of muscle glycogen resynthesis (Ivy et al. 1988). Lambert et al. (1992) showed that the effectiveness of a carbohydrate solution of as high as 10 % (v/v) in replacing body water deficit was comparable to that of non-carbohydrate water. Similarly, the fermented coconut water in this study contained high amounts of residual sugar (34.5–35.6 g/l) after 2 days of fermentation by the two probiotic cultures (Fig. 2a). Further work should be conducted to investigate the degree of hydration and carbohydrate replacement of the fermented coconut water to confirm its hydration and restoration effects relative to unfermented coconut water.

Overall, the two probiotic cultures showed a capability for utilising the coconut water constituents for growth, lactic acid production and volatile compound formation. The viable cell counts of both cultures reached approximately 10^8 CFU/ml after a 2-day fermentation at 37 °C and survived under conditions of high acidity and 4 °C for at least 26 days. The changes in most of the non-volatile and volatile compounds after fermentation were similar between the two cultures, although the final concentrations of some compounds differed significantly at the statistical level. No substantial changes were observed in mineral content. Hence, probiotic fermentation could provide an alternative outlet for coconut water utilisation and may produce a novel probiotic beverage for consumers, especially for sports nutrition.

Declaration of interest None.

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