



EFFECTS OF CELLULASE HYDROLYSIS AND CARBONATES ON THE PROBIOTIC FERMENTATION OF NONI (MORINDA CITRIFOLIA)

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Key words: noni, cellulase, antioxidant capacity, probiotics.

ABSTRACT

In typical fermentation of noni (*Morinda citrifolia*), nutrients loss, along with unfavorable taste and odor frequently occurred due to the time-consuming fermentation. To improve the quality of fermented noni, cellulase hydrolysis and probiotic fermentation were employed. Four hours of hydrolysis with 100 U/mL of cellulase at 50°C significantly increased the reducing sugar, total phenolic content, reducing power, and trolox equivalent antioxidant capacity. No probiotics tested in this study could grow on the noni slurry and its hydrolysate, except for *Pediococcus pentosaceus* (BCRC 14053), which was able to grow on noni with 0.6% calcium carbonate. The probiotic count increased to 8.55 log CFU/mL after 18 h of cultivation at 37°C. The fermentation time shortened the typical processing method (approximately 2 months) to 18 h, and the taste and flavor were significantly improved.

I. INTRODUCTION

Fermentation with excessive length of time (Newton, 2002; Nelson, 2006; Yang et al., 2007) frequently causes nutrient loss and unfavorable taste and odor, and is an obstacle to the marketing of noni products. Accordingly, in many countries, the fruit is often commercialized fresh or as a juice in both formal and informal markets; it is also produced as a pasteurized juice, either in pure form or in combination with other

juices (usually grape or blackberry juice), to increase its palatability (Pawlus and Kinghorn, 2007; Krishnaiah et al., 2012). The noni fruit contains fruitful nutrients including amino acids, minerals, vitamins, and polysaccharides. It is also rich in polyphenols such as coumarins (containing scopoletin and esculetin), flavonoids (containing rutin, quercetin, and quercetin derivatives), phenolic acid (containing vanillic acid), vanillin, and iridoids (containing asperulosidic acid and deacetylasperulosidic acid) (Dussossoy et al., 2011; West et al., 2011). A research team led by Professor Chi-Tang Ho at Rutgers University (USA) successfully identified several new flavonol glycosides in the *M. citrifolia* plant, an iridoid glycoside from the leaves, and a trisaccharide fatty acid ester, rutin, and an asperulosidic acid in the fruit. Two novel glycosides {6-O-(β -D-glucopyranosyl)-1-O-octanoyl- β -D-glucopyranose and asperulosidic acid} and a new unusual iridoid named citrifolinoside have been shown to have an inhibitory effect on activator protein-1 transactivation and cell transformation in the mouse epidermal JB6 cell line (Wang et al., 1999; Wang et al., 2000; Liu et al., 2001; Sang et al., 2001; Sang et al., 2003; Wei et al., 2011). Many studies have indicated that the noni has antioxidation and angiotensin converting enzyme inhibition activities (Yamaguchi et al., 2002; Chan-Blanco et al., 2006; Pawlus and Kinghorn, 2007). It is therefore considered capable of serving as an inhibitor of membrane lipid peroxidation and as a peroxy radical scavenger (Yamaguchi et al., 2002; Zin et al., 2002). How to improve its fermentation condition, taste, and odor is therefore a concern of noni industrialists and scientists.

Cellulase plays a major role in the effective biological hydrolysis of cellulose into glucose through synergistic actions of endo- β -1,4-glucanase (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91), and β -D-glucosidase (EC 3.2.1.21) (Perez et al., 2002). The action mode for cellulases on polymers is either exo- or endo-cleavage, and all cellulases target the specific cleavage of β -1,4-glycosidic bonds (Wood and McCrae, 1979). Glucose is the main carbohydrate derived from cellulase hydrolysis and it can be used by probiotics (Kimoto-Nira et al., 2010; Hernandez-Hernandez et al., 2012).

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Lactic acid bacteria (LAB) are Gram-positive and nonspore forming, and they use anaerobic respiration and lack catalase (Medina et al., 2004; Slover and Danziger, 2008; Pringsulaka et al., 2012; Hwanhlem et al., 2014). They can improve intestinal microflora, reduce the risk of cancer (John et al., 2007; Zhang et al., 2014), increase immunity (Pawlus and Kinghorn, 2007; Gupta and Patel, 2013), reduce oxidative stress (Wang et al., 2014), increase antimicrobial activity (Pawlus and Kinghorn, 2007; Gupta and Patel, 2013), and improve texture, taste, and odor during fermentation (De Martinis and Freitas, 2003; Pal et al., 2005; Dalié et al., 2010; Jiang et al., 2012; Cizeikiene et al., 2013). LAB are generally recognized as safe and their bacteriocins have long been employed to inhibit the growth of pathogens in the food industry (Rogers, 1928; Miao et al., 2014). Wang et al. (2009) reported that *Bifidobacterium longum* and *Lactobacillus plantarum* could be optimal probiotics for the fermentation of noni juice. According to the published literature, no other study on the fermentation of a noni slurry with probiotics has been conducted.

This study aimed to optimize the conditions for the cellulase hydrolysis and probiotic fermentation of noni. A limited amount of probiotics can grow in a noni slurry. This study also reported the functionality change of noni after cellulase hydrolysis and the improvement of probiotic growth on noni hydrolysates with the addition of calcium carbonate.

II. MATERIALS AND METHODS

1. Raw Material Preparation

Fresh noni (*Morinda citrifolia*), purchased from a noni farm in Southern Taiwan, was homogenized using a homogenizer (Vitamix TNC5200, Vitamix, Cleveland, Ohio, USA) and used as a raw material in this study. Commercial noni juice was purchased from a local market. Cellulase (1400 U/g) was purchased from Kwang Hwa Trading Co. Ltd. The following strains were obtained from the Bioresource Collection and Research Center (BCRC) at the Food Industry Research and Development Institute (Hsinchu, Taiwan): *Lactobacillus plantarum* subsp. *plantarum* (BCRC 10069), *Lb. plantarum* (BCRC 12251), *Streptococcus salivarius* subsp. *thermophilus* (BCRC 12268), *Lb. casei* (BCRC 12272), *Lb. helveticus* (BCRC 12296), *Lactococcus lactis* subsp. *cremoris* (BCRC 12304), *Lc. lactis* subsp. *lactis* (BCRC 12322), *Lb. plantarum* (BCRC 12327), *Pediococcus pentosaceus* (BCRC 14053), *Lb. helveticus* (BCRC 14076), *Bifidobacterium adolescentis* (BCRC 14608), *B. pseudolongum* subsp. *pseudolongum* (BCRC 14673), and *Lb. johnsonii* (BCRC 17010). A de Man, Rogosa, and Sharpe (MRS) agar was purchased from Becton, Dickinson and Company (Franklin Lakes, New Jersey). The bacterial strains were stored at -80°C until use. They were activated through cultivation on a Lactobacilli MRS broth for 12 h and then transferred twice before use.

2. Preparation of the Noni Hydrolysate

The hydrolysis of 100 g of homogenized noni (noni to

water ratio of 1 : 4) was conducted with various amounts of cellulases (0, 25, 50, 75, 100, 125, 150, 175, and 200 U/mL) for 12 h at 50°C , with shaking. During hydrolysis, sampling was performed at a 2-h interval. After samples were centrifuged at $5000 \times g$ for 30 min, the supernatants were subjected to the following assays.

3. Assays

The degree of hydrolysis of noni was evaluated by measuring the release of reducing sugar, the total phenolic content, the reducing power, and the trolox equivalent antioxidant capacity (TEAC).

1) Release of Reducing Sugar

The reducing sugar was determined using the dinitrosalicylic acid method and calculated using the standard curve constructed with glucose as the standard (Miller, 1959).

2) Total Phenolic Content

The total phenolic content (TPC) of each sample was estimated using the Folin-Ciocalteu colorimetric method. Fifty microliters of sample and 200 μL of 5% Na_2CO_3 were added to 50 μL of 10% Folin-Ciocalteu's phenol reagent, and allowed to react for 1 h in the dark at room temperature. The absorbance at 750 nm was measured using a spectrophotometer. The TPC was calculated according to the standard curve calibrated with gallic acid (Singleton et al., 1999).

3) Reducing Power

The reducing power was determined by mixing 1.0 mL of sample with 1.0 mL of 200 mM phosphate buffer solution (PBS, pH 6.6) and 1.0 mL of 1% $\text{K}_3\text{Fe}(\text{CN})_6$. After 20 min at 50°C , the reaction was terminated by adding 1.0 mL of 10% trichloroacetic acid. After 10 min of centrifugation at $10000 \times g$, 100 μL of supernatant was uniformly mixed with 100 μL of distilled water and 20 μL of 0.1% ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), and then incubated at room temperature for 10 min in the dark. The absorbance at 700 nm was measured using a spectrophotometer. The reducing power was determined on the basis of a standard curve constructed with vitamin C (Oyaizu, 1988).

4) Trolox Equivalent Antioxidant Capacity

TEAC was determined through the reaction of 2 mM 2,2'-azono-bis (3-ethylbenz-thiazoline-6-sulfonic acid, ABTS) in 0.01 M PBS (pH 7.4) containing 0.818% NaCl and 0.0015% KCl. To 2.0 mL of 2 mM ABTS, 0.1 mL of 70 mM $\text{K}_2\text{S}_2\text{O}_8$ was added to form $\text{ABTS}^{\cdot+}$ and stored at room temperature for 16 h before use. The $\text{ABTS}^{\cdot+}$ solution was prepared to a 1:20 dilution by using PBS (pH 7.4). To a 10- μL sample, 0.99 mL of $\text{ABTS}^{\cdot+}$ was added and mixed uniformly. The resultant samples were incubated at room temperature for 6 min in the dark, and the absorbance at 734 nm was measured using a spectrophotometer. The ABTS radical scavenging ability (%) was calculated as $([\text{control absorbance} - \text{sample absorbance}]/$

control absorbance) $\times 100\%$ (Miller et al., 1993).

4. Fermentation of the Noni

Thirteen probiotic strains were screened by inoculating 3% of probiotic cultivation broth into pH 3.0 or pH 6.0 PBS and cultivated at 37°C for 0, 6, 12, 18, and 24 h. The probiotic strains that managed to grow were further cultivated in a noni slurry made with different types of salt (CaCO₃, MgCO₃, Na₂CO₃, Ca₃(PO₄)₂, Mg₃(PO₄)₂, Na₃PO₄, CaCl₂, MgCl₂, NaCl, CaSO₄, and MgSO₄). The viable counts and pH were monitored during cultivation.

5. Assays for the Fermented Noni Slurry

The release of reducing sugar, the total phenolic content, the reducing power, and the trolox equivalent antioxidant capacity of the fermented noni slurry were measured as mentioned in section II(3).

6. Determination of Viable Microbial Counts

The viable microbial count was measured to evaluate whether the noni slurry hydrolysate could be used by probiotics. The microbial growth during fermentation was measured using a plate count on the MRS agar after serial decimal dilutions from an initial 0.5 mL of sample in 4.5 mL of sterile 0.85% NaCl solution. The plates were incubated at 37°C for 48 h and then the viable probiotics were counted (Tamang, 1996; Darmayanti, 2014).

7. Storage Stability of the Fermented Noni

After 18 h of fermentation at 37°C, the noni slurry product was refrigerated at 4°C for 8 wk. The viable microbial count was determined weekly and expressed as colony forming units (log CFU/mL).

8. Sensory Evaluation

The sensory characteristics of the fermented noni hydrolysate and commercial fermented noni juice (typically fermented for 2 months) were evaluated. Mineral water was used as a palate cleanser during the evaluation of each sample. Consumer tests were conducted on the campus of National Kaohsiung Marine University (Kaohsiung, Taiwan). All consumers were recruited from the National Kaohsiung Marine University area. This sensory evaluation was intended to collect the opinions of ordinary consumers, not experts in food science. The sensory test was conducted in a single session on a single day by 126 untrained consumers composed of 67% females and 33% males aged 19-42 years old. The color, flavor, taste, and overall acceptability were assessed by consumers using a 9-point scale (1 = *extremely dislike*, 9 = *extremely like*).

9. Statistical Analysis

The Duncan multiple range test was employed to determine the significance of differences within treatments. For each treatment, 3 determinations were used and the mean values

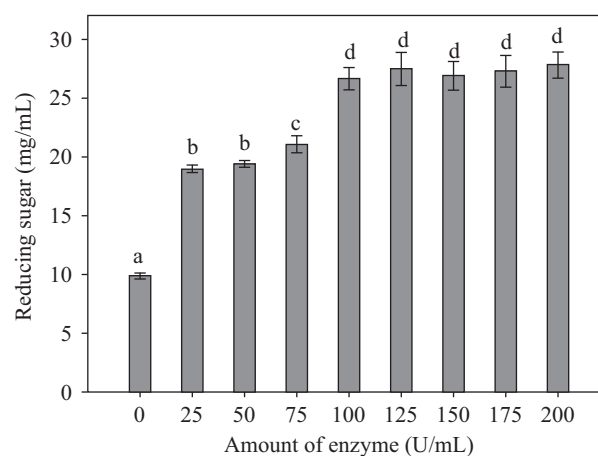


Fig. 1. Change in reducing sugar of noni after 4 h hydrolysis with different amounts of cellulase.

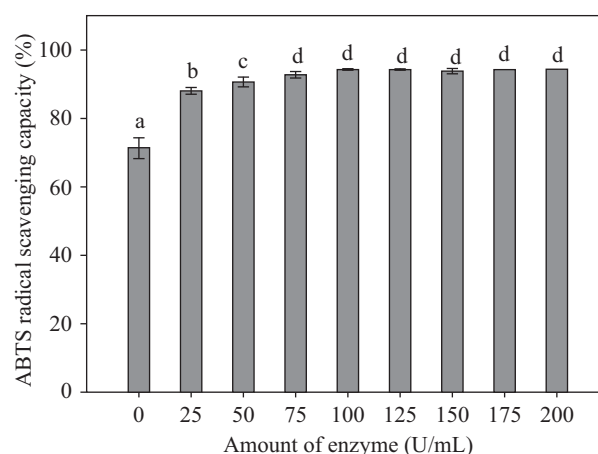


Fig. 2. Change in ABTS^{•+} scavenging ability of noni after 4 h hydrolysis with different amounts of cellulase.

were calculated. Values were considered to be significantly different at $p < 0.05$ (Norušis, 1993).

III. RESULTS AND DISCUSSION

1. Optimum Hydrolysis Conditions

After 4 h of hydrolysis with cellulases at 50°C, the reducing sugars increased 2-3 times that of the nonhydrolyzed sample, depending on the amount of enzyme added (Fig. 1). It increased to a steady state (from 9.88 mg/mL to 26.7 mg/mL), when the amount of cellulase was higher than 100 U/mL (Fig. 1). The ABTS^{•+} scavenging ability increased with an increase in the enzyme amount, and approached a steady state when the enzyme was higher than 75 U/mL (Fig. 2). No significant differences in the total phenolic content and reducing power were observed after 4 h of cellulase hydrolysis with various amounts of enzyme (data not shown). According to the data obtained, the optimal cellulase concentration for the biohydrolysis of *M. citrifolia* was 100 U/mL.

Table 1. Change of probiotics count in noni slurry hydrolysate after 24 h fermentation with different probiotics.

Probiotics counts (log CFU/mL)		
Fermentation time (h)	F0*	F24
Not adjusted the pH	ND**	
Commercial noni juice	4.94 ± 0.20cd***	3.16 ± 0.03a
Adjusted the pH to 6.5 (1 N Ca(OH) ₂)		
<i>Pediococcus pentosaceus</i> BCRC 14053	7.47 ± 0.23g	5.04 ± 0.02d
<i>Lactobacillus helveticus</i> BCRC 12296	6.59 ± 0.19e	3.62 ± 0.10b
<i>Lactobacillus casei</i> BCRC 12272	7.39 ± 0.20g	3.36 ± 0.32ab
<i>Streptococcus salivarius</i> subsp. <i>thermophilus</i> BCRC 12268	7.08 ± 0.09f	4.70 ± 0.20c

*F0: 0 h fermentation; F24: 24 h fermentation.

**ND: not detected.

*** a~g: Values with different letters at the same column are differ significantly ($P < 0.05$).

2. Optimum Fermented Strains

Three percent of the 13-strain probiotic broth were first inoculated into a PBS (pH 3.0 or pH 6.0) and cultured for 0, 6, 12, 18, and 24 h at 37°C. It was found that *S. salivarius* subsp. *thermophilus* (BCRC 12268), *Lb. casei* (BCRC 12272), *Lb. helveticus* (BCRC 12296), and *P. pentosaceus* (BCRC 14053) had a higher acid tolerance than the other strains (data not shown). These 4 strains were further employed to ferment the noni slurry and the commercial noni juice. *P. pentosaceus* (BCRC 14053) showed the highest microbial counts in the noni slurry (Table 1) and was therefore used in the following experiments.

3. Effect of Various Types of Salts on the Fermentation of Noni Hydrolysate

To investigate the effect of salts on the growth of *P. pentosaceus* (BCRC 14053) in noni, 3% *P. pentosaceus* (BCRC 14053) was inoculated in the noni hydrolysate with 0.4% of various metal (calcium, magnesium, and sodium) carbonates, phosphates, chlorides, and sulfates. As indicated in Table 2, *P. pentosaceus* survived a 48-h cultivation in those media with CO₃²⁻ and PO₄³⁻ salts.

To further investigate the effect of salt concentrations, various concentrations (0.2%, 0.4%, and 0.6%) of carbonates and phosphates were added to the noni hydrolysate and then fermented with *P. pentosaceus* (BCRC 14053) at 37°C for 48 h. According to regulations by the Food and Drug Administration in Taiwan, the magnesium carbonate content in drinks cannot be over 5000 ppm. Only 0.2% and 0.4% of magnesium carbonate were tested. From the viable probiotic counts, only the strain with 0.6% CaCO₃ could maintain a count of 8.36 log CFU/mL after 24-h fermentation, but this significantly decreased to 8.13 log CFU/mL after 48 h of fermentation ($p < 0.05$) (Table 3). The noni hydrolysate and commercial noni juice without the addition of carbonates could not be used by

Table 2. Change of probiotics count in noni slurry hydrolysate with 0.4 % of different types of salt during 48 h fermentation with *Pediococcus pentosaceus* BCRC 14053.

Probiotics counts (log CFU/mL)			
0.4 % salts	F0*	F24	F48
CaCO ₃	8.88 ± 0.04dIII**	8.45 ± 0.05fII	7.86 ± 0.01fI
MgCO ₃	8.86 ± 0.04dIII	7.81 ± 0.25eII	6.76 ± 0.08eI
Na ₂ CO ₃	7.98 ± 0.07cIII	6.32 ± 0.03dII	4.67 ± 0.03cI
Ca ₃ (PO ₄) ₂	7.57 ± 0.07aIII	5.95 ± 0.05cII	4.93 ± 0.02dI
Mg ₃ (PO ₄) ₂	7.70 ± 0.07bIII	5.46 ± 0.05bII	4.50 ± 0.05bI
Na ₃ PO ₄	7.86 ± 0.10cIII	4.70 ± 0.11aII	3.71 ± 0.04aI
CaCl ₂	ND***	ND	ND
MgCl ₂	ND	ND	ND
NaCl	ND	ND	ND
CaSO ₄	ND	ND	ND
MgSO ₄	ND	ND	ND

*F0: 0 h fermentation; F24: 24 h fermentation; F48: 48 h fermentation.

** a~f: Values with different letters at the same column are differ significantly ($P < 0.05$). I~III: Values with different letters at the same row are differ significantly ($P < 0.05$).

*** ND: not detected.

Table 3. Change of probiotics count in noni hydrolysate with different concentrations of salts during 48 h fermentation with *Pediococcus pentosaceus* BCRC 14053.

Probiotics count (log CFU/mL)			
	F0*	F24	F48
0.2 % CaCO ₃	8.12 ± 0.08cdIII**	7.42 ± 0.16eII	6.43 ± 0.03eI
0.4 % CaCO ₃	8.22 ± 0.01deII	8.29 ± 0.07gII	7.60 ± 0.12gI
0.6 % CaCO ₃	8.33 ± 0.06eII	8.36 ± 0.04gII	8.13 ± 0.07hI
0.2 % Ca ₃ (PO ₄) ₂	7.81 ± 0.03aIII	3.92 ± 0.38aII	2.85 ± 0.08aI
0.4 % Ca ₃ (PO ₄) ₂	7.72 ± 0.15aIII	5.64 ± 0.19bII	4.95 ± 0.18cI
0.6 % Ca ₃ (PO ₄) ₂	7.94 ± 0.02bIII	6.30 ± 0.08cII	5.23 ± 0.11dI
0.2 % MgCO ₃	8.22 ± 0.09deIII	6.68 ± 0.05dII	4.66 ± 0.24bI
0.4 % MgCO ₃	8.00 ± 0.04bcII	7.94 ± 0.09fII	6.70 ± 0.13fI

*F0: 0 h fermentation; F24: 24 h fermentation; F48: 48 h fermentation.

** a~f: Values with different letters at the same column are differ significantly ($P < 0.05$). I~III: Values with different letters at the same row are differ significantly ($P < 0.05$).

the tested probiotics. The addition of CaCO₃ in the noni hydrolysate before fermentation showed that CaCO₃ is crucial to successful lactic acid fermentation. According to Chae et al. (2009), an MRS with 0.1% CaCO₃ could substantially improve the growth of probiotics during the fermentation of dongchimi-kimchi because of the buffering effect. Therefore,

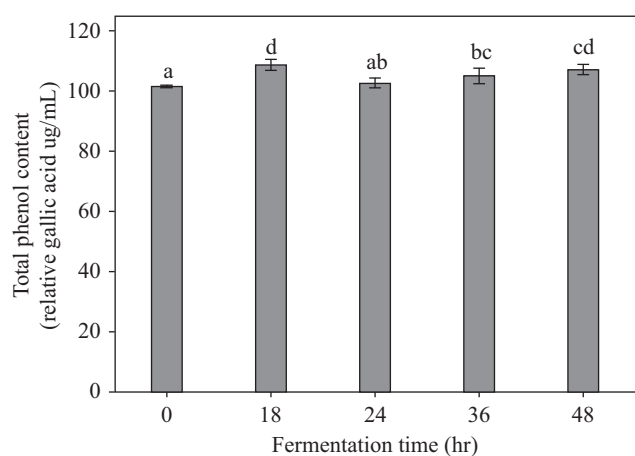


Fig. 3. Change in total phenolic content of noni slurry hydrolysates with 0.6 % CaCO_3 after 18 h fermentation with 0.05 % *Pediococcus pentosaceus* BCRC 14053.

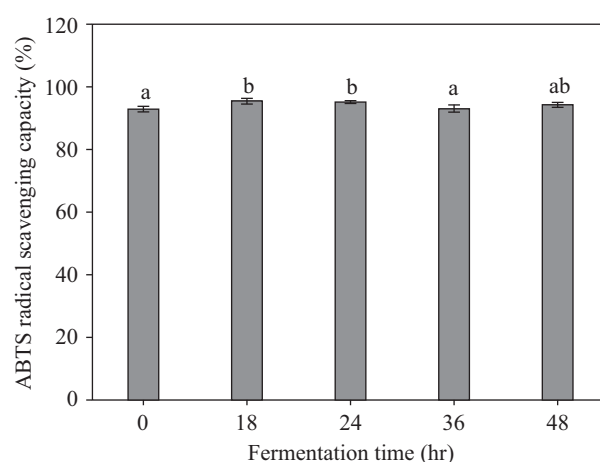


Fig. 5. Change in ABTS⁺ scavenging ability of noni slurry hydrolysates with 0.6 % CaCO_3 after 18 h fermentation with 0.05 % *Pediococcus pentosaceus* BCRC 14053.

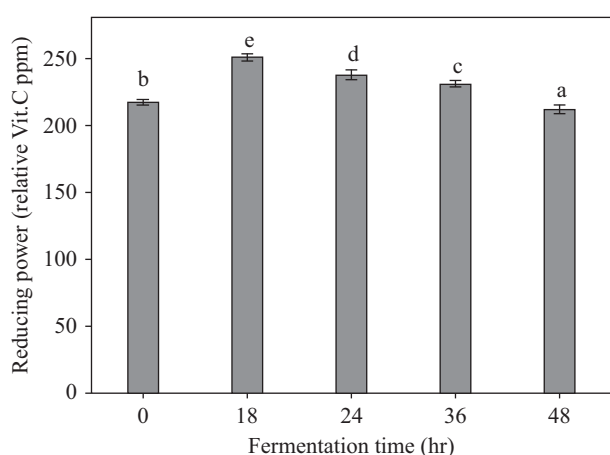


Fig. 4. Change in reducing power of noni slurry hydrolysates with 0.6 % CaCO_3 after 18 h fermentation with 0.05 % *Pediococcus pentosaceus* BCRC 14053.

during lactic acid fermentation, the inhibition appears with the lactic acid. It was hypothesized that the undissociated lactic acid passes through the bacterial cytoplasmic membrane and dissociates inside the cell, causing the acidification of the cytoplasm and the failure of proton motive forces, which consequently reduces the amount of energy needed for cell growth (Markovic et al., 2011).

4. Effect of the Inoculum Amount on the Fermentation of Noni Slurry Hydrolysate

A 0.05-0.5% volume of *P. pentosaceus* (BCRC 14053) culture broth was inoculated to the noni slurry hydrolysate with 0.6% CaCO_3 , and fermented at 37°C. During the 48-h fermentation, viable probiotic counts increased from 6.26 to 8.55 log CFU/mL, while the pH decreased from 6.16 to 5.33 (Table 4). After 18 h of fermentation with 0.05% *P. pentosaceus* (BCRC 14053), the total phenolic content, reducing power, and ABTS⁺ scavenging ability significantly increased

from 101.67 µg/mL, 217.85 ppm, and 93.05% to 108.79 µg/mL, 251.46 ppm, and 95.63% ($p < 0.05$), respectively (Figs. 3-5). The similar final probiotic counts and pH values by different probiotic inoculums (Table 4) might be due to the bacteria growth being in the log phase and stationary phase after 18 h and 24 h of fermentation, respectively. The ABTS⁺ scavenging ability of fermented noni hydrolysate (7.89 ± 0.07 mM trolox) was higher than that in many other fresh fruit (apple: 5.47 ± 0.04 , banana: 3.44 ± 0.29 , grape: 3.95 ± 0.11 , papaya: 2.92 ± 0.06 , pineapple: 5.93 ± 0.05 , orange: 5.13, and strawberry: 3.41 mM trolox), but lower than that of guava (15.18 ± 0.81 mM trolox) (Fu et al., 2011).

5. Storage Stability of the Viable Probiotic Counts of the Probiotic Fermented Noni

The probiotics count of the hydrolyzed noni was 8 log CFU/mL right after 18 h fermentation. It decreased gradually, but still maintained at > 6 log CFU/mL during 8 wk of storage at 4°C (Fig. 6). Compared to the data obtained by Wang et al. (2009), the probiotics counts of *Lb. casei*, *B. longum* and *Lb. plantarum* decreased to 0, 5 and 5 log CFU/mL, respectively, after 4 wk storage at 4°C. *P. pentosaceus* revealed higher resistance to the refrigerated storage than *Lb. casei*, *B. longum* and *Lb. plantarum* in the fermented noni.

6. Sensory Analysis

As shown in Fig. 7, the color, flavor, taste, and overall acceptability of the fermented noni hydrolysate were competitive with those of the commercial noni juice. The taste and overall acceptability were rated higher in the fermented noni hydrolysate than in the commercial noni juice.

IV. CONCLUSION

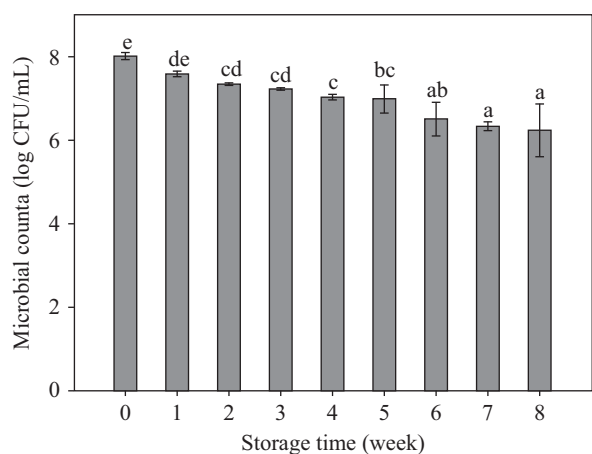
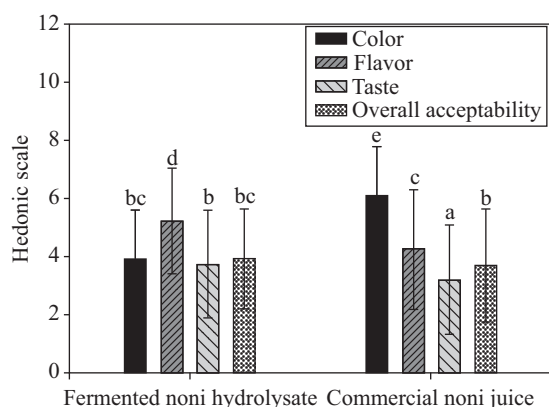
The data shows that a 4-h cellulase hydrolysis and an 18-h *P. pentosaceus* fermentation with 0.6% CaCO_3 produced a

Table 4. Changes of pH value and probiotics count of noni slurry hydrolysate with 0.6% CaCO₃ during 48 h fermentation with 0.5, 0.1 and 0.05% inoculum of *Pediococcus pentosaceus* BCRC 14053.

Amounts of inoculum	F0*	F18	F24	F36	F48
pH value					
0.50%	6.16 ± 0.03aIV**	5.20 ± 0.03aIII	5.06 ± 0.02aII	4.93 ± 0.04aI	4.89 ± 0.03bI
0.10%	6.26 ± 0.04bV	5.27 ± 0.04bIV	5.15 ± 0.04bIII	4.99 ± 0.02aII	4.82 ± 0.04aI
0.05%	6.16 ± 0.01aV	5.33 ± 0.04bIV	5.19 ± 0.05bIII	4.94 ± 0.04aII	4.82 ± 0.03aI
Probiotics count (log CFU/mL)					
0.50%	7.22 ± 0.03cI	8.54 ± 0.08aIV	8.48 ± 0.08aIV	8.31 ± 0.03aIII	8.04 ± 0.06aII
0.10%	6.44 ± 0.01bI	8.58 ± 0.01aV	8.42 ± 0.06aIV	8.30 ± 0.05aIII	8.08 ± 0.08aII
0.05%	6.26 ± 0.01aI	8.55 ± 0.03aV	8.46 ± 0.02aIV	8.32 ± 0.02aIII	8.04 ± 0.08aII

*F0: 0 h fermentation; F18: 18 h fermentation; F24: 24 h fermentation; F36: 36 h fermentation; F48: 48 h fermentation.

**a-c: Mean values with different letters at the same column differ significantly ($P < 0.05$). I-V: Mean values with different letters at the same row differ significantly ($P < 0.05$).

**Fig. 6. Change in probiotics count of noni slurry hydrolysate after 18 h fermentation with *Pediococcus pentosaceus* BCRC 14053 during 8 wk storage at 4°C.****Fig. 7. Sensory evaluation of fermented noni products and commercial noni juice.**

noni product that was competitive in quality (including total phenolic content, reducing power, ABTS⁺ scavenging ability, and sensory quality) to typical fermented (> 2 months fermentation) noni juice. Four hours of cellulase hydrolysis and

the addition of CaCO₃ to the medium substantially shortened the noni fermentation time from approximately 2 months to 18 h, which is a result that would highly benefit the noni processing industry.

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