Development of the Novel Functionality of Probiotics Fermented Noni Hydrolysates

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ABSTRACT

To improve the typical fermentation of noni, this study investigated the effects of the cellulases hydrolysis on the antioxidation of noni slurry and utilization by probiotics. Various amounts of cellulases (0, 25, 50, 75, 100 and 125 U/mL) were used to hydrolyze noni slurry (noni: water=1: 4) at 50°C for 10 h. The resulted hydrolysate was further fermented by *Pediococcus pentosaceus* BCRC 14053 with the addition of 10% glucose and 0.2% $CaCO_3$. The LAB counts of *Pediococcus pentosaceus* BCRC 14053 increased to 7.09 log CFU/mL, while the pH decreased to 4.93 after 24 h cultivation. These data suggested that cellulase hydrolysis could increase its antioxidative ability and its hydrolystaes could be utilized by *Pediococcus pentosaceus* BCRC 14053.

Keywords: *Morinda citrifolia*, Noni, cellulase, Probiotics, Antioxidant capacity

1 INTRODUCTION

Noni fruit contains fruitful nutrients including amino acids, minerals, vitamins and polysaccharides. It is also rich in polyphenols such as coumarins (contain scopoletin and esculetin), flavonoids (contain rutin, quercetin and quercetin derivative), phenolic acid (contain vanillic acid), vanillin and iridoids (contain asperulosidic acid and deacetylasperulosidic acid) [1,2]. Many studies indicated that noni has antioxidation and angiotensin converting enzyme (ACE) inhibition activities. It is, therefore, considered to be able to serve as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers [3,4]. However, the time-consuming and high costs of typical noni fermentation greatly limit its commercial value. This study aimed to investigate the effects of the cellulases hydrolysis on the antioxidation of noni slurry and utilization by probiotics.

2 MATERIALS AND METHODS

2.1 Raw material preparation

Fresh noni, purchased from a orchard in southern Taiwan, was homogenized (noni: water=1: 4) as raw material using in this study. *Pediococcus pentosaceus* BCRC 14053, *Lactobacillus helveticus* BCRC 12296, *Lactobacillus casei* BCRC 12272, and *Streptococcus salivarius* subsp. *thermophilus* BCRC 12268 was obtained from Bioresource Collection and Research Center at the Food Industry Research and Development Institute (Hsinchu, Taiwan). The bacterial strains was stored at -80℃. When activating, the bacterial strain was placed on Lactobacilli MRS broth and transferred twice before use.

2.2 Preparation of *Morinda citrifolia* (Noni) hydrolysate

The homogenized noni was hydrolyzed for 0, 2, 4, 6, 8, and 10 h with various amounts of cellulases (0, 25, 50, 75, 100, 125 U/mL) at 50°C. After 30 min centrifugation at 7500 ×g, the supernatants were subjected to following fermentations and various assays.

2.3 Fermentation conditions of *Morinda citrifolia* (Noni)

Thirteen probiotic strains were screened by inoculating 3% of probiotics into a pH 3.0 or pH 6.0 PBS and culturing at 37°C for 0, 6, 12, 18, 24 h. The probiotic strains being able to grow, were further cultivated on noni slurry or puree with pH 6.5. The viable counts and changes in pH were monitored.

2.4 Determination of reducing sugars

The reducing sugar was determined by DNS method and calculated using the standard curve constructed by glucose as standard [5].

2.5 Determination of total phenolic content

The total phenolic content (TPC) of each sample was estimated using the Folin-Ciocalteu colorimetric method. To 50 μL 10% Folin-Ciocalteu's phenol reagent, 50 μL sample was added. About 200 μL 5% Na_2CO_3 were then added and allowed to stand for 1 h in the dark at room temperature. The absorbance at 750 nm was measured with a spectrophotometer. Quantification was done on the basis of a standard curve with gallic acid [6].

2.6 Determination of flavonoids content

The total flavonoids content was determined by mixing 0.5 mL sample with 1.5 mL distilled water, 0.1 mL 10% $Al(NO)_3$ and 0.1 mL of 1M CH_3COOK. After 40 min standing at room temperature in dark, the absorbance at 415 nm was measured. The flavonoids content was calculated on the basis of a standard curve calibrated by quercetin [7].

2.7 Determination of total anthocyanins content

The total anthocyanins was determined by mixing 0.5 mL sample with 0.5 mL acidified MeOH (1% HCl), and then vortexed for 5 min at room temperature in dark. After 15 min centrifugation (10000 ×g), the absorbance at 530 and 657 nm of the supernatant was measured. The extinction coefficient of 31.6 M^{-1} cm^{-1} was used to calibrate the absorbance values into anthocyanin concentration, using the following equation [8]:

$$\text{Anthocyanin conecntration } (\mu g/g) = \frac{A530 - 0.33 \times A657}{31.6} \times \frac{\frac{\text{volume (mL)}}{\text{weight (g)}}}{\text{Molecular Weight}} \quad (1)$$

2.8 Determination of reducing power

The reducing power was determined by mixing 1.0 mL of sample with 1 mL 200 mM phosphate buffer (pH 6.6) and 1.0 mL of 1% $K_3Fe(CN)_6$ after 20 min reaction at 50°C. Cooled down rapidly and the reaction was terminated by adding 1.0 mL of 10% TCA. After 10 min centrifugation (9100 ×g), 100 μL supernatant was uniformly mixed with 100 μL distilled water and 20 μL of 0.1% ferric chloride ($FeCl_3 \cdot 6H_2O$) and then incubated at room temperature for 10 min in dark. The absorbance at 700 nm was measured. The reducing power was determined on the basis of a standard curve constructed with Vit. C [9].

2.9 Determination of trolox equivalent antioxidation capacity (TEAC)

TEAC was determined by 2 mM of 2,2'-azono-bis (3-ethylbenz-thiazoline-6-sulfonic acid, ABTS) in 0.01 M phosphate buffer (pH 7.4) containing 0.818% NaCl and 0.015% KCl. To 2.0 mL of 2 mM ABTS, 0.1 mL of 70 mM $K_2S_2O_8$ was added to form $ABTS^{\cdot+}$ and stored at room temperature for 16 h before use. The $ABTS^{\cdot+}$ stock solution was diluted (20×) using phosphate buffer (pH 7.4). To 10 μL sample, 0.99 mL of $ABTS^{\cdot+}$ stock solution was added and mixed uniformly. The resultant samples were incubated at room temperature for 6 min in dark and measured the absorbance at 734 nm [10].

$$\text{ABTS radical scavenging ability } (\%) = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\% \quad (2)$$

2.10 Determination of microbial count

Microbial growth during fermentation was measured by plate-count on 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 counted the viable LAB.

2.11 Statistical Analysis

Duncan's multiple range test was employed to determine the significance of differences within treatments. For each treatment, 3 determinations were performed and the mean values were calculated. Values were considered significant different when $p < 0.05$.

3 RESULT

3.1 The optimum hydrolysis conditions of *Morinda citrifolia* (Noni)

Reducing sugars increased from 3.86, 3.53, 2.77 and 4.25 mg/mL to 50.12, 44.63, 36.42, 45.23 mg/mL (calibrated by glucose, galactose, arabinose and fructose), while the total phenolic compounds and flavonoids contents increased from 80.43 and 24.72 to 92.03 and 41.85 μg/mL, respectively, after 8 h hydrolysis with 75 U/mL cellulase at 50°C (Table 1). The reducing power and $ABTS^{\cdot+}$ scavenging ability also increased from 139.28 and 42.32 to 164.12 ppm and 88.99%, respectively (Table 2).

3.2 The optimum fermented strains of *Morinda citrifolia* (Noni)

Three % of bacteria were, firstly, inoculated into a pH 3.0 or pH 6.0 PBS and cultured 0, 6, 12, 18, 24 h at 37°C. It was found that *Pediococcus pentosaceus* BCRC 14053, *Lactobacillus helveticus* BCRC 12296, *Lactobacillus casei* BCRC 12272 and *Streptococcus salivarius* subsp. *thermophilus* BCRC 12268 had better acid tolerance. These strains were further to ferment noni puree (noni: water=1: 3) and found that the optimum fermentation strains was

Pediococcus pentosaceus BCRC 14053 (Table 3). Therefore, the resulted hydrolysate was further fermented by 3% *Pediococcus pentosaceus* BCRC 14053 with the addition of 10% glucose and 0.1-0.2% $CaCO_3$ at 37°C, 24 h on noni puree (noni: water=1: 4).

3.3 The optimum fermented conditions of *Morinda citrifolia* (Noni)

Use of the above resulted hydrolysate with 0.2% $CaCO_3$ as medium for 3% *Pediococcus pentosaceus* BCRC 14053 fermentation, the LAB counts increased to 7.09 log CFU/mL, while the pH decreased to 4.93 (Table 4). The total phenolic, flavonoids and anthocyanins contents were 96.28 μg/mL, 37.06 μg/mL, 118.18 μg/g, and reducing power and $ABTS^{\cdot+}$ scavenging ability were 144.58 ppm and 82.30%, respectively, after 24 h cultivation (Table 5).

Table 1 Change in reducing sugar of noni after 8 h hydrolysis with different amounts of cellulase

	Reducing sugars (mg/mL)			
Amount of enzyme (U/mL)	glucose	galactose	arabinose	fructose
0	3.86	3.53	2.77	4.25
25	22.34	19.95	16.22	20.54
50	35.48	31.62	25.77	32.22
75	50.12	44.63	36.42	45.23
100	62.90	55.99	45.71	56.59
125	74.13	65.97	53.88	66.57

Table 2 Change in antioxidant composition and antioxidant capacity of noni after 8 h hydrolysis with different amounts of cellulase

	Amount of enzyme (U/mL)					
	0	25	50	75	100	125
Total phenolic (relatively gallic acid μg/mL)	80.43	89.28	90.30	92.03	90.00	89.96
Flavonoids (relatively quercetin μg/mL)	24.72	32.17	38.25	41.85	45.79	50.48
Reducing power (relatively Vit. C ppm)	139.28	164.52	164.23	164.12	164.07	161.13
ABTS radical scavenging capacity (%)	42.32	73.25	84.16	88.99	91.62	94.05

Table 3 Change in microbial counts of noni hydrolysate (noni: water=1: 3) after 24 h fermentation by different probiotics

	Microbial counts (log CFU/mL)	
Fermentation time (h)	F0	F24
Not adjusted the pH	n.d.	
Adjusted the pH to 6.5		
Pediococcus pentosaceus BCRC 14053	7.83	5.59
Lactobacillus helveticus BCRC 12296	7.18	5.05
Lactobacillus casei BCRC 12272	6.35	3.82
Streptococcus salivarius subsp. *thermophilus* BCRC 12268	3.72	n.d.

n.d.: not detected

Table 4 Change in pH and microbial count of noni hydrolysate after 24 h fermentation by *Pediococcus pentosaceus* BCRC 14053

	Fermentation time (h)		
	F0	F18	F24
pH value			
0.1% $CaCO_3$	4.53	4.57	4.54
0.2% $CaCO_3$	4.98	5.03	4.93
Microbial count (log CFU/mL)			
0.1% $CaCO_3$	2.41	n.d.	n.d.
0.2% $CaCO_3$	7.14	7.09	7.09

n.d.: not detected

Table 5 Change in antioxidant composition and antioxidant capacity of noni hydrolysate after 24 h fermentation by *Pediococcus pentosaceus* BCRC 14053

Fermentation time (h)	F0	F24
Total phenolic (relatively gallic acid μg/mL)	89.66	96.28
Flavonoids (relatively quercetin μg/mL)	39.21	37.06
Anthocyanin (μg/g)	122.95	118.18
Reducing power (relatively Vit. C ppm)	140.81	144.58
ABTS radical scavenging capacity (%)	83.96	82.30

4 CONCLUSION

The noni hydrolysate was fermented by *Pediococcus pentosaceus* BCRC 14053 with the addition of 10% glucose and 0.2% $CaCO_3$, the LAB counts increased to 7.09 log CFU/mL, while the pH decreased to 4.93 after 24 h cultivation. These data suggested that noni hydrolystae could be utilized by *Pediococcus pentosaceus* BCRC 14053.

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