Enhancement of antioxidant effect of pitaya by lactic acid bacteria fermentation

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Abstract. Pitaya has received a great deal of consumer interest for its large amount of micronutrients. This study investigated the changes of total phenols and total flavonoids of fermented pitaya, as well as the antioxidant abilities. The results showed that fermentation could significantly increase the counts of water-soluble phenols and flavonoids. After being fermented for 24h, the DPPH free radical, hydroxyl radical and superoxide anion scavenging ability raised significantly. Reducing power of fermented pitaya also increased remarkably. Thus, fermentation could increase the antioxidant ability of pitaya, and could be a potential process for pitaya products.

1. Introduction
Oxidative stress, caused by excess oxidants, results in damage to protein and DNA, and relates to a series of diseases, such as neurological decline, cardiovascular disease and cancer. In addition, the imbalanced reactive oxygen species (ROS) also lead to DNA mutation, cell apoptosis, lipid peroxidation, alteration of gene expression, and modification of cell signal transduction [1-2]. Increasing investigations, however, have suggested that consumption of fresh fruits and vegetables could reduce risk of some chronic diseases caused by oxidative stress.

Pitaya, belonging to cactus species, originates from Latin America and widely cultivated in southern China. Recent years, pitaya fruit have drawn attention worldwide because of its sensorial properties and its high antioxidant activity [3]. Due to the large size of pitaya, the public usually consume for fresh-cuts and juice. However, the fresh-cuts or juice easily goes bad. Fermentation is potential process for fruit productions. Therefore, we investigated the effect of lactic acid bacteria (LAB) fermentation on antioxidant ability of pitaya.

2. Materials and methods

2.1. Preparation of fermented pitaya
Fresh pitayas were peeled manually, cut into cubes, and ground into puree. Sucrose and glucose (8% w/v, 4:1) were into puree. The mixture was thermally treated at 68 °C for 30 minutes and chilled immediately down to 4 °C. A *Lactobacillus plantarum* strain isolated from pickled vegetable was inoculated (5% v/v) into mixture and incubated aerobically at 37 °C for 24h.

2.2. Determination of pH value and viable count
The pH value of fermented pitaya was measured by pH meter immediately. And viable count of fermented pitaya was determined by dilution method of plate counting.
2.3. Determination of total phenolics and total flavonoids
The amount of total phenolics contents was determined by the Folin-Ciocalteu method with slight modifications [4]. Fermented pitaya of 0.5g was diluted with distilled water to 10mL, centrifuged and collected supernate as sample and used in following tests. A mixture of 0.5mL sample (previously diluted 20-fold with distilled water), 5mL distilled water and 1mL Folin-Ciocalteu reagent was stood at 25°C for 10 minutes. 3mL of sodium carbonate (150g/L) solution was added into the mixture. After 2h at 25°C in dark, absorbance was measured at 765 nm, and results were expressed as gallic acid equivalents.

The amount of total flavonoids contents was determined by colorimetric method with slight modifications [5]. Sample was adjusted to neutral pH by 0.1M NaOH. 2mL sample was added to 3mL 60% ethanol into a 10mL scaled tube. 0.3mL NaNO₂ (5 g/L) was added following by a 6 minutes’ standing. 0.3mL AlNO₃ (10 g/L) was followed with another 6 minutes’ standing. 4 mL 1M NaOH was added into the tube, diluted with 60% ethanol (v/v) to the volume, and incubated at room temperature for 15 min. The samples were measured at 510 nm and expressed as rutin.

2.4. Determination of antioxidant effects of fermented pitaya

2.4.1. DPPH radical scavenging ability
Reaction system consisted of 0.1 mL of supernate and 3 mL of DPPH solution (0.5 mmol/L) prepared with ethanol reacted at 25 °C in dark for 0.5 h. Ethanol replacing the sample in the reaction system was used as control. The absorbance values were determined at 515 nm and antioxidant ability was calculated by the following formula:

\[ \text{DPPH radical scavenging ability (\%)} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100 \]

A₀ and A₁ refer to the absorbance of control and samples, respectively.

2.4.2. Hydroxyl radical scavenging ability
In glass tubes, 2 mL sample, 2 mL ethanol-salicylic acid solution (9 mmol/L), 1 mL ferrous sulfate (9 mmol/L) and 2 mL hydrogen peroxide (8.8 mmol/L) were added in given order. Then cultivated the mixture at 37 °C for 30 min and detected absorbance at 510 nm. Hydroxyl radical scavenging ability was calculated by the following formula:

\[ \text{Hydroxyl radical scavenging ability (\%)} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100 \]

A₀ and A₁ refer to the absorbance of control and samples, respectively.

2.4.3. Superoxide anion scavenging ability
Sample (0.25mL) and Tris-HCl (0.1 mol/L, pH 8.2, 4.5mL) mixed and reacted at 37 °C for 20 min; added 0.4mL pyrogallic acid (50 mmol/L), reacted at 37 °C for 5 min. Then added 0.1mL hydrochloric acid (8 mol/L) to stop the reaction, and detected absorbance at 320 nm. Superoxide anion scavenging ability was calculated by the following formula:

\[ \text{Superoxide anion scavenging ability (\%)} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100 \]

A₀ and A₁ refer to the absorbance of control and samples, respectively.

2.4.4. Reducing power
Mixed 1mL sample, 2.5mL phosphate buffer (pH 6.6) and 5 mL potassium ferricyanide (1%, w/v). Added 5mL trichloroacetic acid (10%, w/v) after reacting at 50 °C for 20 min, and centrifuge the mixture to get supernate. Mixed the supernate (2.5mL), water (5.65 mL) and ferric trichloride (0.5mL, 0.1%, w/v), and detected the absorbance at 700nm.
3. Results and Discussions

3.1. pH value and viable count of fermented pitaya
As shown in Figure 1, the viable count of LAB significantly increased from 4.79 lg(CFU/mL) to 9.35 lg(CFU/mL) (p<0.05). Meanwhile, pH value was influenced by the physiological activity that significantly decreased from 5.38 to 4.03 (p<0.05). These results suggested that pitaya is a suitable medium for LAB.

![Fig. 1 Changes of pH value and viable count after fermentation](image)

3.2. Content of total phenolics and total flavonoids
For now, studies mainly focus on the red pitaya for their higher contents of flavonoids and phenolics [6]. In our study, both the contents of water-soluble phenolics and flavonoids gradually increased with the fermentation time, and increased significantly for 24h fermentation (p<0.05) (Figure 2). Previous studies have showed that bind polyphenols can be hydrolyzed to free polyphenols by enzymes produced during fermentation [7]. Some studies also suggested that polyphenols might aggregate into macromolecules and form precipitation that reduces the content of soluble polyphenols [8]. Our results, however, suggested that fermentation may release the conjugated flavonoids and phenolics that increased the contents of water-soluble phenols and flavonoids.

![Fig. 2 Content changes of total phenols and total flavonoids](image)

3.3. Antioxidant effects of fermented pitaya
Results in this section showed that antioxidant effects of pitaya have been improved (p<0.05) by fermentation for 12h- and 24h-fermented pitaya.
Fig. 3 Changes of DPPH free radical scavenging ability of fermented pitaya

The DPPH approach is simple and usually applied for the detection of antioxidant activity. DPPH ethanol solution will turn from purple into light yellow when hydrogen given by antioxidant combine with DPPH and the fading degree positively correlated with antioxidant capacity [9]. In Figure 3, pitaya showed a significant up-going tendency of DPPH radical scavenging activity that from 17.89% to 26.32% (24h).

Fig. 4 Changes of hydroxyl radical scavenging ability of fermented pitaya

Hydroxyl radical (·OH) is the most active radical, formed by superoxide and hydrogen peroxide, which can accelerate the oxidation of sugars, proteins and lipids through electron transfer [10]. As illustrated in Figure 4, hydroxyl radical scavenging ability was found in pitaya, and it significantly increased from 12.08% to 25.96% and 28.32% (24h) respectively, and there was no significant difference between fermentation groups (p>0.05).

Fig. 5 Changes of superoxide anion scavenging ability of fermented pitaya
Superoxide anions (O2⁻) produced by mitochondrial electron transport system that. Superoxide anion shows a strong biological function that easily inactivate glutathione peroxidase, cause DNA damage, cell membrane damage and other toxic effects. By fermentation, superoxide anion scavenging ability of pitaya climbed from 3.81% to 6.62% (12h) and 7.68%(24h) respectively ($p<0.05$). Moreover, it showed a similar tendency compared with hydroxyl radical scavenging ability that fermentation time showed no significant influence on the superoxide anion scavenging ability ($p>0.05$).

Antioxidants can interrupt free radical chain reactions by providing electrons that transform free radicals into stable substances [11]. The total reduction capacity is an important index to evaluate the electron supply capacity of antioxidants, and an increase in absorbance at 700 nm indicates an enhanced reduction capability [12]. Fermentation also increased the reducing power of pitaya (24h) ($p<0.05$) (Figure 6). While shorter fermentation time (12h) showed no remarkable effect on the reducing power ($p>0.05$).

Antioxidant abilities was related to the amount of polyphenols and flavones. In the study, we found that fermentation significantly increased the contents of water-soluble phenolics and flavonoids. At the same, we also observed an increased antioxidant capacity of pitaya. These results suggested that fermentation may release the bioactive constituents and enhance the health promoting effects of some plant materials.

4. Conclusion
Based on the results and discussions presented above, the conclusions are obtained as below:

(1) It is shown Lactobacillus planetarium strain can grow well in pitaya and the viable count could reach at 9.35 lg(CFU/mL).
(2) Fermentation could convert the existing form of phenolics and flavonoids and significantly increased both the contents of free phenolics and flavonoids.
(3) Fermentation noticeably enhanced the antioxidant ability of pitaya that can be a potential process for new health fruit products.

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