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Lactobacillus fermentum LLB3 Improves Antioxidant Activity of Bitter Melon (*Momordica charantia*) Juice

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Momordica charantia (bitter melon) contains substances with antidiabetic properties such as charantin, vicine, and polypeptide-p, as well as other unspecific bioactive components such as antioxidants. It is suitable for functional drink and need further studies to elaborate its functional properties. *Lactobacillus fermentum* LLB3 isolated from bamboo shoot pickle was used to ferment bitter melon juice. The aim of this study was to evaluate changes in antioxidant activity of bitter melon juice during fermentation. Study has been carried out by fermenting bitter melon juice with *L. fermentum* LLB3. The free radical scavenging activity of the phenolics were done using 2,2-diphenyl- 1-picrylhydrazyl (DPPH). Antioxidant activity of bitter melon juice increased during 24 hours fermentation. In addition, the sugar content and pH decreased compared with the baseline value. The fermentation of bitter melon juice by *L. fermentum* LLB3 increased its antioxidant activity. These result suggest that fermented bitter melon juice is a promising agent for diabetes management.

Key words: antioxidant activity, bamboo shoot pickle, bitter melon, diabetes management, *Lactobacillus fermentum* LLB3

Momordica charantia (pare) mengandung zat dengan khasiat antidiabetes seperti *charantin, vicine,* dan *polypeptide-p*, serta komponen bioaktif spesifik lainnya seperti antioksidan, sehingga cocok untuk diolah menjadi minuman fungsional. *Lactobacillus fermentum* LLB3 yang diisolasi dari acar rebung digunakan untuk memfermentasi jus pare. Tujuan dari penelitian ini adalah untuk mengevaluasi perubahan aktivitas antioksidan jus pare selama fermentasi. Penelitian dilakukan dengan memfermentasi jus pare dengan *L. fermentum* LLB3. Aktivitas antioksidan diukur menggunakan 2,2-difenil-1-pikrilidrazil (DPPH). Aktivitas antioksidan jus pare meningkat selama 24 jam fermentasi. Selain itu, kadar gula dan pH menurun dibandingkan dengan nilai awal. Fermentasi jus pare oleh *L. fermentum* LLB3 dapat meningkatkan aktivitas antioksidannya. Hasil ini menunjukkan bahwa jus pare fermentasi merupakan produk yang menjanjikan untuk manajemen diabetes.

Kata kunci: acar rebung, aktivitas antioksidan, *Lactobacillus fermentum* LLB3, manajemen diabetes, pare

Foods such as fruits, vegetables and grains are reported to contain a wide variety of antioxidant components, including phytochemicals. Phytochemicals, such as phenolic compounds, are considered beneficial for human health, decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation (Karovicova and Kohajdova 2003).

Bitter melon (*Momordica charantia* L.) is an important vegetable grown in tropical and sub-tropical regions (Aboa *et al.* 2008; Wu and Ng 2008). The fruits are eaten while still green and unripe. They have been used for generations by indigenous populations in Africa, China, India, Japan, and Latin America for food and folk medicine (Khan and Anderson 2003; Aboa *et al.* 2008; Matsuda *et al.* 1998). Bitter melon has been the subject of intensive investigations for biologically active compounds and for its functional properties (Majekodunmi *et al.* 1990; Matsuda *et al.* 1998; Begum *et al.* 1997). The fruit extract had the highest value of antioxidant activity and that gallic acid was the predominant phenolic compound in the fruit extract (Kubola and Siriamornpun 2008). The folk medicinal properties associated with bitter gourd includes treatment for various chronic and degenerative diseases, including, diabetes mellitus (Nerurkar *et al.* 2006; Aboa *et al.* 2008; Nerurkar *et al.* 2010) coronary heart disease and cancer (Anilakumar *et al.* 2015).

Lactic acid fermentation is considered as one of the most suitable tool to exploit the biogenic/functional potential of plant matrices and to enrich them with bioactive compounds (Pellati *et al.* 2004). The fermentation by selected lactic acid bacteria was largely used to enhance the antimicrobial, antioxidant and immune-modulatory features of several cereal, pseudo-cereal and leguminous flours (Coda *et al.* 2012) as well as of medicinal plants like Echinacea spp. (Rizello *et al.*

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2013). Lactic acid fermentation can help to improve the safety, shelf life, and nutritional and sensory properties of vegetables. Strains of several *Lactobacillus* species have been proven to exert a range of health-promoting activities such as immunomodulation, enhancement of resistance against pathogens and reduction of blood cholesterol levels and are used as probiotics (Karovicova and Kohajdova 2003).

In this study, bitter melon juice was fermented using *Lactobacillus fermentum* BLL3 over a period of 72 h and investigated the changes in antioxidant activity, sugar content, and pH. The aims of this research is to evaluate changes in antioxidant activity of bitter melon juice during fermentation.

MATERIALS AND METHODS

Microbial Strains. Probiotic lactic acid bacteria (*Lactobacillus fermentum* LLB3) was isolated from pickled bamboo shoots based on the previous study of Hartayanie *et al.* (2016). Bacterial culture was stored frozen at-20 ºC in MRS medium (Merck, Germany) containing 20% glycerol. The strains were reactivated by means of double passage on MRS when needed.

Bitter Melon Juice Preparation. Immature Bitter melon (*Momordica charantia*) was obtained from pasar Bandungan, Ambarawa. The fruits were washed with deionized water, drained at ambient temperature and cut in halves, lengthwise. The seeds were removed and flesh was extracted using a commercial food processor (Philips) under room temperature. The bitter melon juice was placed into bottle and then pasteurized for 5 min at 80 ºC.

The Fermentation of Bitter Melon Juice. *Lactobacillus fermentum* LLB3 was grown in the MRS broth incubated at 37 ºC for 24 h and reached an OD_{600} of 1.0, which is equivalent to 10^6 CFU ml^{-1}. Twenty mililiter of the cultivated MRS broth was added aseptically into 180 mL of pasteurized bitter melon juice. The fermentation process took place in a incubator (Memmert, Germany) at 37 ºC for 72 h. The fermented bitter melon juice was harvested at 24, 48, and 72 h and compared with those of their unfermented control (un-inoculated) counterparts denoted as 'fermentation time 0'.

DPPH Free Radical Scavenging Activity. The DPPH assay was conducted according to (Brand-Williams et al. 1995) with some modifications. Stock solution of 0.6 mol L^{-1} DPPH (Sigma-Aldrich) in methanol (Merck) was prepared and kept at −20 ºC until used. Fresh working solution was prepared for each assay by mixing 10 mL of stock solution with 45 mL of methanol to obtain an absorbance of 1.1 ± 0.01 units at 515 nm. Each appropriately diluted bitter melon extract and standard solution (100 µL) was mixed and incubated with 3900 µL of working solution for 30 min in dark. All of the samples and blank solution (3,9 ml DPPH + 0,1 ml methanol) were measured at 515 nm wavelength by using spectrophotometer. The percentage of free radical scavenging activity was calculated as follows:

$$\text{Scavenging effect (\%)} = [1 - \frac{\text{A517 nm sample}}{\text{A517 nm blank}}] \times 100\%$$

Chemical Analysis. The pH of the samples were measured by using digital pH meter (EC-pHTestr30-KIT). The Total Soluble Solid analysis for determining the sugar concentration of the samples were determined by using brix refractometer (Hanna Instruments Sucrose Refractometer).

Statistical Analysis. The experiment was done in triplicate for each substance. The results were expressed as percentage decrease with respect to control values and compared by one-way ANOVA and Duncan test. A difference was considered statistically significant if $p \leq 0.05$.performed in accordance to SNI 01-2891-1992.

RESULTS

The percentage of DPPH scavenging activity in bitter melon juice (0 h) was 72.46% and increased to 82.44% at 24 h. Afterwards, it remained constant (Table 1).

The fermentation process is characterized by a decrease in sugar content and pH of the foods due to the production of organic acids. The pH decreased from 5.55 to 4.8 and the sugar content also decreased from 2.7% to 2.3% (Table 2).

DISCUSSION

DPPH assay has been widely used to determine the free radical scavenging activity of various plant extracts (Gil *et al.* 2000; Pellati *et al.* 2004; Rizello *et al.* 2013). Antioxidant activity was measured by DPPH-linked free radical scavenging activity assay at 0, 24, 48, and 72 h of fermentation time. Antioxidant molecules in the sample scavenge the free radical DPPH and the color from the DPPH assay solution becomes light yellow resulting in a decrease of the absorbance at 517 nm. *L. fermentum* LLB3 can

Table 1 Change in scavenging effect during fermentation of bitter melon juice

Time (hour)	Scavenging Effect (%)
0	72.46 ± 2.86 [a]
24	82.44 ± 1.73 [b]
48	82.48 ± 3.28 [b]
72	80.47 ± 3.74 [b]

* Values with different superscripts showed significant differences between treatments ($p < 0.05$) based on the One-Way Anova test. The differences among different treatments were determined by Duncan's multiple range test at the 0.05 probability level.

Table 2 Change in pH and sugar content during fermentation of bitter melon juice

Bitter gourd juice	pH	°brix
0 h fermentation	5.55 + 0.01[c]	2.72 + 0.08[c]
24 h fermentation	4.61 + 0.04[a]	2.55 + 0.10[b]
48 h fermentation	4.81 + 0.02[b]	2.37 + 0.05[a]
72 h fermentation	4.81 + 0.04[b]	2.36 + 0.08[a]

* Values with different superscripts showed significant differences between treatments ($p < 0.05$) based on the One-Way Anova test. The differences among different treatments were determined by Duncan's multiple range test at the 0.05 probability level.

increased antioxidant activity of bitter melon juice. Lactic acid bacteria are used as starter culture in many fruit and vegetable fermentations due to their ability to produce β-glucosidase enzyme. This enzyme is an important catalyst in the liberation of aromatic compounds from glucoside precursors present in fruits and their fermentation products (Michlmayr and Kneifel 2013; Denkova *et al.* 2013). As shown in Table 1, the DPPH scavenging effect of bitter melon juice increased significantly after 24 h fermentation.

The fermentation process is characterized by a decrease in sugar content and pH of the foods due to the production of organic acids. *L. fermentum* LLB3 was able to utilise bitter melon juice for lactic acid production without nutrient supplementation. The reducing sugar concentration rapidly decreased from 2.7 to 2.5 g L^{-1} within 24 h of fermentation period. Between 24 and 72 h of fermentation, the rate of sugar utilization slowed down and its concentration decreased slightly to 2.3 g L^{-1}. Between 24 and 72 hr fermentation, slightly changes in sugar concentration was observed (Table 2). The *bitter melon* fermented juice is safe for diabetic consumption because no sugar adding in fermentation process.

L. fermentum LLB3, used in this study was found capable of rapidly utilising sugar in vegetable juice medium for lactic acid production. Rapid decrease in pH was also observed during the first 24 hr of fermentation period where pH decreased from 5.55 to 4.61 (Table 2). Sligthly pH changes were observed from 24 to 72 h fermentation period. The decrease in pH was concomitant to the increase in viable cell count during the first 24 h of fermentation. A rapid decrease of pH during the early stage of fermentation is an important indicator of end product quality. The increase in acidity during lactic acid fermentation can minimizes the activities of spoilage bacteria and contributes to the pleasant taste and desirable aroma (Breidt *et al.* 2013).

From this study, it can be concluded that the fermentation of bitter melon juice by *L. fermentum* B3 increased its antioxidant activity. This result suggest that fermented bitter melon juice is a promising agent for diabetes management.

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