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Increase in the free radical scavenging capability of bitter gourd by a heat-drying process

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Bitter gourd (*Momordica charantia* Linn.) is widely regarded as one of the best remedy foods for diabetes. The positive effect of bitter gourd on diabetes has been attributed in part to the remarkable free radical scavenging activity of its boiled water extract from sun-dried fruits. It is well known that a heat process significantly influences the antioxidant activity of fresh fruits. However, the heat drying processes of bitter gourd have not been studied so far. Here, we show that the free radical scavenging capability of bitter gourd extract significantly increases after the heat drying process, while the content of flavonoids and phenols, which are generally regarded as the main antioxidant components in bitter gourd, remain unaffected. Furthermore, the content of free amino acids and the total reducing sugar were found to decrease with increasing browning index, indicating the progression of the Maillard reaction, products of which are known to possess significant antioxidant activity. Therefore, it suggests that Maillard reaction products may be the main contributors to the increase in antioxidant capability. Finally, the bitter gourd extract with the higher antioxidant activity, was shown to manifest a corresponding higher proliferation activity on NIT-1 beta-cells. These results suggest that controllable conditions in the heat-drying processing of fresh bitter gourd fruit is of significance for enhancing the total free radical scavenging capacity, beta-cell proliferation activity and possibly the anti-diabetic activity of this fruit.

Introduction

Bitter gourd (*Momordica charantia* L.) is a common vegetable that is often used as a folk remedy for diabetes in Asia, South America and North Africa. Its anti-diabetic effect has been proved by an increasing number of studies.[1,2] Several different mechanisms for its anti-diabetic activity have been proposed.[3,4] Among them, free radical scavenging is a plausible mechanism since diabetes mellitus was reported to be associated with an increased production of free radicals.[5] In fact, bitter gourd extract has been identified to possess a significant free radical scavenging activity *in vitro* with flavonoids and phenols as the main antioxidant components.[6,7]

It is well established that the heating process can intensify the total antioxidant activity of many fresh fruits and herbs, such as tomatoes,[8] soybeans[9] and ginsengs.[10,11] It is also reported that the hot water extract of bitter gourd possesses higher antioxidant activity than the cold water extract.[12] These results suggest that the heating process can influence not only the physical properties of fresh materials but also their biological activities. In fact, bitter gourd commonly undergoes a sun-drying process before it is utilized as the remedy for diabetes. Theoretically, such a heating process could therefore greatly influence the antioxidant activity of the bitter gourd extract, but this has not been studied so far. There is a lack of understanding about the influence of the processing conditions on the biological activities of the bitter gourd extracts, which may result in a lack of product quality control, and thus the effectiveness of the remedy.

The objective of the present study was to evaluate the effect of the heat-drying process on the antioxidant activity and its mechanism. The correlation between the antioxidant activity and proliferation activity on NIT-1 beta-cell was also investigated to confirm the anti-diabetic mechanism of the bitter gourd extract.

Materials and methods

Preparation of fresh bitter gourd extract

Fresh mature bitter gourds were collected from Yinan country (Shandong Province, China). Fruits without seeds were sliced to 2 mm thick. 50 g of the fruit slices were homogenated with pulprefiner and added to sodium phosphate buffer solution (0.02 M, pH 7.2) to a final volume of 100 mL. The mixture was stored at 4 °C for 24 h. The mixtures were then filtered through gauze and centrifuged at 21 400*g* at 4 °C for 15 min. Sodium phosphate buffer solution (0.02 M, pH 7.2) was added to the supernatants to bring the final volume up to 100 mL. The prepared samples were stored at −20 °C for further experiments.

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Preparation of heat-dried bitter gourd extracts

50 g of the fruit slices were heat-dried at 40 °C, 50 °C, and 60 °C in loft driers for 2 h, 4 h, 8 h, 16 h, 32 h, and 64 h. Extracts from the fruit slices, heated at different temperatures for various lengths of time, were prepared according to the same procedures for fresh fruit extract preparation and stored at −20 °C for further experiments.

Determination of DPPH free radical scavenging activity

DPPH free radical scavenging activity was determined according to the method of Marsden, S. B.[13] Briefly, 1 mL of 0.1 mM DPPH in methanol was mixed with 3 mL of 4-fold diluted bitter gourd extract samples with the same phosphate buffer solution. The mixture was then vortexed vigorously and left at 40 °C for 30 min in the dark. For the baseline control, a mixture of 3 mL PBS (pH 7.2) and 1 mL of methanol was used. The absorbance was measured at 517 nm. The percent inhibition was calculated by the following equation,

$$\text{Percent inhibition (\%)}: [1 - (A_i - A_j)/A_0] \times 100\% \quad (1)$$

where, A is the absorbance at 517 nm of different solutions: A_i: 2 mL DPPH solution + 2 mL sample; A_j: 2 mL sample + 2 mL ethanol; A_0: 2 mL DPPH solution + 2 mL ethanol.

Determination of ABTS free radical scavenging activity

ABTS free radical scavenging activity was estimated by the method of Re, R.[14] ABTS was dissolved in water to a concentration of 7 mM. ABTS free radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45 mM of potassium persulfate (final concentration). The ABTS solution was diluted with PBS (pH 7.2) to an absorbance of 0.70 (±0.02) at 734 nm at 30 °C. After addition of 4 mL of diluted ABTS solution to 1 mL of 10-fold diluted bitter gourd extract sample in PBS, the absorbance at 734 nm was determined at 30 °C from exactly 1 min to 6 min. After the initial mixing, appropriate solvent blanks were run in each assay. The inhibition percent was calculated by the following equation.

$$\text{Percent inhibition (\%)}: [1 - (A_i - A_j)/A_0] \times 100\% \quad (2)$$

where A is the absorbance at 734 nm of different solutions: A_i: 4 mL ABTS solution + 1 mL sample; A_j: 1 mL sample + 4 mL 80% ethanol; A_0: 4 mL ABTS solution + 1 mL 80% ethanol.

Determination of total flavonoid and total phenol content

The total flavonoid content was determined using a colorimetric method.[15] 0.25 mL of the bitter gourd extract sample was mixed with 1.25 mL of distilled water followed by the addition of 75 μL of a 5% $NaNO_2$ solution. After 6 min, 150 μL of a 10% $AlCl_3 \cdot 6H_2O$ solution was added and allowed to stand for another 5 min before 0.5 mL of 1 M NaOH was added. The mixture was brought to 2.5 mL with distilled water and mixed well. The absorbance was measured immediately against the blank at 510 nm using an UV-1900 spectrophotometer (Hitachi, Japan). The total flavonoid content was calculated by comparing the absorbance to a standard curve using catechin as a standard.

The total phenol content was determined by the Folin–Ciocalteau method.[16] Samples (0.5 mL of different dilutions) were mixed with 2.5 mL of 0.2 N Folin–Ciocalteau reagents (Sigma-Aldrich) for 5 min and 2.0 mL of 75 mg mL^{-1} sodium carbonate was then added. The absorbance of the reaction was measured at 760 nm with a UV-1900 spectrophotometer (Hitachi, Japan) after 2 h of incubation at room temperature. The total phenol content was calculated by comparing the absorbance to a standard curve made using the standard chemical gallic acid, which is a common reference phenolic compound.

Evaluation of the extent of browning

Absorption of the bitter gourd extract was measured at 420 nm using a UV-1900 spectrophotometer (Hitachi, Japan) and the absorption was designated as the browning index in this study.

Analysis of free amino acids and reducing sugars

The free amino acid content of both fresh and heat dried bitter gourd was determined. 4 mL 5-Sulfosalicylic acid solution was added to 1 mL of the bitter gourd extract sample to a final concentration of 5%. The mixture was kept at room temperature for 30 min and then centrifuged at 21 000g for 10 min. The supernatant was filtrated through Φ 0.45 μm of filter membrane and applied to an automatic amino acid analyzer (Hitachi-8350, Japan).

The reducing sugar content of the bitter gourd extract was estimated according to the method of Miller, G. L.[17]

Assay of proliferation activity on pancreatic beta-cells

The proliferation activity of bitter gourd extracts on pancreatic beta-cells was tested. 50% confluent monolayer cultures of NIT-1 cells in 96-well plates were washed twice with phosphate-buffered saline (PBS) and cultured in DMEM culture medium with 15% fetal calf serum (Gbico, USA) with and without the presence of bitter gourd extract samples. The cultures were incubated at 37 °C, 5% CO_2 for 48 h and cell viability was evaluated by an MTT (Amresco, USA) assay. Proliferation percent was calculated by the following equation.

$$\text{Proliferation percent (\%)}: [A_i/A_0 - 1] \times 100 \quad (3)$$

where A_i: absorbance at 490 nm of the culture well in the presence of bitter gourd extract; A_0: absorbance at 490 nm of the control group without bitter gourd extract.

Statistical analysis

Results were the means ± standard deviations from three replicates of independent experiments of the same treatments. Data were subjected to one-way analysis of variance with the treatment as the fixed factor. Significant differences were detected at the 0.05 level.