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Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species

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Abstract

The study was aimed to determine the antioxidant activity (total antioxidant and free radical-scavenging activities) and total phenolic content of *Amaranthus* sp. The effects of different blanching times (10 and 15 min) on antioxidant activity and phenolic content were also studied. Four types of *Amaranthus* species locally known as spinach, namely 'bayam putih' (*Amaranthus paniculatus*) (BP), 'bayam merah' (*Amaranthus gangeticus*) (BM), 'bayam itik' (*Amaranthus blitum*) (BI) and 'bayam panjang' (*Amaranthus viridis*) (BPG), were selected. Total antioxidant activity of water-soluble components in raw spinach was in the order of BI ≈ BM ≈ BPG > BP, whereas free radical-scavenging activity was in the order of BI > BPG > BM > BP. The total phenolic contents of BM and BP were significantly higher ($p < 0.05$) than other samples. All the studied spinach species possessed different antioxidant activities and phenolic contents. Antioxidant activities and phenolic contents of all the spinach were in the order of raw > blanched 10 min > blanched 15 min. Blanching up to 15 min may affect losses of antioxidant activity and phenolic content, depending on the species of spinach.

Keywords: Spinach; Blanching; Total antioxidant activity; Scavenging activity; Phenolic content

1. Introduction

Overwhelming scientific data, from epidemiological studies, indicate that diets rich in fruit, vegetables and grains are associated with a lower risk of several degenerative diseases, such as cancers (Steinmetz & Potter, 1996) and cardiovascular diseases (Rimm et al., 1996). This association is often attributed to different antioxidant components, such as vitamin C, vitamin E, carotenoids, lycopenes, polyphenols and other phytochemicals.

Food composition data, necessary for epidemiological and nutritional studies, are merely representative of foodstuffs consumed in the raw state. Many food composition databases never take into consideration the fact that concentrations of nutrients and their activity may change through cooking practices such as blanching. This is of great importance, considering that only a small amount of vegetables is consumed in the raw state, whilst most need to be processed for safety and quality.

Amaranthus sp., locally known as spinach or "bayam", is one of the most popular leafy vegetables consumed in Malaysia. Five types of spinach species can be found in Malaysia; 'bayam putih' (*Amaranthus paniculatus*), 'bayam merah' (*Amaranthus gangeticus*), 'bayam itik' (*Amaranthus blitum*), 'bayam duri' (*Amaranthus spinosus*), and 'bayam panjang' (*Amaranthus viridis*). However, only four species of spinach, namely 'bayam putih', 'bayam merah', 'bayam itik' and 'bayam panjang', are abundantly available in the market, and commonly consumed by urban or rural Malaysians. The vegetable has been reported to have a high concentration of antioxidant

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components (Hunter & Fletcher, 2002). This leafy vegetable is generally cooked before being consumed. Losses of antioxidant components from vegetables during cooking have been reported elsewhere (Chu, Chang, & Hsu, 2000; Yadav & Sehgal, 1995). However, no research has been carried out to investigate the antioxidant properties of these four species of spinach. This study was first initiated to determine the antioxidant activity and polyphenol content of these vegetables. However, our previous study found that the total antioxidant activity and phenolic content in 'bayam putih' significantly decreased after 1 min of blanching (Amin, Zamaliah, & Chin, 2004). In practice, spinach is cooked with water for quite some time before being consumed. Hence, the effects of blanching times (10 and 15 min) on the loss of antioxidant activity and polyphenol content were also estimated.

2. Materials and methods

2.1. Samples

2.1.1. Material

Four types of healthy and fresh spinach species, namely 'bayam putih' (*Amaranthus paniculatus*), 'bayam merah' (*Amaranthus gangeticus*), 'bayam itik' (*Amaranthus blitum*) and 'bayam panjang' (*Amaranthus viridis*), were randomly selected and purchased from several retailers in the wholesale market at Seri Kembangan, Selangor, Malaysia.

2.1.2. Preparation of sample

Spinach was cleaned under running tap water and excessive water was drained off. The spinach (1 kg) was chopped into small pieces and divided into three portions (raw, blanching for 10 min, and blanching for 15 min). Blanching was done by simmering the vegetables in boiling water in the ratio of 1 to 5, draining the sample and leaving it to cool at room temperature. All the raw and blanched samples were lyophilized using a bench top freeze-dryer (Labconco, Freezone 4.5, USA). The lyophilized sample was homogenised using a blender (National; MX-291N, Kuala Lumpur, Malaysia) before being transferred into an air-tight container, and kept at −20 °C for further analysis.

2.1.3. Preparation of extract

The ground sample was extracted with distilled water in the ratio of 1 to 10. The mixture was placed in a conical flask (wrapped with an aluminium foil) and agitated at 100 rpm with the aid of an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for 1 h. The mixture was then filtered through a Whatman No. 4 filter paper to obtain a clear extract. The water extract was used for all analysis.

2.2. Measurement of antioxidant activity

2.2.1. Total antioxidant activity

β-Carotene bleaching assay was carried out according to the method developed by Wettasinghe and Shahidi (1999). Briefly, 2 ml β-carotene solution (0.2 mg/ml chloroform) were pipetted into a round-bottom flask containing 20 μl linoleic acid and 200 μl Tween 20. The mixture was then evaporated at 40 °C for 10 min using a rotary evaporator (Laborata 4000, Heidolph Instruments GmbH & Co. KG, Germany) to remove chloroform. After evaporation, the mixture was immediately added to 100 ml of distilled water. The mixture was vigorously agitated to form an emulsion.

Five millilitre aliquots of the emulsion were transferred into different test tubes containing 200 μl of extract. The mixture was then gently mixed and placed in a water bath at 50 °C for 2 h. Absorbance of the sample was measured every 15 min for 2 h at 470 nm using a Spectronic® Genesys™ 5 spectrophotometer (Milton Roy Company, New York, USA). Blank solution was prepared, containing the same concentration of sample without β-carotene. All determinations were performed in triplicate. The total antioxidant activity was calculated based on the following equation:

$$\mathrm{AA} = \left[1 - \frac{A_0 - A_t}{A_0^{\circ} - A_{\mathrm{t}}^{\circ}}\right] \times 100,$$

where AA is antioxidant activity, A_0 and A_0° are the absorbance values measured at initial time of the incubation for samples and control, respectively, while A_{t} and A_t° are the absorbance values measured in the samples or standards and control at $t = 120$ min.

2.2.2. Free radical-scavenging activity

Effect of extract on DPPH free radical was measured, based on Lee, Park, and Choi (1996). Positive control was prepared by mixing 4 ml of ascorbic acid (0.05 mg/ml) and 1 ml of DPPH (0.4 mg/ml), whereas negative control was prepared by mixing distilled water with 1 ml of DPPH.

Four millilitre of the extract (a final concentration of 20 mg/ml) were added to 1 ml DPPH. The mixture was gently homogenized and left to stand at room temperature for 30 min. Absorbance was read using a spectrophotometer at 520 nm. The ability of extract to scavenge DPPH free radical was calculated using the following equation.

$$\text{Scavenging activity}(\%) = \left[\frac{A(-\mathrm{ve}) - A_{\mathrm{s}}}{A(-\mathrm{ve}) - A(+\mathrm{ve})}\right] \times 100,$$

where, As is the absorbance of the sample, $A(-\mathrm{ve})$ and $A(+\mathrm{ve})$ are the absorbance values of negative and positive controls, respectively.