Food Chemistry 135 (2012) 2942–2946

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Identification of the flavonoids in mungbean (*Phaseolus radiatus* L.) soup and their antioxidant activities

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ARTICLE INFO

Article history:
Received 5 June 2010
Received in revised form 7 May 2012
Accepted 3 July 2012
Available online 17 July 2012

Keywords:
Mungbean soup
Antioxidant
Vitexin
Isovitexin

ABSTRACT

Mung bean soup (MBS) has been traditionally taken as a kind of health food in China. To learn the mechanisms underlying its health benefits, antioxidant capacities of the soup prepared with three cultivars of mung bean were measured. The highest DPPH radical scavenging or ferric reducing activity was observed in soup of mung bean cv. *Huang*. The MBS of cv. *Huang* and *Mao* exhibited higher $ABTS^{\cdot+}$ reducing activities than MBS of cv. *Ming*. The two major flavonoids in the MBS were purified and identified as vitexin and isovitexin, respectively. Modeling samples containing vitexin and isovitexin at the same levels as them in the MBS were prepared to assess their antioxidant contributions in the MBS. Our results showed that antioxidant capacities of the MBS mainly derived from vitexin and isovitexin, these flavonoids accounted for the most of total DPPH radicals scavenging, ferric reducing and $ABTS^{\cdot+}$ reducing scavenging activities in MBS of all the three cultivars.

1. Introduction

Mung bean (*Phaseolus radiatus* L.) is a leguminous species grown primarily in Asia. Mung bean soup (MBS) that usually prepared by boiling a small mount of mung bean with considerable water (the ratio of mung bean in 30 g to water in 1000 mL) is the most popular beverage in China and some other Asian countries. It is best-known that MBS can help people avoid and eliminate heatstroke.

In general, heat stress, including heat exhaustion and heat stroke, may result in enhanced metabolic rate (Gupta, Lahiri, Sultana, Tulsawani, & Kumar, 2010) which is usually accompanied with excessive formation of reactive oxygen species, thus causing a redox imbalance between free radicals and antioxidant defense system (Chang, Chang, Liu, & Lin, 2007; Lord-Fontaine & Averill-Bates, 2002). Flavonoids and phenolic acids in plant foods can reduce oxidative damages that may cause various diseases, such as cancer, cardiovascular diseases, diabetes and ageing (Middleton, Kandaswami, & Theoharides, 2000; Thompson, Brick, McGinley, & Thompson, 2009; Yao, Chen, Wang, Wang, & Ren, 2008).

Extracts from skins of mung bean with methanol have been shown to have antioxidant properties (Duh, Du, & Yen, 1999; Duh, Yen, Du, & Yen, 1997). However, little information is available to explain the health beneficial properties of MBS.

Objectives of this work were to evaluate antioxidant ability of MBS and identify the major antioxidant components in MBS.

2. Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), butylated hydroxytoluene (BHT), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), Folin–Ciocalteu reagent and 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox, a hydrophilic derivative of tocopherol) were purchased from Sigma–Aldrich Chemical Co. (United States), DMSO, methanol and acetic acid of HPLC grade were from Fluka Chemical Co. (Germany), Potassium persulfate, $FeSO_4{\cdot}7H_2O$ and other reagents were analytical grade.

2.2. Plant materials

Huang, *Ming* and *Mao* are the major cultivars of Mung bean in China. In this study, mung bean of cv. *Huang*, *Ming* or *Mao* was obtained from city of Siping in Jilin-province, Zhangjiakou in Hebei-province or Wuhan in Hubei-province in China, respectively.

2.3. Preparation of mung bean soup (MBS)

The mung bean soup (MBS) was prepared according to a traditional cooking method in China. The whole bean (30.0 g) with 1000 mL deionised water was boiled for 30 min, then filtered though a filter paper (Whatman No. 1). The filtrate was collected and added with deionised water to 1000 mL, which was used as standard MBS for the subsequent analysis.

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http://dx.doi.org/10.1016/j.foodchem.2012.07.048

2.4. Determination of DPPH radical scavenging capacity

The antioxidant activity was determined by DPPH assay according to Brand-Williams, Cuvelier, and Berset (1995) with some modifications. The sample was prepared at a concentration of 20%, 40%, 50%, 60%, 80% or 100% of the MBS, which was equal to 6, 12, 15, 18, 24 or 30 mg mung bean/mL, respectively. A reference sample of BHT was prepared at a concentration of 50, 100, 125, 150, 200 or 250 μg/mL, respectively. An aliquot of 200 μL sample mixed with 3.8 mL DPPH (200 μM in methanol) solution was incubated in dark at room temperature for 60 min, then its absorbance at 517 nm was measured by a spectrophotometer. The DPPH scavenging activity of the sample was calculated using the following equation:

$$\text{DPPH scavenging effect}\,(\%) = \left(1 - \frac{\text{absorbance of sample at 517 nm}}{\text{absorbance of control at 517 nm}}\right)$$

EC_{50} was also used for measuring DPPH scavenging capacity in the result, which is defined as the antioxidant necessary to decrease the initial DPPH radical concentration by 50%. It was acquired from the plotted graph of decrease concentration of DPPH against the levels of MBS. The analyses were measured in triplicate.

2.5. Determination of ferric reducing antioxidant power (FRAP)

The Ferric reducing antioxidant power of sample was determined according to the procedure described by Benzie and Strain (1996) with some modifications. The FRAP reagent was prepared by mixing 10 mM TPTZ solution in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$ and 300 mM acetate buffer (pH 3.6) in a ratio of 1:1:10 (v/v/v). This reagent should be prepared freshly and warmed at 37 °C prior to use. Aliquots of 100 μL MBS were mixed with 1.7 mL deionised water and 1.8 mL FRAP reagent, and then the mixture was blended and incubated in water bath at 37 °C for 30 min in order to achieve the reactive equilibrium. The increase in the absorbance was determined by a spectrophotometer at 593 nm. The ferric reducing antioxidant power of MBS is expressed as mM of Fe^{2+} reduced based on the $FeSO_4$ standard curve plotted using at a concentration range between 100 and 600 μM. The analyses were conducted in triplicate.

2.6. Determination of $ABTS^{\cdot+}$ radical scavenging capacity

The experiments were carried out according to the procedure of $ABTS^{\cdot+}$ scavenging assay described by a literature with some modifications (Re et al., 1999). The ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting 7 mM stock solution of ABTS (in 20 mM acetate, pH 4.5) with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Prior to assay, the working $ABTS^{\cdot+}$ solution was diluted in acidic medium of 20 mM sodium acetate buffer (pH 4.5) (1:75, v/v) to achieve an absorbance at 734 nm of 0.700 ± 0.02. Then, 500 μL MBS was well-distributed with 2 mL of the $ABTS^{\cdot+}$ working solution, respectively. All the assays were performed at the absorbance at 734 nm, and the reaction lasted for 6 min to achieve the reactive equilibrium. Trolox was a water soluble analogue of vitamin E, and was used as a reference standard. A standard curve was plotted based on measuring the reduction in absorbance of the $ABTS^{\cdot+}$ solution against a concentration range between 100 and 600 μM of Trolox. TEAC (Trolox equivalent antioxidant capacity) of MBS represents that the concentration of Trolox solution that has the same antioxidant capacity as MBS. The analyses were conducted in triplicate.

2.7. HPLC detection

As UV–vis spectra (UV–vis T6, Persee Co., China) of the filtrate of MBS was corresponded with that of flavone, high performance liquid chromatography equipped with DAD (diode array detector) (LC-Prominence-20AT and SPD-M20A, Shimadzu Co., Japan) was employed. Spectra were recorded from 210–600 nm; UV absorbance was maintained at 280 and 337 nm. Two hundred and eighty nanometres was used for observing various phenolic compounds, while 337 nm which was one of maximum UV absorbance was better to quantify flavonoid. A analytical column C18 (Shim–pack VP-ODS 15 cm × 4.6 mm ID, 5 μm, Shimadzu Co., Japan) was used and kept at 30 °C. A gradient event of mobile phase solvent A: water (acetic acid 1%, v/v) and B: methanol (acetic acid 1%, v/v) was as follows: 10–35% B (10 min), 35–42% B (15 min), 42–75% B (10 min), 75% B (5 min), 75–10% B (5 min), 10% B (5 min), and at a flow rate 1.0 mL/min. One injection volume of sample was 10 μL. All chromatograms were analysed using system LC solution software.

2.8. XAD-16 macroporous resin separation

The column chromatography (30 cm × 28 mm ID) filled with XAD-16 macroporous resin (polystyrene resin, 0.7 mm particle size, Rohm and Haas Co., America) was employed to enrich flavonoid. Pretreatment for activating resin: soaked by ethanol overnight, the chromatographic column of resin was flowed by 500 mL ethanol at 6 mL/min, and 1200 mL deionised water was used for removing ethanol at 6 mL/min; then the column was flowed by 360 mL of 4% (v/v) hydrochloric acid at a flow rate of 10 mL/min, and equilibrated with deionised water to a neutral pH; similarly, the column was flowed by 450 mL of 5% (v/v) sodium hydroxide at a flow rate of 10 mL/min, and equilibrated with deionised water to a neutral pH.

In a preliminary experiment, we found compound-1 and compound-2 were the dominant phenolic compounds in all the mungbean soups of the three cultivars. Hence, Mao MBS was chosen randomly as a sample for purification and analysis of compound-1 and compound-2. After pretreated, *Mao* MBS solution was injected into the column and adsorbed statically for 4 h in dark. Subsequently, the column was flowed by 900 mL of deionised water. Then, the flavonoids were eluted with 900 mL of 60% (v/v) ethanol solution at a flow rate of 6 mL/min. This procedure was repeated 5 times in order to accumulate enough flavonoids. All portions of flavonoid elution were concentrated by rotatory evaporator under vacuum (RE-52, Shanghai Yarong Co., China), and redissolved with 2 mL methanol for further separation.

2.9. Separation and enrichment of the flavonoids by semi-preparative HPLC

To obtain enough amount of the flavonoids for the subsequent analysis, semi-preparative HPLC with a column C_{18} (capcell pak UG 25 cm × 10 mm ID, 10 μm, Shiseido Co., Japan) was used and performed at 30 °C. The mobile phase consisted of 35% methanol was used under isocratic condition at a flow rate of 4 mL/min. One injection volume of MBS sample was 100 μL. UV absorbance was maintained at 337 nm. According to retention time of the compound-1 and compound-2, their outflows were carefully collected. This performance was repeated for 10 times. The collected fractions were first concentrated by rotatory evaporating, and then lyophilised (LGJ-10, Henan Brother Co., Ltd., China) for 24 h. Purity of the compound-1 or the compound-2 was examined by HPLC at UV absorbance wavelength of 230, 260, 280, 300, 320, 337, 360 nm. Sequentially, 7 chromatographs were generated for quantifying compounds in the samples by peak area normalisation method.