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Original Research Article

Analysis of water-soluble bioactive compounds in commonly consumed soymilk in China

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Genistin (PubChem CID: 5281377)
Daidzein (PubChem CID: 5281708)
Daidzin (PubChem CID: 107971)
Raffinose (PubChem CID: 439242)
Stachyose (PubChem CID: 439531)

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ABSTRACT

Soybean, an important source of many bioactive compounds, is of interest because of the health benefits it confers. We compared the contents of different bioactive components, such as peptides, amino acids, isoflavones, saponins, and oligosaccharides, in five commonly used soymilk types in China, using an amino acid analyzer, high-performance liquid chromatography, and an ultraviolet spectrophotometer. The concentrations of almost all bioactive compounds, except for stachyose and raffinose, were significantly higher in homemade soymilk (HSM) samples than in ready-made soymilk (RSM) samples. HSM created using non-genetically modified soybeans contained the most amino acids (21.7%), water-soluble proteins (25.6%), peptides (11.90%), and water-soluble isoflavones (1175 μg/mL). HSM made using genetically modified soybeans contained the most saponin (36.7 mg/mL). RSM samples contained the most stachyose and raffinose. These findings provide basic information for evaluating the function of soymilk with regard to its bioactive ingredients.

1. Introduction

The consumption of soybean products has been shown to confer potential health benefits (Murphy et al., 2008). Some essential amino acids present in soybeans cannot be synthesized by the human body (Goerke et al., 2012; Trindade et al., 2001). Soybean peptides show antioxidant activity, inhibit the accumulation of free radicals, and can be used to treat many diseases (Cao et al., 2011). Interest in soy isoflavones has also increased in recent decades, due to their potential preventive activity against human chronic diseases, such as cardiovascular disease, cancer, and osteoporosis (Masilamani et al., 2012). Soy saponins have many biological functions, including hypocholesterolemic, hemolytic, immunostimulatory, and antitumorigenic activities (Hu et al., 2002). Raffinose and stachyose are two major soybean oligosaccharides that have been reported to have prebiotic properties in intestinal environments (Grmanová et al., 2010). However, the concentrations of bioactive compounds in soybean products vary depending on the soybean sources and processing conditions.

Asians have been consuming soybean products for more than 1000 years (Conroy et al., 2013; Mueller et al., 2012). Soymilk is one of the most popular and traditional products, especially in

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China, and contains eight essential amino acids (Setchell and Cassidy, 1999). Soymilk also contains peptides, saponins, isoflavones, and oligosaccharides, among others. The many types of soymilk can be divided into two main groups: traditional homemade soymilk samples (HSMs), made using a household soymilk machine, and modern ready-made soymilk samples (RSMs), purchased from a market. Many kinds of flavors can be added to RSM to improve its taste, and these additions modify the composition and concentrations of bioactive compounds. RSMs are becoming popular, especially among young people, due to their ready availability. However, no research has been conducted to investigate the composition of nutrients and bioactive compounds in these two main types of soymilk.

Generally, more water-soluble bioactive compounds in soymilk are absorbed more easily by the body and provide more beneficial health effects (Gao and Hu, 2010; Liu et al., 2010; Trindade et al., 2001). The distribution and concentrations of bioactive compounds have been studied in some soybean products, including some soymilk samples (Nachaisin et al., 2011); however, the concentrations of water-soluble bioactive compounds have not been determined for different types of soymilk. Thus, the present study established methods to detect the main water-soluble bioactive compounds in soymilk and compared the concentrations of the main compounds in five different types of soymilk.

2. Materials and methods

2.1. Sources of soymilk samples

Five different types of soymilk were assessed in this study. The first type was homemade soymilk made using non-genetically modified (non-GM) soybeans, which is one of the most frequently consumed soymilks in China; the non-GM soybeans were from Jiamusi, Heilongjiang, China and brought from Zhihaishanggu Trading Company, Ltd. (Beijing China). Another type was homemade soymilk made using GM soybeans (Liangyou Ltd., Shanghai, China), the use of which has raised some controversy due to safety concerns about consumption of genetically modified soy. The soybeans used to make this milk contained a gene for 5-enolpyruvylshikimate-3-phosphate synthase (*epsps*). The other types of soymilk evaluated were three of the most popular types of commercial soymilk, including one sugar-free and two with added sugar (Yon Ho Ltd., Shanghai, China).

2.2. Chemicals

Amino acid standard AAS18, genistein, genistin, daidzein, daidzin, oleanolic acid, cytochrome C, trasylol, bacitracin, L-glutathione oxidized, reduced glutathione tablets, and Gly-Leu (≥98%) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Raffinose and stachyose (≥98%) were procured from TCI Chemical Co. (Tokyo, Japan). Ninhydrin, trisodium citrate, citric acid, sodium borohydride, acetic acid, absolute ethanol, 2-methoxyethanol, acetic acid sodium salt, acetic acid ethyl ester, perchloric acid, trifluoroacetic acid, and vanillin were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Methanol and acetonitrile (high-performance liquid chromatography grade) were bought from Dikma Chemical Co. (Beijing, China). Milli-Q water was used as a control during the study.

2.3. Soymilk sample preparation

GM or non-GM soybeans were placed in water heated to 100 °C and ground using a soymilk maker to produce homemade soymilk at a final concentration of 0.07 g dry weight/mL. Sugar or sugar-free soymilk powder (0.7 g) was dissolved in 10 mL of water heated to 100 °C to make the commercial samples. All the prepared samples were then centrifuged at 14,000 × *g* for 30 min. The supernatants were filtered using a 0.22-μm microporous membrane for sample detection.

2.4. Determination of water-soluble proteins

The concentrations of water-soluble proteins were determined with a modification method using an ATN-2300 Kjeldahl Azotometer (FOSS Ltd., Hillerød, Denmark) (Junsomboon and Jakmunee, 2006). Briefly, 10-mL samples were digested and distilled in a hooded combination Kjeldahl unit. The performance of individual heating units was adjusted by boil tests to ensure equal heating throughout the whole sample. The digestions were performed in 12 mL of concentrated H_2SO_4, to which 2 FisherTab CT-50 Kjeldahl tablets [containing 0.3 g of copper (II) sulfate catalyst and 10 g of potassium sulfate] were added. The samples were heated to obtain a clear solution, which was allowed to boil for a further 1 h. The digested solution was cooled, diluted with distilled water, rendered basic with 48 mL concentrated NaOH until the pH was 5.10, and distilled into a saturated boric acid (H_3BO_3) solution containing a pH indicator. Ammonium (NH_4^+) in the distillates was titrated with 0.1 mol/L HCl. Finally, the protein concentrations were calculated according to the nitrogen quantity with a conversion factor of 5.71.

2.5. Determination of amino acids

An L-8800 automatic amino acid analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan) with a 4.6 mm (ID) × 60 mm ion exchange column (Hitachi High-Technologies Corporation, Tokyo, Japan) was used to analyze the amino acid profiles in soymilk, using a modified method (Kim et al., 2013). The following analyzer settings were used: buffer flow rate of 0.4 mL/min, reagent flow rate of 0.35 mL/min, reactor heater temperature of 135 °C, column temperature of 75 °C, auto-sampler temperature of 5–8 °C, sample injection volume of 20 μL, and detection wavelength of 570 nm (for proline) or 440 nm (for all other amino acids). For amino acid quantification and calibration, an amino acid standard of cysteic acid at 0.1 μmol/mL and other standards at 0.2 μmol/L were prepared. An external standard was used to calculate the concentration of each amino acid and the concentrations were reported as percentages of soymilk samples.

2.6. Determination of peptides

A Waters E2695 high-performance liquid chromatography (HPLC, Waters Co., Ltd., Milford, MA, USA) was performed to detect small peptides and isocratic elution was used. The chromatographic conditions were as follows: TSK gel G2000SWXL column of 7.8 mm × 300 mm (TOSOH Co., Ltd., Tokyo, Japan), mobile phase containing 0.1% (v/v) trifluoroacetic acid in ultrapure water, flow rate of 1.0 mL/min, column temperature of 30 °C, UV detector at 220 nm and the injection volume was 20 μL. The peptides selected were cytochrome C (molecular weight, MW: 12384), bacitracin (MW: 6511.4), aprotinin (MW: 1422.69), reduced glutathione (MW: 612), and Gly-Leu (MW: 188.23) were selected as the molecular weight standards to establish the linear equations for the retention time and molecular weight, as shown in Fig. 1.

2.7. Determination of isoflavones

Isoflavone detection was performed by a waters E2695 HPLC (Waters Co., Ltd., Milford, MA, USA) analysis system. Gradient elution was used to minimize the analysis time and get an