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Optimization of Isoflavone Extraction from Soy Germ

– Research Note –

Sang-Moon Bae[1], Chan Ho Jang[1], Jang-Hoon Kim[1], Hyun Ae Lim[1], Joo-Ryong Kim[1], Jeong Hwan Kim[2] and Jong-Sang Kim[1†]

[1]Department of Animal Science and Biotechnology, Kyungpook National University, Daegu 702-701, Korea
[2]Department of Food Science and Technology, Geongsang National University, Jinju 660-701, Korea

Abstract

Soy isoflavones have drawn much attention due to their potential to prevent breast and prostate cancers, osteoporosis, heart disease, and other postmenopausal symptoms. Soy germ is one of the richest sources of isoflavones, and thus has good potential to be used as the ingredient of health foods. This study examined the extraction rate of isoflavones from soy germ at various conditions. After the effect of extraction temperature and duration on isoflavone extraction from soy germ was examined, the optimum concentration of ethanol as extraction solvent was determined. When ethanol concentration was fixed at 60% (v/v), the maximum isoflavone extraction was achieved at 2 hrs and 30°C. Among various concentrations of ethanol tested, 80% (v/v) ethanol showed the highest extraction efficiency. In conclusion, the maximum extraction of isoflavones was obtained using 80% (v/v) ethanol as a solvent, at 30°C of temperature, and 2 hrs of extraction time.

Key words: isoflavones, soy germ, HPLC analysis, extraction

INTRODUCTION

Soy isoflavones belong to a class of compounds called phytoestrogens, naturally occurring plant chemicals that mimic or interact with human estrogen (1). Soy contains three primary isoflavones, genistein (4′,5,7-trihydroxy-isoflavone), daidzein (4′,7-dihydroxyisoflavone), and glycitein (7,4′-dihydroxy-6-methoxyisoflavone), as well as their β-glycosides, genistin, daidzein and glycitin (2). The glycosides can have an attached acetyl or malonyl group. Soy foods are major source of isoflavones and traditionally consumed in relatively high amounts by some Asian populations, such as Korean, Chinese and Japanese, and in low amounts by Western populations, such as North American and European. Many studies (3) demonstrated that there was a strong correlation between the abundant intake of soy foods and low incidence of several chronic diseases such as sex hormone dependent cancers, coronary heart disease, osteoporosis and postmenopausal discomfort. Isoflavones present in soy foods appear to be major components responsible for the preventive action against these chronic diseases. As isoflavones are similar to mammalian estrogens (1) in chemical structure, they act like sex hormone or exert antagonistic action against endogenous sex hormones depending on the concentration of the compounds.

Soy germ is one of the richest natural sources of isoflavones and contained 7~8 times more isoflavones than soybean cotyledon (4). Accordingly, it has been widely used as a source of isoflavones by health food companies where processed soy germ into various products such as cookies, capsules, and other supplement. In many cases, soy germ itself has been used as an ingredient, but it is also subjected to extraction to produce more purified isoflavones. Although extraction rate is an economically important issue, the study on improving extraction efficiency is limited. This study presents the effect of extraction conditions including temperature, time, and ratio of solvent to water, on the extraction efficiency of isoflavones from soy germ. Similar study was performed by Choi et al. (4). However, the optimum extraction conditions appeared to be different from our data.

MATERIALS AND METHODS

Materials

Soybean germ (hypocotyl) was obtained from Dr. Chung's Food Co. Ltd. (Cheongju, Korea). Ethanol was of HPLC grade and purchased from Merck Co. (Whitehouse Station, NJ, USA). Other reagents were of reagent grade. Isoflavone standards including genistein, daidzein, glycitein were obtained from Sigma Co. (St Louis, MO,

†Corresponding author. E-mail: vision@knu.ac.kr
Phone: +82-53-950-5752, Fax: +82-53-950-6750

USA) and Indofine Chemical Co. (Hillsborough, NJ, USA).

Extraction of isoflavones

Isoflavones were extracted from soy germ using aqueous ethanol at various temperatures and for different periods. Soy germ was ground for 1.5 min at intervals of 15 sec using a laboratory blender (FM 909T, Hanil, Seoul). Two grams of the pulverized soybean germ was mixed with 20 mL of ethanol/water in 50 mL conical tube, followed by shaking (200 rpm) for various time periods at different temperatures. The mixture was then centrifuged at 3,000×g for 15 min, and the supernatant obtained was made into 60% ethanol (v/v) by either adding pure water or 100% ethanol. The supernatant was filtered through 0.45 μ nylon syringe filter (Nunc, Rochester, NY, USA) and used for HPLC analysis.

Sample preparation

Sample for HPLC analysis was prepared with a modification of the method described previously (5). In brief, isoflavone extract was mixed with 10 volumes of 1 M HCl in a glass tube covered with bead, and incubated for 90 min in 98°C heating block (Model 17600, Thermolyne, Dubuque, Iowa, USA). After digestion, 8 mL pure methanol was added to 0.2 mL of sample, vortexed for about ten seconds, and filtered through a syringe filter. The filtered sample was used for isoflavone analysis in HPLC.

HPLC quantification of isoflavones

HPLC analysis was carried out using reversed-phase separation of the compounds on a C18 column (Capcell, Shiseido, Japan) and using methanol/1 mM ammonium acetate (60/40) as isocratic eluent. A high-pressure liquid chromatography (Jasco PU 1580) equipped with UV/Visible detector (Jasco UV-2077, Japan). Elution was monitored at 254 nm, and spectral data were recorded and stored over the time of the run on all samples (4). Injection volume was 20 μL.

RESULTS AND DISCUSSION

This study was undertaken to determine the optimum condition for isoflavones extraction from soy germ that is normally produced during soymilk processing and is high in glycitin, daidzin and genistin. Apparent total isoflavone content in the germ was variable depending on extraction conditions. The maximum yield was obtained when extraction was performed at 30°C for 2 hrs using 80% (v/v) ethanol, and the total isoflavone content was 56.47 mg/g dry matter (Fig. 1). After converted into aglycones, the contents of total genistein and daidzein plus glycitein in soy germ were 8.47 and 48 mg/g, respectively. Although our analytical condition poorly

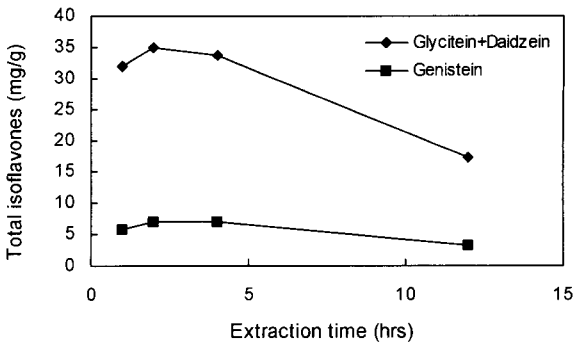

Fig. 1. Total genistein and daidzein plus glycitein concentrations in soy germ as extracted at 30°C using 60% ethanol for various times.

resolved glycitein from daidzein, it was estimated that soy germ contained 25.92 mg of total glycitein, 22.08 mg of total daidzein, 8.47 mg of total genistein per one gram, consistent with other reports that glycitein is the most abundant isoflavone in soy germ. Choi et al. (4) reported that soy hypocotyls contained 10.51 mg/g of total glycitein and 34.07 mg/g of total isoflavones. Soy germ represents a soy matrix containing concentrations 6- to 10-fold higher than found in other soy foods (6). The ratio of genistein to glycitein forms is quite different from that in soy foods derived principally from the cotyledons. According to the study by Murphy et al. (6), different solvents including acetonitrile (ACN), acetone, ethanol and methanol, have different abilities to extract the different isoflavone forms. The hydrophobicity of the isoflavone forms is aglucone > acetyl-β-glucoside > malonyl-β-glucoside > β-glucoside based on their chromatographic behavior on reversed-phase columns in the presence of an acid in the mobile phase to protonate the malonyl forms. However, there was no significant difference among 4 kinds of solvents in the extraction rate of isoflavones from soy germ, although acetonitrile was the superior solvent in extracting most of the isoflavone forms from the other soy products such as soy flour, tempeh, tofu, and TVP.

When isoflavones were extracted at 30°C using 60% (v/v) ethanol, 2 hrs of extraction appeared to be most efficient became extended extraction over 2 hrs tended to decrease the yield of isoflavones. Meanwhile, when the solvent concentration and extraction time were fixed at 60% (v/v) and 2 hrs, extraction at 30°C resulted in the highest extraction efficiency (Fig. 2). Finally, isoflavone extraction was carried out at 30°C and 2 hrs using various ethanol concentrations. Among ethanol concentrations tested (0, 20, 40, 60, 80, 100% (v/v)), 80% (v/v) ethanol showed the highest isoflavone extraction rate (Fig. 3). Choi et al. (4) reported that isoflavones was most efficiently extracted from soy hypocotyls using

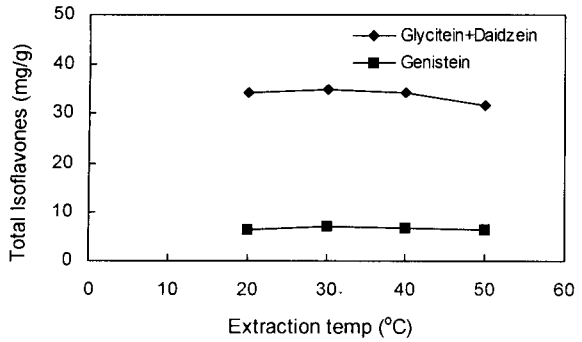

Fig. 2. Total genistein and daidzein plus glycitein concentrations in soy germ as extracted for 2 hrs using 60% ethanol at various temperatures.

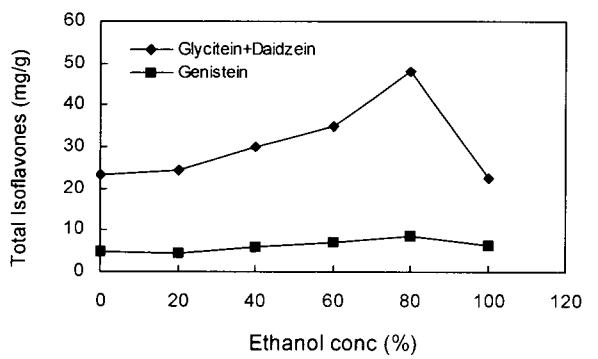

Fig. 3. Total genistein and daidzein plus glycitein concentrations in soy germ as extracted at 30°C for 2 hrs using various concentrations of ethanol as solvent.

60% ethanol at 40~60°C, and extraction yield was not significantly improved as the time was increased over 1 hr. Our study showed that extended extraction appeared to decrease the yield of isoflavones probably due to the loss of the compounds during extraction. The discrepancy between these data and ours in extraction rate of isoflavones even in similar experimental conditions might be caused by different analysis methods. We analyzed total isoflavones after converting all forms of isoflavones in the samples into aglycones while the other study measured intact isoflavones which mainly exist in glucoside forms.

Murphy (7,8) and Eldridge (9,10) compared several extraction solvents in the early 80s. The former found that acidified acetonitrile was the best extraction solvent among the ones they had examined while the latter reported that 80% methanol was the best. As a result of those studies, 80% methanol and acidified 83% acetonitrile (10 mL of acetonitrile plus 2 mL of 0.1 N HCl) have became the most commonly used extraction solvents in isoflavone analysis. While most soyfoods were extracted efficiently with acidified aqueous ACN, these authors noted that each soy food type must be initially evaluated to determine proper ACN/water ratios. Murphy et al. (11,12) established the optimum condition for extracting isoflavones from various food matrices for HPLC analysis. According to their data, the best extraction of isoflavones from dry foods was achieved by using 53% (v/v) acetonitrile (10 mL ACN plus 2 mL 0.1 N HCl plus 7 mL of water). Recently, Klump et al. (13) have proposed an Association of Official Analytical Chemists' method for soy isoflavone analysis that requires only six standards. The extracted samples are treated with heat and alkaline conditions to convert all the 6″-O-acetyl- and 6″-O-malonyl-glucosides to β-glucosides. The six standards, three aglucones and three β-glucosides are commercially available. As far as extraction of isoflavones for analysis is concerned, acetonitrile appears to be the best solvent (6). However, the attempt for improving isoflavone extraction as a use of food supplement is limited. Several studies examined the optimum condition for extracting isoflavone from soy hypocotyls or soybeans using aqueous ethanol (4,14,15).

Since the beneficial effect of isoflavones on sex hormone-associated diseases and various postmenopausal symptoms, the demand for isoflavones as health food supplement is expected to increase world-widely. Accordingly, maximizing the extraction of isoflavones from the various raw materials merits further study. In conclusion, this study presents that the extraction of isoflavones from soy germ could be efficiently performed using 80% aqueous ethanol at 30°C for 2 hrs.

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