Research Article

Extraction and Determination of Isoflavones in Soybean Seeds

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ABSTRACT

Soybeans and soya products are the main source of isoflavones. Several methods and different kinds of soybeans have been published to quantify soy isoflavones, but the amount of isoflavones was variable each other.. The purpose of this study was to extraction, quantify and compare levels of three major isoflavones in soybean seeds from three sources. Isoflavones have been extracted from soybean seeds using two different solvents (ethanol, acetonitrile) then they have been analyzed by high performance liquid chromatography (HPLC). The results showed that soybean seeds contain high percentage of genistin.

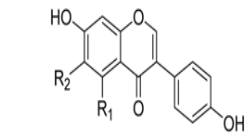
Keywords: Isoflavones, Extraction, Soyabean Seeds, Genistin.

INTRODUCTION

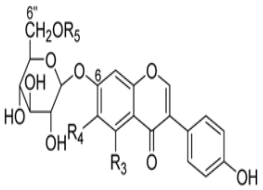
Interest in soybeans and soy based products has grown significantly in the last decade due to their reported nutritional and health-promoting benefits. Researchers have credited phytochemicals in soybeans, especially isoflavones, for some of these beneficial health effects.

Soy Isoflavones are reported to play a role in the prevention of osteoporosis, and several hormonally influenced cancers and to act as phytoestrogens in humans. Their ability to act as antioxidants may also serve to prevent oxidative damage in living tissue.[1]

Isoflavones are polyphenolic compounds which exist in twelve differents chemical forms (Lee et al., 2004) (figure 1). Daidzein, glycitein and genistein are the aglycone forms of isoflavone In conjuction with sugars, they build the β-glucosides (daidzin, glycitinandgenistin),the most dominant form in soybean.[2]



Aglycones	Symbol	R_1	R_2
Daidzein	De	H	H
Glycitein	Gle	H	OCH_3
Genistein	Ge	OH	H



Glucosides	Symbol	R_3	R_4	R_5
Daidzin	Di	H	H	H
Glycitin	Gly	H	OCH_3	H
Genistin	Gi	OH	H	H
Acetyldaizin	AcDi	H	H	$COCH_3$
Acetylglycitin	AcGly	H	OCH_3	$COCH_3$
Acetylgenistin	AcGi	OH	H	$COCH_3$
Malonyldaidzin	MDi	H	H	$COCH_2COOH$
Malonylglycitin	MGly	H	OCH_3	$COCH_2COOH$
Malonylgenistin	MGi	OH	H	$COCH_2COOH$

Figure 1: Chemical structures of 12 isoflavone isomers

The content of isoflavones in soybean is about 0.1–0.4%, and the amount and composition of isoflavones are different according to the year, growing district, and growing environment [3]. Wang and Murphy reported total isoflavone contents from 1176 to 3309μg/g across years, and from 1176 to 1749μg/g across sites within the same year for single soybean cultivars.

Actually many laboratories have reported analytic values for isoflavones in soybeans and soybean products but the amount of isoflavones was variable each other[4]. The objective of this study was to extraction, analyze and compare the content of three main isoflavones (genistin, daidzin and glycitin) from three sources.

MATERIALS AND METHODS

Reagents

The standard chemicals of daidzin, glycitin andgenistin were obtained from Sigma. Co. (USA). The HPLC grade solvent ofethanol, acetonitrile and acetic acid was purchased from DuksanCo. (Seoul, Korea). Water was filtered by a Milford ultra-pure water system (Milford, Bedford, MA, USA).

Samples preparation

Twenty soybean seeds test samples were purchased from three deferent sources.

The first source A includes A1-A2-A3-A4.

The second one H includes H1-H2-H3-H4-H5.

The third one I includes I1-I2-I3.

Isoflavone standards

Isoflavone stock solutions were prepared by dissolvingthe standards in methanol to give a 100 μg/mL concentration.

Calibration curves were made for each standard withsix concentrations (20-40-60-80-100µg/mL

Isoflavone extraction

Isoflavones were extracted from the soybean samplesusing two different solvents (ethanol 80%, acetonitrile 80%) Samples (1 g) were dispersed in 10 mL of each solvent and vigorously mixed at room temperature for 2 h using Snijders shaker. The dispersions were then centrifuged for 30 min at 3000 rpm, using a Labofuge centrifuge. The extracts were dissolved in 80% methanol and filtered through a 0.45µm filter unit prior to HPLC analysis[5].

Chromatographic conditions

A standardized analytical technique to quantify these compounds in foods has, therefore, become essential. High performance liquid chromatography (HPLC) using reversed phase C18 stationary matrices, mostly with mixtures of methanol or acetonitrile, has proved to be the method of choice for the analysis of isoflavones. [6]

Column: ODS 15 cm x 4.6mm, 5 µm particles.

Mobile phase: A: Acetonitrile+ 0.1 glacial acetic acid

B: water + 0.1 glacial acetic acid

(A:B) 50%:50%

Flow rate: 1 mL/min.

Temp: 25°C

Detector: UV, 260 nm

Injection: 1 µL.

RESULTS AND DISCUSSION

The HPLC chromatogram of the isoflavone standards is represented in fig.2. The proposed method resulted in a good resolution of the standards within 5 min.

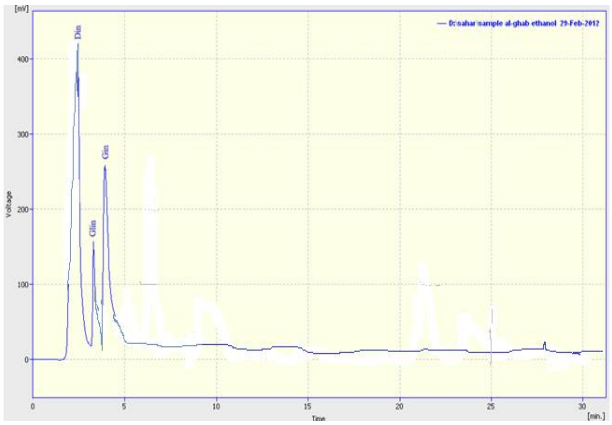


Figure 2: HPLC chromatograms of isoflavonestandards mixture

Calibration curve

The calibration curves were constructed to measure theamount of three isoflavones. Thecurves of isoflavones werelinearly plotted fig (3)(4)(5)

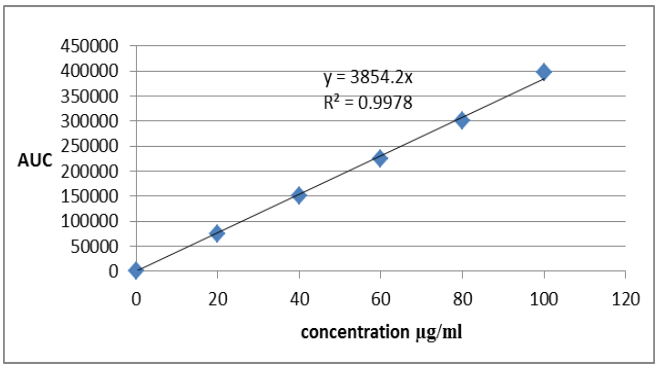


Figure 3: Calibration curve of genistin

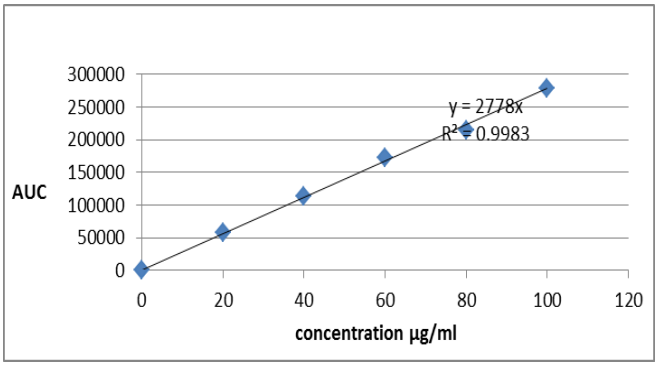


Figure 4: Calibration curve of daidzin

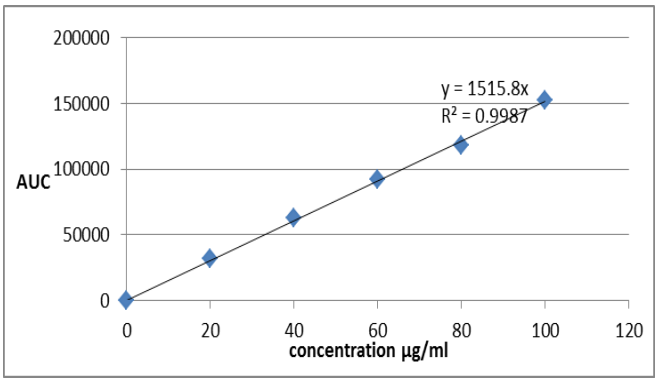


Figure 5: Calibration curve of glycitin

Effect of solvent

Closer examination of the results, however, showed that theextraction efficiency of ACN was significantly higher than the other solvent in extracting the Isoflavones from soybeans samples as shown in fig(6)(7)(8) .

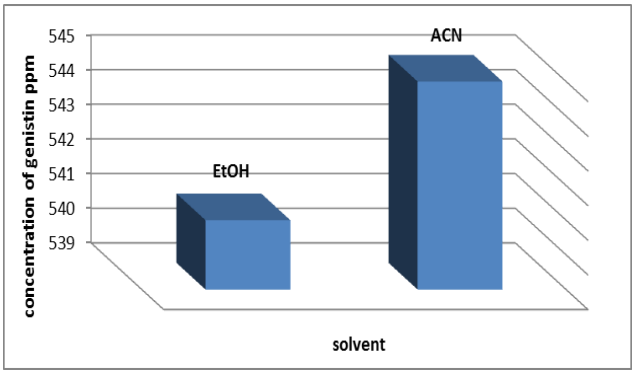


Figure 6: Effect of solvent on extraction of genistin

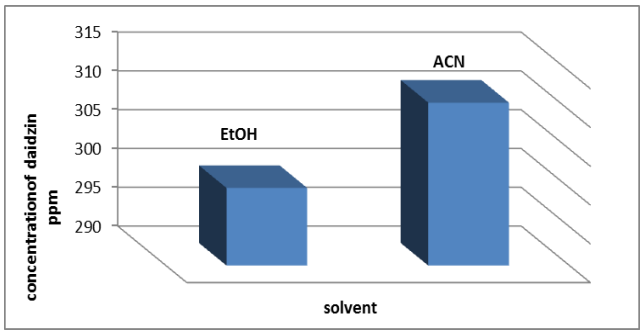


Figure 7: Effect of solvent on extraction of daidzin

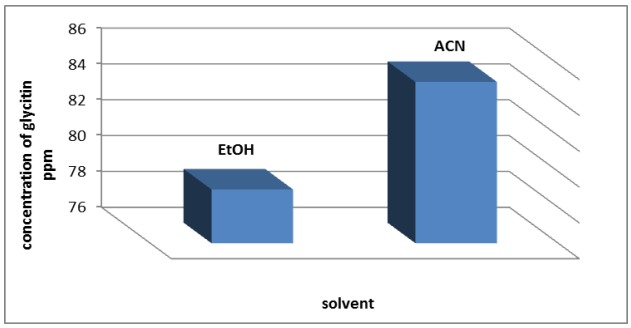


Figure 8: Effect of solvent on extraction of glycitin

Extraction with acetonitrile was, however, preferred because this system resulted in fast settling of suspending sample particles and less interfering impurities in the extract[7][8].

Quantitation of daidzin, genistin and glycitin

As shown in the fig (9)(10)(11) the average of daidzin in the samples of our experiment ranged from 298.72-390.8 µg/g, genistin from 539.9-661.14µg/g , glycitin from 78.1-130.8 µg/g.

The H samples contain high percentage of the three studied isoflavones.

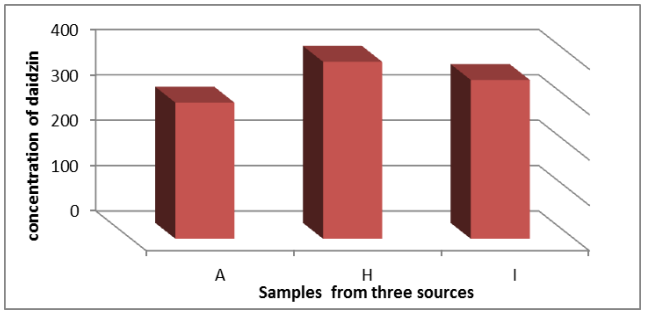


Figure 9: Comparison of daidzin content

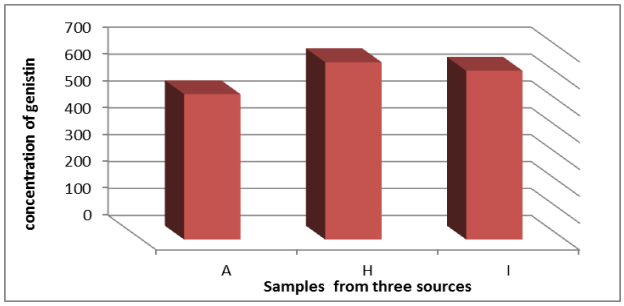


Figure 10: Comparison of genistin content

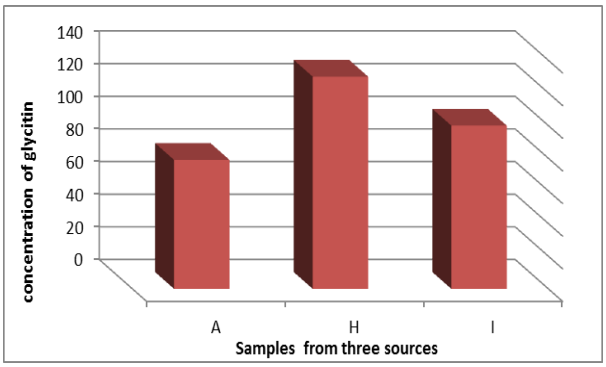


Figure 11: Comparison of glycitin content

CONCLUSION

The results presented in this paper could prove helpful in selecting analytical method for the extraction and determination of isoflavones in soybean seeds.

The soybean seeds contain high percentage of genistin, then daidzin and glycitin.

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