ISOFLAVONES, PHENOLICS CONTENT AND ANTIOXIDANT ACTIVITY OF THREE EGYPTIAN SOYBEAN CULTIVARS

BY

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Abstract

Soybean (*Glycine max* L.) is an important worldwide crop, mainly used as raw material for oil production, and the residue is mainly used as feedstuff. The medicinal functions of consuming soybean have been shown to be effective against number of chronic diseases such as cancer, heart disease, osteoporosis and menopausal symptoms. The objective of this work was to evaluate isoflavone and phenolic content and their antioxidant activity in three Egyptian soybean cultivars Giza 22, Giza 35 and Giza 111. Significant differences among the studied cultivars were found for genistein, daidzein, glycitein and total isoflavones due to genetic effects since all cultivars were collected at the same location. The total isoflavones ranged from 470.6 mg/100 g dry soybean (Giza 22) to 200.3 mg/100 g (Giza 111). The rank of total phenolic content (TPC) was Giza 22 > Giza 35 > Giza 111. At a concentration of 10 mg/mL, the free radical scavenging (antioxidant) capabilities were 79.34, 65.97 and 59.35 % for the extracts of Giza 22, Giza 35 and Giza 111, respectively. In conclusion, the present study or related studies are of great importance to identify the best soybean cultivar with the highest isoflavone content to be used as source for production of nutraceutical products rather than as a nutritional source of protein.

Keywords: HPLC, soybean cultivars, isoflavone, phenolic, antioxidant, *Glycine max* L.

Introduction

Soybean is recognized as an oil seed crop containing several useful nutrients including isoflavones, proteins, carbohydrates, vitamins and minerals. It can substitute for meat and to some extent for milk. It is a crop capable of reducing protein malnutrition as soybean protein provides all the eight essential amino acids in the daily amounts needed for human health. Past several years of scientific and clinical evidences had revealed the medicinal benefits of the soybean components against metabolic disorders (Hartman *et al.*, 2011).

Consumption of soybean had been linked to promote many health conditions, especially in reducing the risk of various cancers (Messina, 1997; Johnson, 2011). It was confirmed that unique components of soybean, such as isoflavones and phenolic compounds play an important role in protecting against oxidative stress that causes the development of some chronic diseases (Pusparini *et al.*, 2013). Soybean isoflavones have the potential to directly scavenge oxidants such as superoxide and nitric oxide or via indirect mechanisms, such as induction of antioxidant-scavenging enzymes (Yoon and Park, 2014).

Daidzein, glycitein and genistein are the three basic isoflavone aglycones, and daidzin, glycitin and genistin are the main corresponding isoflavone glycosides. The malonyl derivatives of these glucosides are also present in soybean (Kudou *et al.*, 1991). Malonyl isoflavones are thermally unstable and can be easily decarboxylated to produce acetyl glycosides during sample preparation and analysis. In total, there are twelve isoflavones identified in soybean (Griffith and Collison, 2001).

Several studies indicate that genetic variability affects isoflavone content of soybean cultivars. Isoflavone content variations can also be attributed to annual changes in environmental conditions from different locations such as temperatures, humidity, light intensity and rainfall (Wang and Murphy, 1994a; Tsukamoto *et al.*, 1995; Carrão-Panizzi *et al.*, 1998). Therefore, the objective of this work was to evaluate isoflavone concentration in three Egyptian soybean cultivars Giza 22, Giza 35 and Giza 111 using HPLC equipped with a photodiode array detector, in addition to determination of phenolic content and antioxidant activity.

Materials and methods

Authenticated soybean seeds of Giza 22, Giza 35 and Giza 111 were obtained from Agricultural Research Center (ARC), Giza, Egypt. Ground seeds (100 g) were defatted overnight with *n*-hexane (1:10), then washed two times with *n*-hexane (2 X 300 mL) and allowed to dry. The defatted samples were then extracted overnight with 80% methanol (2 X 500 mL) followed by evaporation of the combined extracts under reduced pressure. Samples were prepared by dissolving 8 mg extract in 3 mL methanol by sonication.

Genistein, daidzein and glycitein were purchased from Sigma-Aldrich (Steinheim, Germany), several concentrations (0.2 to 1 mg/mL) of each isoflavone were prepared to construct the calibration curves. The test sample and external standard solutions were filtrated through 0.22 µm syringe filter before injection into HPLC apparatus.

The isoflavone concentrations were determined according to Carrão-Panizzi *et al.* (2009) on HPLC Ultimate 3000 system equipped with a photodiode array detector (Bremen, Germany) running under Hystar software on a bounded silica C18 column (4.6 X 150 mm, 1.8 µm), column oven set at 30 °C. The mobile phases are composed of 0.2% formic acid/water (A) and 0.2% formic acid/methanol (B). The column was equilibrated with 10% B in A. The injection volume was 5 µL at a flow rate of 0.5 mL/min. After injection, the column was run for 1 min at the initial conditions, then developed with a linear gradient from 10% to 100% B/A in 35 min, 100% B for 4 min and from 100% to 10% B in 10 min (equilibration). The detecting wavelength was 285 nm. Isoflavone peaks were identified by their spectra and retention time and confirmed by comparing with data obtained from previous analyses of soybean isoflavone mixtures. Concentrations of the isoflavones were calculated as their respective aglycones, expressed as means for three replications in mg/100 g soybean powder.

The TPC was assessed in triplicate using gallic acid as the standard by Folin-Ciocalteu assay as described by Singleton *et al.* (1999). The absorbance was measured in triplicate at 765 nm against a reagent blank. The TPC was expressed as milligrams of gallic acid equivalents (mg of GAE/100 g soybean powder).

The *in vitro* free radical scavenging activity was determined using DPPH assay according to the method described by Burda and Oleszek (2001) with ascorbic acid as a standard.

Results and discussion

The twelve expected isoflavones are well resolved from one another and from background peaks (**Figure 1**). The total isoflavone content of Giza 22, Giza 35 and Giza 111 cultivars were 470.6, 377.9 and 200.3 mg/100 g soybean powder, respectively. For daidzein, the highest average value was found in Giza 22 (201.3 mg/100 g), while, Giza 35 cultivar had the highest value of glycitein (172.8 mg/100 g). For genistein, the highest average value was observed in Giza 22 (159.9 mg/100 g). In addition, Giza 111 cultivar showed the lowest content of daidzein and glycitein (**Table 1**).

Mujić *et al.* (2011) reported that total isoflavone content in different Croatian soybean cultivars was in the range from 80.7 to 213.6 mg/100 g. Several authors considered the isoflavones as the major phenolic compounds and their concentration in different soybean varieties varied from 126.1 to 409.2 mg/100 g of soybean (Wang and Murphy, 1994b; Carrão-Panizzi and Kitamura, 1995). The total isoflavone content in five Japanese and Chinese soybean varieties were reported by Yamabe *et al.* (2007) in the range from 221 to 444 mg/100 g. Soybean isoflavones have a positive impact on human health including prevention of chronic diseases such as cancer, heart disease, osteoporosis and menopausal symptoms (Zaheer and Humayoun, 2017).

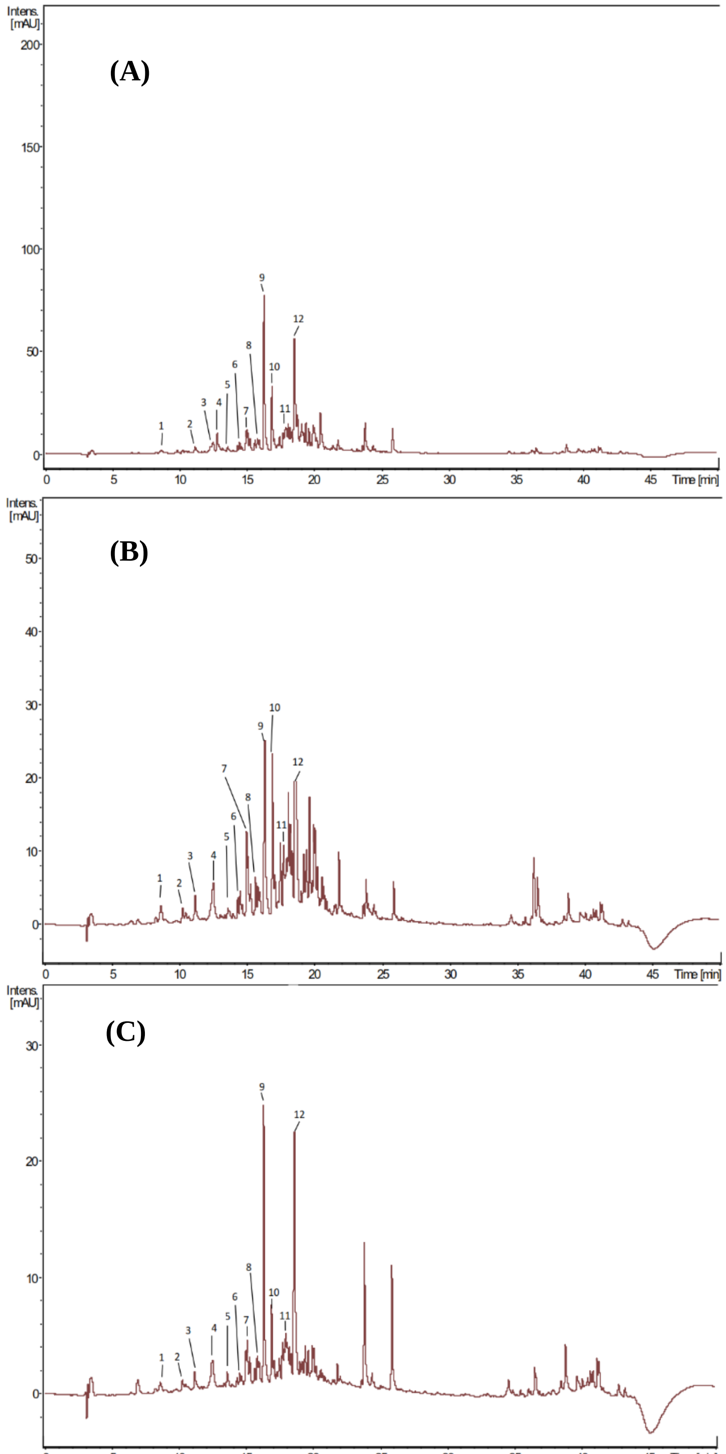

Figure 1. HPLC analysis of isoflavones in (A) Giza 22, (B) Giza 35 and (C) Giza 111

1: daidzin; 2: glycitin; 3: genistin; 4: malonyl daidzin; 5: malonyl glycitin; 6: acetyl daidzin; 7: acetyl glycitin; 8: malonyl genistin; 9: daidzein; 10: glycitein; 11: acetyl genistin; 12: genistein

Table 1. Mean contents of daidzein, glycitein, genistein and total isoflavones of the three studied cultivars

Cultivars	**Content of isoflavone (mg/100 g of soybean)**			
	Daidzein	Glycitein	Genistein	**Total**
Giza 22	201.3	109.4	159.9	**470.6**
Giza 35	143.7	172.8	61.4	**377.9**
Giza 111	88.5	33.4	78.4	**200.3**

The TPC of the three investigated cultivars Giza 22, Giza 35 and Giza 111 was 695, 580 and 525 mg GAE/100 g soybean, respectively. Chung *et al.* (2011) estimated the TPC in Korean soybean and it reached 366 mg/100 g soybean. Moreover, Malenčić *et al.* (2007) published results of TPC of different soybean genotypes from Serbia where total phenolic content ranged from 270 to 488 mg/100 g. Guzmán-Ortiz *et al.* (2017) reported similar TPC values for soybean collected from Mexico at concentration of 548 mg/100 g.

Giza 22, Giza 35 and Giza 111 cultivars showed scavenging capabilities of 79.34, 65.97 and 59.35 %, respectively, while ascorbic acid (1 mg/mL) showed inhibition activity of 91.74 %. The DPPH free radical scavenging test has been widely used for evaluating antioxidant activity of grain and cereal extracts (Yu *et al.*, 2002; Masisi *et al.*, 2016). In this study, the order of the DPPH radical quenching capabilities also was in agreement with their concentration of phenolic content. Similar results were published by Mujić *et al.* (2011) where antioxidant activity of soybeans also correlated well with TPC. The uses of natural antioxidants from plant extracts have experience growing interest due to some human health professionals and consumer’s concern about the safety of synthetic antioxidants in foods (Santos-Sanchez *et al.*, 2017).

Conclusion

The rank of antioxidant activity was Giza 22 > Giza 35 > Giza 111. A greater quantity of isoflavone content, phenolic compounds and antioxidant capacity could be provided by Giza 22 cultivar as compared with Giza 35 and Giza 111 soybeans.

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محتوى الايزوفلافونات والفينولات والفعالية المضادة للأكسدة لثلاث انواع زراعية مصرية لفول الصويا

للسادة الدكاترة

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فول الصويا يعتبر كمحصول زراعي هام على مستوى العالم يستخدم كمصدر لإنتاج الزيت وما يتبقى يستخدم للتغذية. كما أن الوظيفة الطبية لاستخدام فول الصويا هي لمنع كثير من الامراض المزمنة. الهدف من الدراسة تحديد مستوى الايزوفلافونات والفينولات والفعالية المضادة للاكسدة لثلاث أنواع زراعية من فول الصويا جيزة ٢٢ وجيزة ٣٥ وجيزة ١١١. ظهرت فروق واضحة في كميات الجينيستن والديدزين والجليسيتين والمحتوى الكلي الايزوفلافوني نتيجة التأثيرات الجينية بما أن المحاصيل الزراعية تم تجميعها من مكان واحد. المحتوى الكلي الايزوفلافوني تراوح بين ٤٧٠.٦ ملجم / ١٠٠ جم صويا (جيزة ٢٢) الى ٢٠٠.٣ ملجم / ١٠٠ جم صويا (جيزة ١١١)، ترتيب المحتوى الكلي الفلافوني كان جيزة ٢٢ > جيزة ٣٥ > جيزة ١١١. بتركيز ١ ملجم / مل الفعالية المضادة للأكسدة كانت ٧٩.٣٤ و٦٥.٩٧ و٥٩.٣٥ ٪ لمستخلص جيزة ٢٢ وجيزة ٣٥ وجيزة ١١١ على الترتيب. الدراسة الحالية تمثل أهمية كبرى لمعرفة أي الانواع الزراعية أفضل بأعلى محتوى ايزوفلافوني من اجل الاستخدام كمستحضر دوائي غذائي بخلاف كونه مصدر للبروتين الغذائي.