

Effect of soybean cultivars on the content of isoflavones in soymilk

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Abstract

The aim was to study the relationship between the isoflavone content in different soybean cultivars and in the soymilk. Seven cultivars of soybean seeds from two locations with different levels of isoflavones were processed to soymilk. The content of total and individual isoflavones was determined by high performance liquid chromatography. The total phenolic content, oil and protein content in soybean cultivars were also determined. Significant differences in the content of individual isoflavones were observed within the soybean cultivars. The strong positive correlations were observed between total isoflavone content in soybean seeds and in soymilk. The total phenolic content in soybean cultivars ranged from 83 to 143.4 mg GAE/100g of soybean. The total isoflavone content in soybean seeds ranged from 71.2 to 133.8 mg/100g of soybean which comprise from 76.7 to 98.8% of the total phenols. In soymilks, total isoflavone content ranged from 16.1 to 61.0 mg/kg of soymilk. The most abundant isoflavone in soybean seeds was genistein while in soymilk it was genistin. There was statistically significant difference ($p < 0.05$) among two locations in total and individual isoflavone contents.

Key words: isoflavone, soybean cultivar, soymilk, total phenolic content, HPLC.

Introduction

Functional food is the most promising field of nutritional science. This food is interesting from the consumer point of view with the prospect of maintaining health and preventing diseases using natural food as part of a regular diet. There are several raw materials that can be used for healthy purposes and soybeans are among those with the greatest potential [1]. The soybean seed is truly unique, as it is the only food to contain nutritionally significant amounts of isoflavones, a well-studied group of phytoestrogens with numerous biological effects [2]. Interest in soy isoflavones is based on data suggesting potential of isoflavones in lowering cholesterol levels, preventing prostate and breast cancers, osteoporosis, cardiovascular disease as well as relieving menopausal symptoms [3-5]. The isoflavone content in soybeans comprise about 72% of the total phenols [6] and are significantly affected by cultivar and environmental factors [7-8]. Genistein and daidzein are the isoflavones that can be found in the highest percentage in food, in the form of conjugated glycosides [9].

With the increasing interest in the potential health benefits of soy isoflavones, knowledge of the effect of thermal processing on the isoflavone content in soymilk is important [10]. Soy products should be made rich in genistein and daidzein according to their

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antioxidant activity [11]. Since the biological effect of two most important aglycones genistein and daidzein are different [12], the distribution of isoflavone in soybean as well in soymilk is important factor, influencing the functional value of soybeans and soy food [11].

The objective of this study was to compare the content of isoflavones in seven soybean cultivars from two different locations and to determine the total and individual isoflavone content of produced soymilks. Furthermore, the total phenolic content, protein and oil content of soybean cultivars were determined as well.

Material and methods

Material

The analysis was performed on seven soybean cultivars: “Sabina”, “Ružica”, “Buga”, “Hrvatica”, supplied from Bc INSTITUT d.d. Zagreb, and three soybean cultivars “os497-97“, “os49-01“, “os36-03“ from Agriculture Institut Osijek in 2008. The soybeans were hand-selected to eliminate those that were cracked or otherwise damaged. The soybeans were analyzed in triplicate for oil and protein content according to the standard methods. Oil was determined by solvent extraction [13] and protein by the Kjeldahl method [14].

Chemicals

Glycoside standards of daidzin, genistin, glycitin, aglycone standards of daidzein and glycitein, and rutin, used as an internal standard, were purchased from Sigma-Aldrich (Steinheim, Germany). Standard of genistein was supplied from Riedel de Haen[®] (Castle Hill, N.S.W., Australia). Genistin, genistein, and daidzein were prepared in HPLC grade methanol, and daidzin, glycitin, and glycitein in ethanol, as they varied in solubility characteristics.

Determination of isoflavone in soybeans

The soybean samples (15 g) were powdered, 5 mL of internal standard (rutin in MeOH), and 50 mL of 80% MeOH were added. The mixtures were sonicated on an ultrasonic bath for 3h at room temperature. The mixture was then filtered through a Whatman No. 1 filter paper, and the volume was reduced to 5 mL under a stream of nitrogen. This was filtered over 0.45 μm syringe cellulose filter, and transferred into HPLC vials. HPLC analysis of the extracts was performed using Agilent 1200 series HPLC with RR Zorbax SB-C18 column (3.5 μm , 30 x 2.1 mm). Mobile phase A was 0.2% formic acid in water, and mobile phase B was acetonitrile. The injection volume was 1 μL , and elution at 0.45 mL/min with gradient program (0-1.24 min 2% B, 1.24-3.70 min 2-29% B, 3.70-8.00 min 29-30% B, 8.00-9.00 min 30-98% B, 9.00-10.00 min 2% B). UV detection was carried out at 260 nm. Four mixed standards containing all six analyzed isoflavones were used for quantification. Rutin (internal standard) was added to each isoflavone standard at a concentration of 25 $\mu\text{g/mL}$. Single standards were also prepared for peak identification. Isoflavone concentrations were calculated as mg of isoflavones per 100 g of soybean. All measurements were conducted in triplicate [15].

Production of soymilk

Soymilk was prepared using different soybean cultivars. For each batch of soymilk, 100 g of dry soybeans were soaked overnight in cold water (soybean : water ratio 1:7, w/v). After 18 h of soaking, the hulls from soybeans were removed manually. After that, the soybeans were well rinsed with water and then immersed in a solution of 0.5% NaHCO_3 . The temperature of 0.5% NaHCO_3 solution was 90 °C. A certain amount of soybeans was left to stand for 30 min in a solution and then washed with cold water. The device for making soymilk (Soylove-Soy Maker, Korea) was filled with 1700 mL cold water, and in filtering barrel were added soaked

and washed soybean. The soymilk production was taken from 28-30 min and process involved grinding of soybeans, extraction with hot water, pressing and filtering.

Determination of isoflavone in soymilk

In 2 g of soymilk sample were added 8 mL MeOH and 0.2 mL of internal standard rutin (C=0.2 mg/mL). The mixtures were sonicated on ultrasonic bath for 30 min at room temperature. The mixture was then filtered through econofilter 25/0.45 microm RC, and transferred into HPLC vials. HPLC analysis was performed on the same way as for the soybean seeds. All measurements were conducted in triplicate.

Total phenolic content in soybeans (TPC)

The soybean samples (about 15 g) were powdered, and 1 g of powder was added in 10 ml of 80% MeOH. The mixtures were sonicated on an ultrasonic bath for 3h at room temperature. This was filtered over 0.45 μ m syringe cellulose filter. Total phenolic content of the soybean extracts was determined using Folin-Ciocalteu reagent. An aliquot (200 μ l) of extract was mixed with 1.6 ml of Folin-Ciocalteu reagent and 0.8 ml of 7.5% sodium carbonate. The absorbance was measured at 740 nm against distilled water as blank after incubation for 120 min at room temperature. Total phenolic content was expressed as gallic acid equivalents (mg GAE/100g of soybean) through standard calibration curve of freshly prepared gallic acid. All measurements were conducted in triplicate [16].

Statistical analysis

One-way analysis of variance (ANOVA) and multiple comparisons (Duncan's *post-hoc* test) were used to evaluate the significant difference of the data at $p < 0.05$. Data were expressed as means \pm standard deviation.

Results and discussion

Data for total phenolic content (TPC) of seven soybean cultivars are presented in Table 1. The maturity group of each soybean cultivar is presented as well. Within each group of soybean, cultivar variation for total phenolic content was significant ($p < 0.05$). Among all the cultivars analysed, the highest value of TPC was observed in cultivar „os36-03“ (143.4 mg GAE/100g of soybean) and the lowest in cultivar „Ružica“ (83 mg GAE/100g of soybean). Kumar *et al.* [17] published average value of total phenol content in Indian yellow soybeans to be 1.04 mg GAE/g of soybean. Soybean cultivars from Agriculture Institut Osijek, exhibited higher values for TPC than soybean cultivar from Bc INSTITUT d.d. Zagreb.

Table 1. Total phenolic content in soybean cultivars

Soybean cultivar	Location	Maturity group	Total phenolic content (mg GAE/100g of soybean)
Ružica	Rugvica	0	83.0 \pm 0.6 ^a
Buga	Rugvica	0	127.9 \pm 0.9 ^b
Hrvatica	Rugvica	0	115.2 \pm 0.3 ^c
Sabina	Rugvica	00	116.6 \pm 1.0 ^d
os497-97	Osijek	0	97.8 \pm 0.7 ^e
os49-01	Osijek	0-I	139.2 \pm 1.0 ^f
os36-03	Osijek	00	143.4 \pm 0.6 ^g

Data are expressed as mean value of replication (n) \pm SD (standard deviation);

The same letter in the same column indicates no significant differences (Duncan's test, $p < 0.05$)

There were significant differences ($p < 0.05$) between locations for protein and oil contents (Table 2). Soybean cultivars „os36-03“ and „os49-01“ had the highest mean protein content, whereas cultivar „os36-03“ had the highest oil content. We attribute differences in protein and oil content between the two locations, where soybean cultivars from location Osijek has much higher oil content compared to soybean cultivars from location Rugvica.

Table 2. Oil and protein content in soybean cultivars

Soybean cultivar	Oil content (%)	Protein (%)
Ružica	17.07 ^a	34.09 ^a
Buga	17.01 ^a	34.08 ^a
Hrvatica	18.06 ^b	35.04 ^b
Sabina	15.06 ^c	37.06 ^c
os497-97	21.55 ^d	39.83 ^d
os49-01	21.5 ^d	40.4 ^e
os36-03	21.73 ^d	40.4 ^e

The same letter in the same column indicates no significant differences (Duncan's test, $p < 0.05$)

This could be explained with the dissimilarity in temperatures during the growing season [18,19]. Protein content in soybeans cultivars from Bc INSTITUT d.d. Zagreb is negatively correlated to seed oil. It has been well documented [20,21] that seed protein content negatively correlated with oil. High negative correlation between protein and oil content in four soybean cultivars developed at the Institute of Field and Vegetable Crops in Novi Sad was also published by Miladinović *et al.* [22]. Variation in both oil and protein content were relatively negligible in soybean cultivars from Agriculture Institut Osijek.

The HPLC chromatograms of soybean cultivar „Hrvatica“ and soymilk produced from the same cultivar are shown in Fig. 1. By comparison with standard daidzin, glycitin, genistin, daidzein, genistein, and glycitein, these isoflavones were detected at retention times of 2.33, 2.39, 2.64, 2.71, 2.96 and 3.03 min, respectively. Retention time for rutin (internal standard) was 2.53 min. Other peaks in the chromatogram could be due to the conjugated forms of soy isoflavones, which could not be identified in this study owing to the lack of standards.

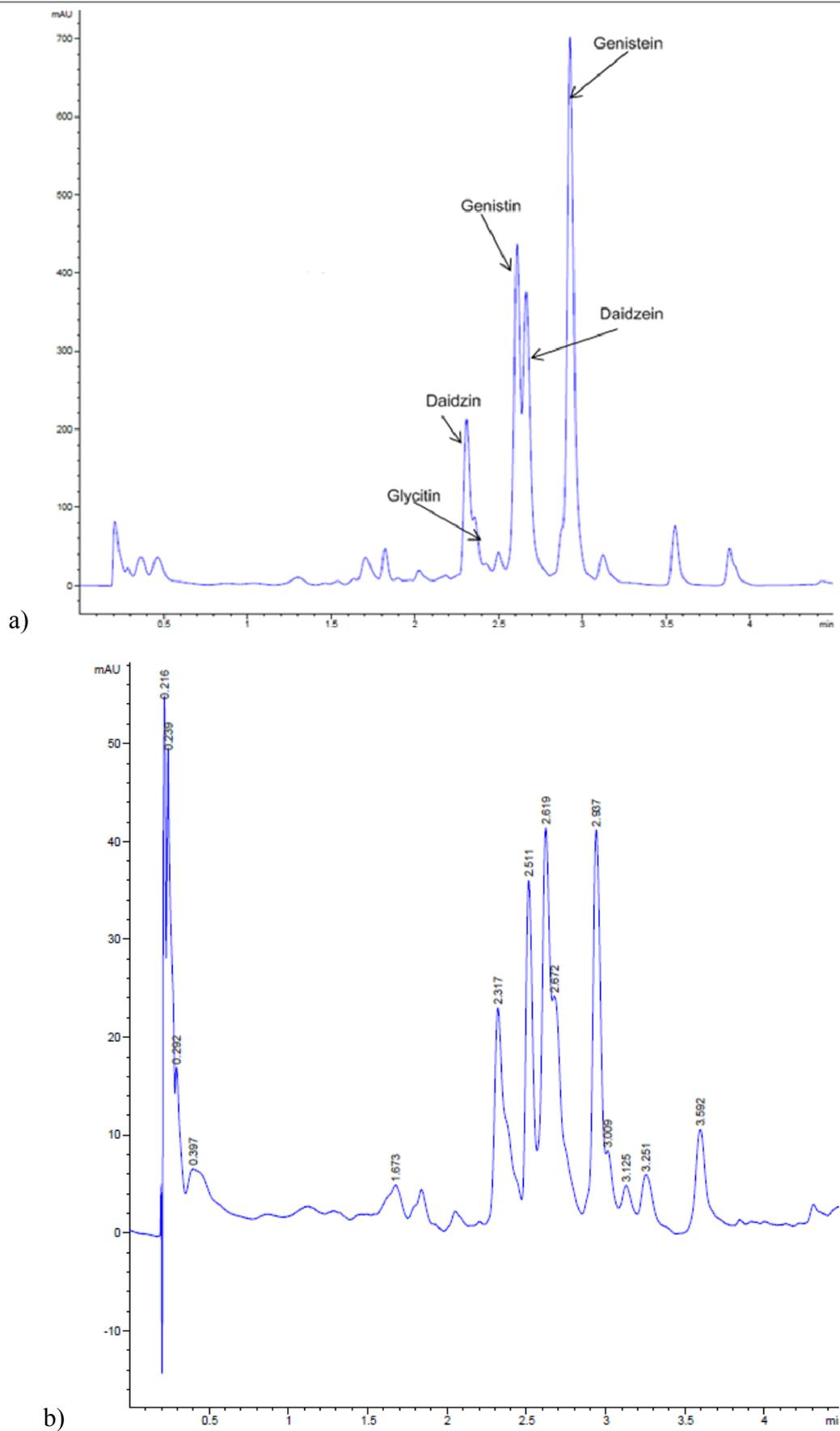


Figure 1. HPLC Chromatogram of isoflavone in (a) soybean cultivar „Hrvatica“ (b) soymilk

The total and individual isoflavone content of different soybean cultivars is presented in Table 3. The composite values for six isoflavones, namely daidzin, genistin, glycitin, daidzein, genistein, and glycitein were analyzed and expressed as total isoflavone content.

Table 3. Isoflavone content of soybean cultivars

Soybean cultivar	Content of isoflavone (mg/100g of soybean)					
	Daidzin	Glycitin	Genistin	Daidzein	Genistein	Total
Ružica	14.2 ± 0.3 ^a	5.7 ± 0.1 ^a	12.3 ± 0.2 ^a	21.9 ± 0.5 ^a	17.2 ± 0.5 ^a	71.2
Buga	17.2 ± 0.3 ^b	7.7 ± 0.2 ^b	20.2 ± 0.4 ^b	27.2 ± 0.5 ^b	27.8 ± 0.5 ^b	100.0
Hrvatica	18.8 ± 0.4 ^b	7.0 ± 0.1 ^c	28.7 ± 0.6 ^c	25.6 ± 0.5 ^c	32.9 ± 0.6 ^c	112.9
Sabina	20.9 ± 0.4 ^c	7.7 ± 0.2 ^b	38.4 ± 0.7 ^d	20.1 ± 0.4 ^d	28.2 ± 0.6 ^b	115.2
os497-97	10.5 ± 0.2 ^d	5.9 ± 0.1 ^a	15.5 ± 0.3 ^e	26.9 ± 0.5 ^b	35.7 ± 0.6 ^d	94.5
os49-01	12.6 ± 0.3 ^a	6.2 ± 0.1 ^d	15.7 ± 0.3 ^e	34.9 ± 0.6 ^e	37.4 ± 0.6 ^e	106.8
os36-03	13.7 ± 0.3 ^a	5.9 ± 0.1 ^a	24.3 ± 0.5 ^f	34.5 ± 0.7 ^e	55.5 ± 0.9 ^f	133.8

Data are expressed as mean value of replication (n) ± SD (standard deviation);

The same letter in the same column indicates no significant differences (Duncan's test, $p < 0.05$)

The total isoflavone content in soybean cultivar was in range from 71.2 to 133.8 mg/100g of soybean. Wang and Murphy [23] reported total isoflavone contents from 1176 to 3309 µg/g across years. In soybean cultivars grown in Canada total isoflavone contents ranged from 360 to 2241 µg/g [8]. Isoflavone content of soybeans in Asia varied from 699.7 to 2581.6 µg/g with cropping year [24]. Devi *et al.* [11] reported total isoflavone content of Indian soybean cultivars from 525 to 986 mg/kg. There were statistically significant differences among the seven soybean cultivars. „os36-03“ cultivar had the highest isoflavone content (133.8 mg/100g of soybean), while „Ružica“ had the lowest (71.2 mg/100g of soybean). Glycitein content in soybean seeds was not reported because it was below limit of detection. There was significant difference among two locations in total and individual isoflavone contents. Wang and Murphy [23] and Hoeck *et al.* [7] also published the differences between isoflavone content among locations within the same year. Seguin *et al.* [8] published that isoflavone content were significantly affected by cultivar, year and location. It can be also noticed that the most abundant isoflavone in soybean cultivars was genistein followed by daidzein. The genistein series has gained most attention in isoflavone research because of its potential positive effects on health [25]. Highly significant positive correlations were observed between genistein content and total isoflavone content ($R^2 = 0.828$). Seguin *et al.* [8] also observed significant positive correlation between individual and total isoflavone. TPC correlated well with total isoflavone content ($R^2 = 0.818$) as expected [11].

The total and individual isoflavone content of soymilk produced from different soybean cultivars is presented in Table 4.

Table 4. Isoflavone content in soymilk

Soybean cultivar	Content of isoflavone (mg/kg of soy milk)						Total
	Daidzin	Glycitin	Genistin	Daidzein	Genistein	Glycitein	
Ružica	3.8 ± 0.2 ^a	1.1 ± 0.1 ^a	3.8 ± 0.1 ^a	2.6 ± 0.1 ^a	3.7 ± 0.1 ^a	1.0 ± 0.1 ^a	16.1
Buga	6.8 ± 0.1 ^b	2.6 ± 0.1 ^b	7.2 ± 0.1 ^b	3.2 ± 0.1 ^b	4.8 ± 0.2 ^b	1.3 ± 0.1 ^b	25.7
Hrvatica	6.3 ± 0.1 ^c	2.7 ± 0.1 ^b	7.3 ± 0.1 ^b	4.2 ± 0.1 ^c	7.1 ± 0.1 ^c	2.3 ± 0.1 ^c	29.8
Sabina	7.0 ± 0.1 ^b	4.0 ± 0.1 ^c	11.4 ± 0.1 ^c	4.6 ± 0.1 ^d	7.9 ± 0.1 ^d	3.2 ± 0.1 ^d	38.1
os497-97	5.4 ± 0.2 ^d	2.1 ± 0.1 ^d	6.6 ± 0.2 ^d	3.7 ± 0.2 ^e	6.4 ± 0.2 ^e	1.5 ± 0.1 ^e	25.8
os49-01	9.3 ± 0.3 ^e	4.5 ± 0.1 ^e	9.4 ± 0.3 ^e	5.8 ± 0.3 ^f	7.0 ± 0.3 ^c	2.8 ± 0.1 ^f	38.8
os36-03	12.7 ± 0.2 ^f	3.7 ± 0.1 ^f	20.6 ± 0.3 ^f	7.7 ± 0.2 ^g	13.3 ± 0.3 ^f	2.9 ± 0.1 ^f	61.0

Data are expressed as mean value of replication (n) ± SD (standard deviation);

The same letter in the same column indicates no significant differences (Duncan's test, $p < 0.05$)

The total isoflavone content in different soymilks was in range from 16.1 to 61.0 mg/kg of soymilk. Isoflavone content in India soymilk was 135 mg/kg of soymilk [11]; in Singapore soymilk ranged from 76 to 199 µg/g [26]; in homemade soymilk from 1574 to 2567 µg according to different soaking, grinding and cooking methods for preparing soymilk [27]. Differences exist according to different soybean cultivars, cropping year, locations as well the method of soymilk production. The most abundant isoflavone in soymilks was genistin. It can be observed that the content of isoflavones in soybean seeds was significantly higher compared to their content in soymilk. Generally, the processed products showed lower levels of isoflavones extracted from the raw product, and the reason for this is soaking and heating processing which caused significant losses of isoflavones [10,12,27] as well as water to soybean ratio, grinding and separation methods, coagulation conditions [21]. The individual isoflavone was destroyed during the processing of soymilk due to the thermal degradation of isoflavone content. Furthermore, glycitein content in soybean seeds were below limit of detection, while in soymilk glycitein content was detected. Kao *et al.* [28] observed that the concentration of aglycone glycitein increased with increasing soaking temperature and time, probably because of conversion from glycitin. The content of isoflavones in soymilk was higher in seeds with higher protein content and such soybean cultivars are better for the production of soymilk.

The total isoflavone content positively correlated ($R^2 = 0.824$) with the total isoflavone content in soymilk (Fig. 2). The strong positive correlation ($R^2 = 0.993$) of total isoflavone content between soybean and soymilk was also observed by Hiroshi *et al.* [29].

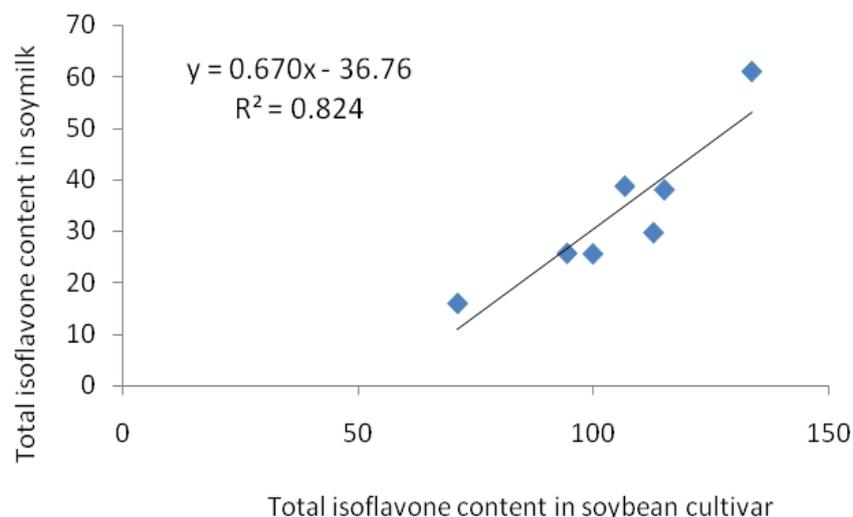


Figure 2. Correlation of total isoflavone content between soybean cultivar and soymilk

Conclusions

This study evaluated the content of individual and total isoflavones in different soybean cultivars and in soymilk. Significant difference was observed in the content of individual and total isoflavone in soybean cultivars and soymilk from two locations. The strong positive correlation of total isoflavone content between soybeans and soymilk was observed. From results in this study it is evident that soaking, grinding and heating decreased the total isoflavone content during the soymilk production. The content of isoflavones in soymilk was higher in soybean cultivars with higher protein content and these cultivars are more suitable for soymilk production. It can be concluded that isoflavones are associated with proteins, and the losses of isoflavones in soymilk may be due to protein-associated isoflavones being released into the soymilk. Further understanding the relationships of the content of isoflavones, oil, and protein with soybean cultivars is essential to soybean growers.

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