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Hydroponic Cultivation Improves the Nutritional Quality of Soybean and Its Products

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ABSTRACT: Hydroponic cultivation allows the control of environmental conditions, saves irrigation water, increases productivity, and prevents plant infections. The use of this technique for large commodities such as soybean is not a relevant issue on fertile soils, but hydroponic soybean cultivation could provide proteins and oil in adverse environmental conditions. In this paper, the compositions of four cultivars of soybean seeds and their derivates, soy milk and okara, grown hydroponically were compared to that of the same cultivar obtained from soil cultivation in an open field. Besides proximal composition, the concentrations of phytic acid and isoflavones were monitored in the seeds, soy milk, and okara. Results demonstrated that, independent from the cultivar, hydroponic compared to soil cultivation promoted the accumulation of fats (from 17.37 to 21.94 g/100 g dry matter) and total dietary fiber (from 21.67 to 28.46 g/100 g dry matter) and reduced isoflavones concentration (from 17.04 to 7.66 mg/kg dry matter), whereas protein concentration was unaffected. The differences found in seed composition were confirmed in the respective okara products, but the effect of cultivation system was not significant looking at the soy milk composition. Data showed that hydroponic cultivation improved the nutritional quality of soybean seeds with regard to fats and dietary fiber. They also suggest that specific cultivars should be selected to obtain the desired nutritional features of the soybean raw material depending on its final destination.

KEYWORDS: *Glycine max (L.) Merr., seeds, soy milk, okara, isoflavones, phytic acid*

INTRODUCTION

Hydroponic cultivation is an emerging technology that allows a better control of water and nutrient supply, improves plant productivity, avoids the need for crop rotation, and reduces the use of pesticides.[1] Hydroponic methods are particularly useful in regions where the soil or climate is not suitable for crop cultivation. As the population increases and arable land declines, hydroponics could replace traditional agriculture. There has already been a great deal of talk in the scientific community for the potential use of hydroponics in Third World areas, and this technique will be important in providing fresh food in space programs for long-term colonization of Mars or the moon.[2]

In these contexts also the cultivation of high nutritional density crops, such as soybean, will be considered. Soybean seeds [*Glycine max* (L.) Merr.] are an important source of protein and oil and contain high amounts of components with health benefits, such as dietary fiber and other biologically active substances such as isoflavones. Despite several health properties, the nutritional value of soybean and soy-based meals is lower than expected: this is due to the presence of different compounds usually known as antinutritional factor, which reduce nutrient availability. The main one is phytic acid, the storage form of phosphorus in seeds, acting as an antinutrient as it chelates various metals (Fe^{2+}, Ca^{2+}, Mg^{2+}, Zn^{2+}) and binds to some proteins, resulting in an overall decrease of protein and mineral bioavailability.[3] On the other hand, many recent studies have suggested that phytate also may have many positive effects, which can be considered more prominent than the reduction of mineral bioavailability for the majority of consumers.[4]

Soybean seeds provide several transformed products with multiple gastronomic uses, particularly in vegetarian nutrition. Soy milk is one of the most popular: it is a water extract of soybean seeds, resembling animal milk in physical appearance. Because of its nutritional profile, it is used as an animal milk complement or substitute. Soy milk is not only a nutritious food but also a product made with a simple process involving a relatively low level of technology. The coproduct of soy milk extraction, named okara or soy pulp, is rich in dietary fiber (50−60%), protein, and fat and contains significant levels of B-group vitamins.[5,6] Okara is used in both animal and human nutrition and also to partially replace wheat flour for breadmaking and as a fermentation stock for the production of seasonings, spices, and tempeh.[7] Recently it has been also used as a functional dietary additive in biscuits and snacks.[6]

Genetic factor and growth environmental conditions have a great impact on the chemical composition and nutrient quality of soybean seeds. Protein content can range from 30 to 44%[8−10] and oil amount from 15 to 22%,[9,11,12] depending on soybean cultivar and soil or climate characteristics. Grieshop and Fahey[13] showed that dietary fiber content differed among cultivars from 11 to 19%, but not among countries of

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cultivation. The isoflavones content of different soybean cultivars is extremely variable, ranging from 64 to 459 mg/100 g of dry matter of seed weight, depending on genetic factors, sowing conditions, geographic location, and temperature during cultivation.[14−17] Also, phytic acid content of soybeans can vary considerably; field-type cultivars, which are the usual items of commerce, fall into the range of 1.0−4.5%.[9,18,19]

Many potential health benefits of isoflavones from soy products have been investigated, particularly on breast cancer, vascular disease, osteoporosis, and menopausal symptoms; however, they can also have negative effects, for instance, on the reproductive system or during lactation.[20]

The aim of this study was to compare the nutritional composition of soybean seeds and of the derived products soy milk and okara in commercial seeds of four soybean cultivars obtained by a traditional cultivation system in soil (open field) and by cultivation in a hydroponic system (Nutrient Film Technique (NFT)).

■ MATERIALS AND METHODS

Plant Materials. Commercial seeds of four soybean cultivars ('Atlantic', 'Cresir', 'Pr91m10', and 'Regir'), field grown in the year 2009, were obtained from Italian sellers (Venturoli Sementi Srl for 'Atlantic' seeds and Pioneer Hi-Bred Italia Srl for 'Cresir', 'Pr91m10', and 'Regir' seeds). Cultivars were selected among not genetically modified varieties admitted for cultivation in the European Union. They were chosen on the basis of agronomical and technological properties (yield, tolerance to biological and abiotic stresses, suitability to industrial uses). Seeds were analyzed and used for soy milk extraction and okara production.

Hydroponic Cultivation. The same varieties above-described were cultivated under hydroponic conditions. The experiment was carried out in a growth chamber with a controlled environment. Light was provided by high intensity discharge lamps (400 W) with a light/dark regimen of 12/12 h. Temperature regimen was established at 26/20 °C (light/dark), and relative humidity was kept within the optimum range of 65−75% using a fog system; air change and dehumidification were guaranteed by two air extractors. Plants were grown in plastic double gullies using a recirculating NFT. The nutrient solution was based on the standard Hoagland recipe half-strength modified by Wheeler et al.,[21] according to the specific requirements of soybean. Electrical conductivity and pH were kept at 2.0 dS m^{-1} and 5.8, respectively, and adjusted every 2 days. Soybean pods were harvested twice a week, starting from 114 days after soaking, when pods had turned a brown color. The obtained seeds were analyzed and used for soy milk extraction.

Soy Milk and Okara Preparation. Figure 1 summarizes the procedure to obtain soy milk and okara. Soybeans were soaked in water (ratio 1:10 weight/volume) at room temperature for 24 h, and soaked soybeans were milled with the same amount of water. The obtained paste was boiled for 30 min and the material filtered to separate soy milk from okara. The last step was soy milk sanitization (15 min boiling). Milk extraction was performed twice for each cultivar.

Proximate Composition. Each sample of seed, soy milk, and okara was freeze-dried, blended, and homogenized by grinding to a fine powder, so results for each analysis were expressed on a dry basis. Fat content was measured by extraction with diethyl ether in a Soxhlet system.[22] Proteins were analyzed as total nitrogen content by the Kjeldahl procedure,[23] and the conversion factor used to transform nitrogen into protein was 5.71. Total fiber content was determined by using the AOAC 985.29 gravimetric method.[24] All compositional determinations were performed three times for each sample.

Phytic Acid Determination. The phytic acid content was measured by Ishiguro and others' method based on phytic acid precipitation as ferric phytate;[25] to extract phytic acid from seeds and

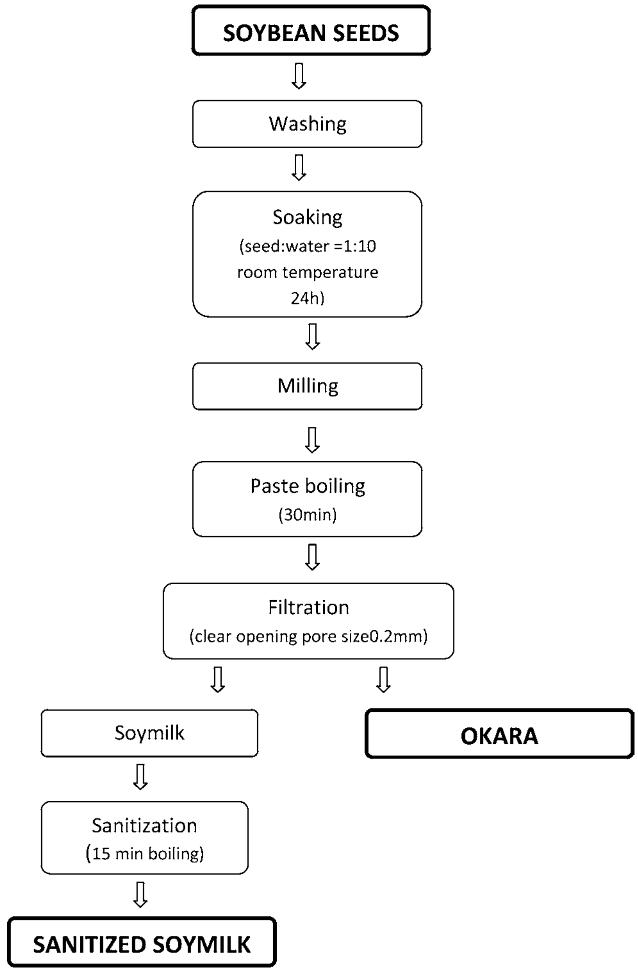

Figure 1. Experimental procedure to obtain soy milk and okara.

from okara, 0.5 g of milled product was mixed in 10 mL of 0.5 M HCl and shaken for 1 h at room temperature, and then the mixture was centrifuged for 6 min at 18000*g*, and supernatant was analyzed. For soy milk samples, an extraction step was not necessary. Phytic acid determination was performed three times for each sample.

Isoflavones Analysis. One gram of materials was extracted by 30 mL of methanol/water (70:30, v/v) and sonicated at room temperature for 30 min. The extraction procedure was repeated twice for each sample. The mixtures were centrifuged at 2500*g*, filtered through a Whatman filter paper, and then used for LC-MS/MS analysis using a method previously described.[26] Chromatographic separation was performed using an HPLC apparatus equipped with two micropumps series 200 (PerkinElmer, Shelton, CT), a UV−vis series 200 (PerkinElmer) detector set at 280 nm, and a Prodigy ODS3 100 Å column (250 mm × 4.6 mm, particle size = 5 μm) (Phenomenex, Torrance, CA). The eluents were (A) water containing 0.2% formic acid and (B) acetonitrile/methanol (60:40, v/v). The gradient program was as follows: 20−30% B (6 min), 30−40% B (10 min), 40−50% B (8 min), 50−90% B (8 min), 90−90% B (3 min), and 90−20% B (3 min) at a constant flow of 0.8 mL/min. The LC flow was split, and 0.2 mL/min was sent to the mass spectrometer. The injection volume was 20 μL. Two injections were performed for each sample. MS and MS/MS analyses of soybean extracts were performed on an API 3000 triple-quadrupole mass spectrometer (Applied Biosystems, Canada) equipped with a TurboIonSpray source working in the negative ion mode. Six glucosides (daidzin, genistin, glycitin, acetyl-genistin, malonyl-daidzin, malonyl-genistin) and only one aglycone (genistein) were identified: LC-MS/MS conditions of identified isoflavones are shown in Table 1. After peak identification, the isoflavones quantification was performed by HPLC as follows:

Table 1. LC-MS/MS Conditions for the Detection of Soybean Isoflavones

compound	precursor ion $[M - H^+]$ (m/z)	product ion
genistein	271	243
		215
		187
daidzin	417	199
		255
genistin	433	215
		271
glycitin	447	285
acetyl-genistin	475	431
		417
malonyl-daidzin	503	417
malonyl-genistin	519	433

filtered extract (20 μL) was injected into an HPLC (Shimadzu LC 10, Shimadzu, Kyoto, Japan) with a photodiode array detector. Separations were achieved on the same column with the same gradient program. The flow rate was 0.8 mL/min, and chromatograms were recorded at 280 nm. Isoflavones were quantified using, as external standard, genistein and genistin for all glucosides. Genistein stock solution was prepared by dissolving standard (Sigma-Aldrich) in methanol, whereas genistin stock solution was prepared by dissolving standard (Sigma-Aldrich) in a mixture of methanol/water (70:30, v/v).

Statistical Analysis. Differences among cultivars were determined by analysis of variance and Duncan's multiple-range test ($P \leq 0.05$). Differences between field-grown and hydroponically grown samples were determined by Student's t test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Proximate Composition. Figure 2 summarizes the variations in proximate composition of soybean seeds determined by cultivation method (hydroponic vs soil). The data shown are the average of the four different cultivars and, despite the variability among cultivars, results are remarkable: hydroponic cultivation in a controlled environment increased fats, total dietary fiber, and phytic acid and reduced the amount of isoflavones compared to soil cultivation in an open field.

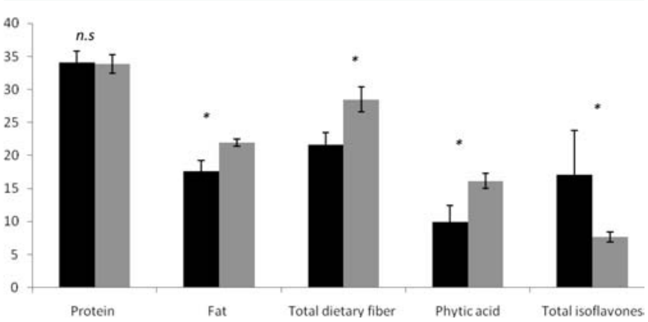

Figure 2. Protein content (%/dry mass), fat content (%/dry mass), total dietary fiber content (%/dry mass), phytic acid content (g/kg dry matter), and total isoflavones content (mg/kg dry matter) in seeds from field-grown (black bars) and in hydroponic grow (gray bars) soybean plants. Data are the mean values of the four analyzed cultivars ± SD. "*" and "n.s." indicate significant and not significant differences between cultivation systems at $P < 0.05$.

The method of cultivation did not affect protein concentration when data of the four cultivars were averaged; however, when results for each cultivar are considered, this is the case only for 'Pr91m10' and 'Regir'. Conversely, protein concentration in hydroponic cultivation was higher ($P \leq 0.05$) in 'Atlantic', whereas it was lower ($P \leq 0.05$) in 'Cresir' compared to seed from field-grown plants (see Table 2). According to Dornbos and Mullen,[27] the higher water availability reduces protein accumulation in soybean seeds, but studies on different pulses, such as peanuts, report that protein concentrations in the seeds harvested from hydroponic system and field cultivation are not different.[33]

The protein concentrations in the seed parallel those observed in soy milk and okara: products from field-grown and hydroponically grown plants did not show differences in 'Pr91m10' and 'Regir', whereas they gave higher values in NTF for 'Atlantic' and in soil for 'Cresir'. Protein content ranges from 35.39 to 39.60 g/100 g in soy milk and from 31.60 to 43.25 g/100 g in okara, confirming previous results reported by other authors for the same products.[4,28−30]

Values of fat content in soybean seeds fall within the range reported in the literature, with no relevant difference among the cultivars (see Table 2). However, it is worth noting that hydroponic cultivation caused an increase in fat content from 17.37 to 21.94 g/100, on the average of the tested cultivars; according to Dornbos and Mullen,[27] this increase can be due to the constant water availability favoring oil accumulation in soybean. Fat contents in soy milk and okara (Table 2) are consistent with previous works.[4,28−30] In agreement with Cai et al.[31] and Mullin et al.,[32] cultivar selection did not affect lipid content in soy milk as it did in okara. This distribution of fats in soybean products is not surprising as fat extractability in water is limited independent from the amount of fats in the starting material. At the same time data from hydroponics showed that the higher the fat content in seeds, the higher the fat content in okara.

Hydroponic cultivation increased dietary fiber content in soybean seeds from 21.67 to 28.46 g/100 g (mean values among the four analyzed cultivars); according to previous works, significant differences among cultivars were detected, and 'Regir' seeds showed the highest value (27.56 g/100 g on average between the two cultivation systems). Dietary fiber content values in okara samples fall within the range reported in the literature, and the same cultivation effect observed in seeds was found in okara.[31]

Phytic acid content was significantly higher in seeds from the hydroponic system than from the field (see Figure 2). This could be due to the better availability of phosphorus in the nutrient solution; in fact, in open-field cultivation, phytic acid concentration has been demonstrated to be positively correlated to the available levels of phosphorus in the soil.[34] The same trend was observed for phytic acid content in okara but not in soy milk, with higher concentration in okara from hydroponics soybean (1.51 vs 1.16 g/100 g dry mass in open field; average of the four cultivars). Significant differences ($P \leq 0.05$) among cultivars were found in both processing products. Omosaiye and Cheryan[35] and Beleia et al.[36] reported slightly different distributions of phytic acid between milk and pulp (1.68 g/100 g dry matter in soy milk and 1.83 g/100 g dry matter in okara, respectively); however, they analyzed market products and not simultaneous experimental production of milk and okara.

Table 2. Proximate Composition and Phytic Acid and Total Isoflavones Content of Field-Grown and Hydroponically Grown Soybean Cultivars and Derivate Products[a]

cultivar	seeds field	seeds hydroponic		soy milk field	soy milk hydroponic		okara field	okara hydroponic	
Protein Content (g/100 g Dry Matter)									
Atlantic	32.48 b	33.92 b	*	35.63 b	37.79 a	*	34.65 b	35.83 a	*
Cresir	35.95 a	34.11 b	*	39.60 a	36.81 ab	*	43.25 a	32.04 bc	*
PR91M10	35.27 a	35.55 a	ns	37.98 a	36.31 ab	ns	33.60 b	33.12 b	ns
Regir	32.52 b	31.96 c	ns	36.25 b	35.39 b	ns	32.86 b	31.60 c	ns
Fat Content (g/100 g Dry Matter)									
Atlantic	17.60 a	21.96 ab	*	18.13 a	18.24 a	ns	11.86 a	19.97 ab	*
Cresir	19.28 a	22.09 a	*	19.09 a	17.06 a	ns	9.45 b	20.98 a	*
PR91M10	16.70 a	21.19 a	*	15.22 a	16.96 a	ns	9.40 a	19.11 b	*
Regir	16.99 a	22.50 b	*	18.90 a	18.12 a	ns	12.59 b	20.80 a	*
Total Dietary Fiber (g/100 g Dry Matter)									
Atlantic	21.65 a	27.51 b	*	nd	nd		50.11 a	64.84 a	*
Cresir	19.26 b	27.32 b	*	nd	nd		42.91 b	55.10 c	*
PR91M10	22.10 a	27.57 b	*	nd	nd		48.78 a	57.61 b	*
Regir	23.68 a	31.44 a	*	nd	nd		50.92 a	65.35 a	*
Phytic Acid Content (g/kg Dry Matter)									
Atlantic	14.04 a	15.71 a	*	14.80 a	16.63 a	ns	12.66 a	15.39 a	*
Cresir	11.47 ab	16.25 a	*	12.33 ab	9.49 b	ns	13.86 a	15.33 a	*
PR91M10	12.12 a	16.84 a	*	12.69 ab	7.93 b	ns	10.48 b	15.25 a	*
Regir	8.94 b	15.69 a	*	8.95 b	9.88 b	ns	9.26 b	14.49 a	*
Total Isoflavones Content (mg/kg g Dry Matter)									
Atlantic	12.09 c	7.67 a	*	44.80 b	48.58 b	ns	13.64 b	18.27 a	*
Cresir	27.08 a	7.27 a	*	65.28 a	44.96 b	*	28.25 a	16.74 b	*
PR91M10	10.39 d	7.27 a	*	37.19 b	45.40 b	ns	11.66 c	15.42 c	*
Regir	18.61 b	8.41 a	*	44.60 b	54.60 a	*	13.44 b	14.91 d	*

[a]Different letters within the same column indicate significant differences at $P < 0.05$; "*" and "ns" indicate significant and not significant differences between cultivation systems at $P < 0.05$.

From a nutritional point of view the increase in dietary fiber, in accordance with all dietary guidelines, would almost inevitably be accompanied by a rise in phytate intake. However, many studies showed that dietary phytate may not be an undesirable component of plant foods, and, except for some specific categories prone to iron deficiency, the importance of increasing dietary fiber consumption should be considered a more important nutritional requirement.[37]

Figure 2 shows significantly lower content of total isoflavones in the hydroponically grown seeds compared to those from the field (17.04 vs 7.66 mg/kg). Data for each cultivar confirmed that this trend was observed in all of the analyzed cultivars (see Table 2); however, it is worth notings that, besides inhibiting isoflavones biosynthesis, hydroponic cultivation eliminated the differences among cultivars observed in field-grown samples.

As observed in previous works,[14,15,17] significant differences among cultivars were detected. Among field-grown cultivars, 'Cresir' showed the best performance in total isoflavones content accumulation in seeds (171.80 mg/100 g) and in both derived products (551.21 and 224.97 mg/100 g in soy milk and okara, respectively). Among hydroponically grown cultivars significant differences were not detected in seeds, and different isoflavones distributions between soy milk and okara were found: the highest value in soy milk was in 'Regir' product, and the highest value in okara was in 'Atlantic' product.

In both transformation products, the effect of cultivation system on isoflavones content was less clear: it depends on cultivar but, on average, variations in seed isoflavones content did not establish significant differences in soy milk (47.97 and 48.39 mg/kg in soy milk from field and from hydroponic, respectively) and in okara (16.75 and 16.34 mg/kg in soy milk from field and from hydroponic, respectively).

Figure 3 shows a representative HPLC chromatogram of soybean seed extracted as reported under Materials and Methods, whereas in Table 3 the isoflavones pattern found in soybean seeds and soybean products is reported. In field-grown seeds, the most abundant component was malonyl-daidzin (36.6% as average of the four cultivars), followed by β-glucosides genistin and daidzin (26.5 and 18.0% as average of

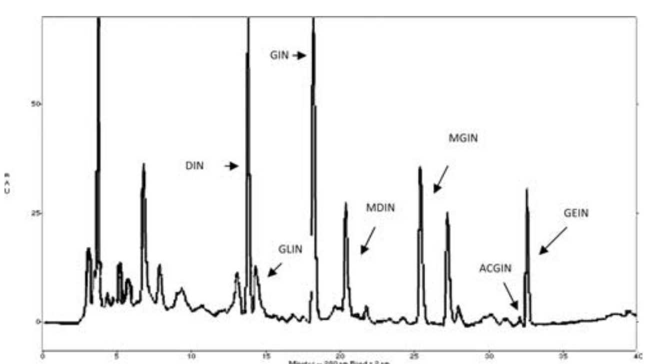

Figure 3. HPLC chromatogram of methanol−water extracts from soybean seeds. UV absorbance at 280 nm was monitored. DIN, daidzin; GLIN, glycitin; GIN, genistin; MDIN, malonyldaidzin; MGIN, malonylgenistin; ACGIN, acetylgenistin; GEIN, genistein. The identification of the seven isoflavones was achieved by LC-MS/MS using the MRM as reported in Table 1.

Table 3. Isoflavones Profile in Field-Grown and Hydroponically Grown Soybean Seeds and Derivate Products (Percent of Total)[a]

	seeds			soy milk			okara		
	field	hydroponic		field	hydroponic		field	hydroponic	
daidzin	18.05	34.08	**	20.79	22.68	ns	13.49	18.61	**
glycitin	5.87	10.03	**	8.61	4.53	**	6.03	6.12	*
genistin	26.50	28.25	**	30.19	31.12	ns	19.15	25.34	**
malonyl-daidzin	36.55	8.97	**	18.17	25.78	**	17.65	23.87	**
malonyl-genistin	10.96	9.00	**	12.88	9.77	**	22.13	14.10	**
acetyl-genistin	nd	1.47		1.38	1.53	ns	4.74	4.18	**
genistein	2.07	8.20	**	7.99	4.59	**	16.81	7.77	**

[a]Data are expressed as mean values of the four analyzed cultivars. "**" and "ns" indicate significant and not significant differences between cultivation systems at $P < 0.01$.

the four cultivars, respectively); acetyl-genistin was not detected. These data are in agreement with those of previous studies.[15,38−40] In hydroponically grown seeds, the percentage of malonyl-daidzin was drastically reduced ($P \leq 0.05$) (9.0% of the four cultivars), and the most abundant forms were β-glucosides daidzin and genistin (34.1 and 28.25% as average of the four cultivars, respectively). In 'Pr91m10' and 'Regir' hydroponically grown seeds, acetyl-genistin was detected at very low levels.

Compared to the seeds, transformation products showed wider distribution of isoflavones forms: according to Jung et al.,[41] heat treatment causes in soy milk a shift toward the β-glucoside and aglycone forms with a corresponding decrease in the malonyl-daidzin content. In soy milk obtained from field-grown seeds, the most abundant component was genistin (30.2% as average of the four cultivars), followed by daidzin (20.8% as average of the four cultivars); these values did not significantly change when soy milk was obtained from hydroponically grown seeds. Soy milk obtained from hydroponically grown seeds showed an increase in malonyl-daidzin content to 25.78% (average of the four cultivars) compared to soy milk from field-grown seeds. In okara from field-grown seeds, the most abundant components were malonyl-genistin and genistin (22.1 and 19.2%, respectively); comparison of these data with okara from hydroponic seeds revealed a decrease in the first constituent and an increase in the second one. Increases in daidzin and malonyl-daidzin were observed, too.

The biological activity of soy isoflavones is not related to the type of glycoside as deglycosylation is a prerequisite for their absorption.[42] On the contrary, isoflavones activity depends on the aglycone moiety: daidzein and glycitein have less estrogenic activity than genistein.[43] In field-grown seeds, the amount of total daidzein was the highest (54.6%), followed by total genistein (39.5%), and total glycitein was the lowest (5.9%), considering the average concentrations of the four cultivars. In derivate products from field-grown seeds the amount of total genistein was the highest (52.4% in soy milk and 62.8% in okara), followed by total daidzein (39.0 and 31.1% in soy milk and okara, respectively), and total glycitein was the lowest (8.6 and 6.0% in the two analyzed products), considering the average concentrations of four analyzed soybeans. This evidence was in agreement with the previous observations that soybeans and soy foods usually contain similar amounts of genistein and daidzein and a much lower amount of glycitein.[15,38] In hydroponically grown seeds, there was a shift toward the content of total genistein (45.4%) and total glycitein (10.0%) at the expense of total daidzein (43.0%), so there was a reduction in total isoflavones content but, on the other hand, an increased percentage of genistein, the form with greater biological activity.

In summary, the data of this paper showed that hydroponic cultivation is not only a method to obtain valuable vegetable productions in adverse environmental conditions but also a system to increase macronutrient content and to improve the nutritional value of soybean products. Hydroponic cultivation promoted seed accumulation of fat and total dietary fiber. On the other hand, the better availability of phosphorus in the nutrient solution increased the phytic acid content. Accurately designed hydroponic systems such as the NFT system do not induce stress in plants and, therefore, the concentration of isoflavones is dramatically reduced. In our experimental conditions a higher percentage of genistein, the isoflavone with greater biological activity, was recovered in seeds from hydroponically cultivated plants. For soybean-derived products, the seed cultivation system did not significantly modify soy milk composition, whereas in okara products the same compositional differences of the seeds were observed.

■ AUTHOR INFORMATION

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