

## Polyphenolic Content in Different Plant Parts of Soy Cultivars Grown under Natural Conditions

ANNALISA ROMANI,<sup>\*,†</sup> PAMELA VIGNOLINI,<sup>†</sup> CARLOTTA GALARDI,<sup>†</sup>  
 CLAUDIO AROLDI,<sup>‡</sup> CONCETTA VAZZANA,<sup>‡</sup> AND DANIELA HEIMLER<sup>§</sup>

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Firenze via G. Capponi,  
 9 50121 Firenze, Italy, Dipartimento di Scienze Agronomiche e Gestione del Territorio  
 Agro-Forestale, Università degli Studi di Firenze, P.le delle Cascine 20, 50144 Firenze, Italy, and  
 Dipartimento di Scienza del Suolo e Nutrizione della Pianta, Università degli Studi di Firenze,  
 P.le delle Cascine 18, 50144 Firenze, Italy

Roots, cotyledons, leaves, stems, pods, and seeds of three soy cultivars were analyzed for their content of isoflavones, flavonols, coumarins, and phenolic acid derivatives with three samplings during a three-month period. The extracts were analyzed by HPLC/DAD and HPLC/MS, allowing us to confirm the presence of daidzein and genistein derivatives as the major isoflavones and to characterize coumarins, most flavonols and phenolic acid derivatives. Seeds exhibited the highest content of isoflavones: 12.61 g/kg of dry weight (DW) in cv. Emiliana; 8.97 g/kg of DW in cv. Elvir; 4.49 g/kg of DW in cv. Kure, and roots are the only part with coumarins, ranging from 4.08 g/kg of DW (cv. Emiliana) to 1.29 g/kg of DW (cv. Elvir) for the longest sampling period. Leaves, pods, and stems have flavonols, and in particular leaves showed: 7.28 g/kg of DW in cv. Emiliana; 6.57 g/kg of DW in cv. Elvir; 7.08 g/kg of DW in cv. Kure. The high content of isoflavones found in the seeds could be ascribed to the natural conditions under which the soy plants were grown.

**KEYWORDS:** Soy parts; polyphenolic content; isoflavones; flavonols; HPLC/DAD; HPLC/MS

### INTRODUCTION

In recent years, due to the importance of isoflavonoids especially in the diet of oriental people, most research on the flavonoid content of soybean has been devoted to this particular group of polyphenols (1–4). Isoflavones are structurally similar to the mammalian oestrogen, oestradiol-17 $\beta$ , and exhibit oestrogenic properties. The role of isoflavones in the diet is claimed to be as a protective compound in cardiovascular diseases, osteoporosis, menopausal symptoms, and breast and prostate cancers (5–6). Almost every group of flavonoids acts as antioxidants, and flavones seem to be the most powerful flavonoids in protecting the body against reactive oxygen species (ROS) (7). With the exception of isoflavonoids, few studies have been carried out for the other polyphenolic classes in soy and soybeans, and these have always been from a physiological point of view (8–9).

The present study investigates the polyphenol content in different plant parts (roots, stems, leaves, cotyledons, pods, and seeds) of three not exhaustively characterized soybean (*Glycine max* L.) varieties, grown under natural conditions and inoculated with *Rhizobium japonicum* [*Bradyrhizobium japonicum*] that fixes N<sub>2</sub> into a form that the plant can use. After the inoculation,

growth of the seedlings was followed for three months to obtain the fully developed parts of the plant, including seeds.

The aim of this work was to obtain information on the polyphenolic composition of all the plant parts, both from a nutritional and physiological point of view, since it may be important to understand which parts of the seedling may be used, and when, for alimentary or pharmaceutical purposes.

### MATERIALS AND METHODS

**Field Experiment.** Seeds of three soybean cultivars (Pioneer “Elvir”, Ciesse “Emiliana”, and Asgrow “Kure”), grown under natural conditions, were germinated at 27 °C and relative humidity 70% in a controlled environment. Four days after germination, seedlings were inoculated with *Bradyrhizobium japonicum*; the microorganism enters into the roots and forms nodules. Subsequently, seedlings were planted in pots (30% sand, 30% loam soil, 30% organic soil) and transferred to the open air. Pots were watered automatically by drip irrigation, twice a day.

The experimental lay out was a randomized block design with four replications (four blocks, 36 plants each, 12 plants per cultivar). Three samplings were performed: 21 days after sowing (sampling I), cotyledons, stems, and leaves were taken from the single plant and weighed; 42 days after sowing (sampling II), root, stem, leaf and (if present) cotyledon samples were obtained; 77 days after sowing (sampling III) the same vegetable material and pods were also collected.

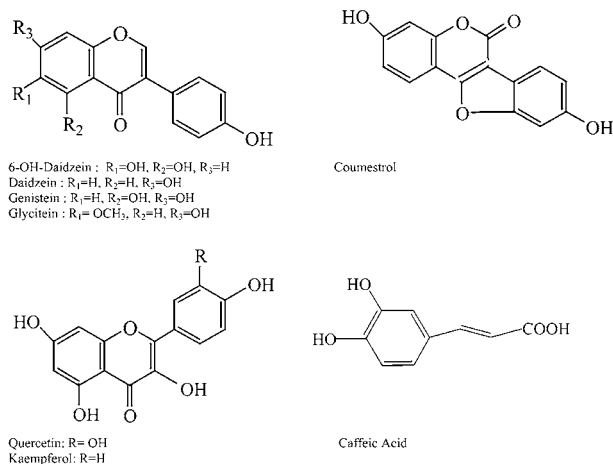
**Sample Preparation.** The different plant parts (roots, cotyledons, leaves, stems, pods, and seeds) of three soy cultivars were analyzed, as follows: the parts were rapidly frozen in liquid nitrogen and stored at –80 °C before analysis. The frozen parts were ground in a mortar

\* Corresponding author: Tel: 0039-55-2757288. Fax: 0039-55-240776.  
 E-mail: annalisa.romani@unifi.it.

<sup>†</sup> Dipartimento di Scienze Farmaceutiche.

<sup>‡</sup> Dipartimento di Scienze Agronomiche e Gestione del Territorio Agro-Forestale.

<sup>§</sup> Dipartimento di Scienza del Suolo e Nutrizione della Pianta.



**Figure 1.** Structures of the principal polyphenolic aglycones detected in soy parts.

with a pestle under liquid nitrogen while the seeds were ground with a mill. A quantity of 1 g of fresh parts was extracted with  $3 \times 30$  mL of ethanol/water (70/30, *v/v*) adjusted to pH 2.0 by HCOOH. The ethanolic extracts were defatted by extraction with  $3 \times 30$  mL of *n*-hexane. The ethanolic extracts were then evaporated to dryness under vacuum with a rotary evaporator at room temperature and finally diluted with ethanol/water (70/30, pH 2) to a final volume of 5 mL according to Romani et al. (10).

Samples of 8  $\mu$ L were analyzed by HPLC/DAD (Diode Array Detector) and HPLC-MS for quali-quantitative evaluation.

**Identification and Quantification of Individual Isoflavones and Flavonols.** Identification of individual polyphenols was carried out using their retention times and both spectroscopic and spectrometric data. Authentic standards of daidzein, genistein, coumestrol, kaempferol-3-*O*-glucoside, and quercetin-3-*O*-rutinoside (rutin) were purchased from Extrasynthèse S. A. (Lyon, Nord-Genay, France).

Individual polyphenols were quantified by a four-point regression curve ( $r^2 \geq 0.9998$ ) operating in the range 0–10  $\mu$ g on the basis of authentic standards, and determination was directly performed by HPLC-DAD. In particular, genistein and glycitein derivatives were determined at 260 nm using genistein as reference compound, while daidzein derivatives were determined at 305 nm using daidzein as

reference compound. Coumestrol derivatives were determined at 330 nm using coumestrol as the reference compound, while flavonols such as kaempferol and quercetin derivatives were determined at 350 nm using kaempferol-3-*O*-glucoside and quercetin-3-*O*-rutinoside (rutin) as reference compounds, respectively. In all cases, actual concentrations of the derivatives were calculated after applying corrections for differences in molecular weight. From each site, three samples were collected, so as to express the analytical results as an average with its standard deviation.

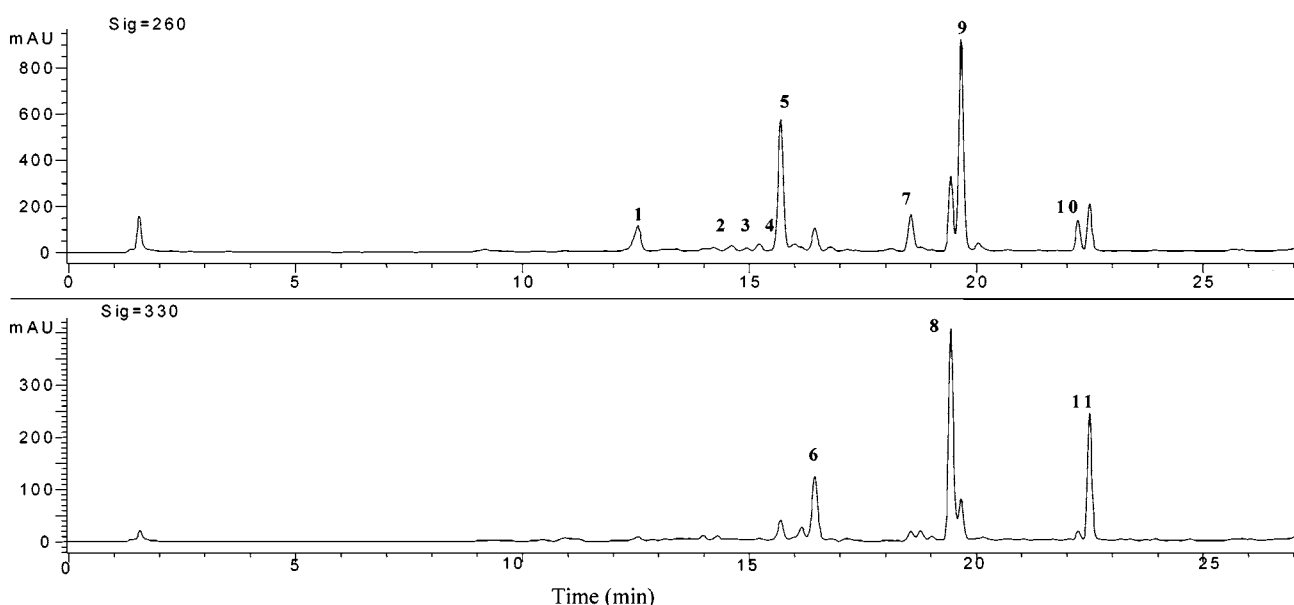
**Analytical Techniques and Equipment. HPLC/DAD Analysis.** The analyses were carried out using a HP 1100L liquid chromatograph equipped with a DAD detector (Agilent Technologies, Palo Alto, USA).

Polyphenolic compounds were separated using a  $150 \times 3.9$  mm i.d., 4  $\mu$ m, Nova Pak C18 column (Waters Corporation, Massachusetts USA) operating at 26 °C. The mobile phase was a three-step linear solvent gradient system, starting from 95% H<sub>2</sub>O (adjusted to pH 3.2 by H<sub>3</sub>PO<sub>4</sub>) up to 100% CH<sub>3</sub>CN during a 27-min period at a flow rate of 0.8 mL/min for the isoflavones, and a 7-step linear solvent gradient system, starting from 100% H<sub>2</sub>O (adjusted to pH 3.2 by H<sub>3</sub>PO<sub>4</sub>) up to 100% CH<sub>3</sub>CN during a 117-min period at a flow rate of 0.8 mL/min for the flavonols following a previous protocol by Romani et al. (10). UV/Vis spectra were recorded in the 190–450 nm range and the chromatograms were acquired at 260, 305, 330, and 350 nm.

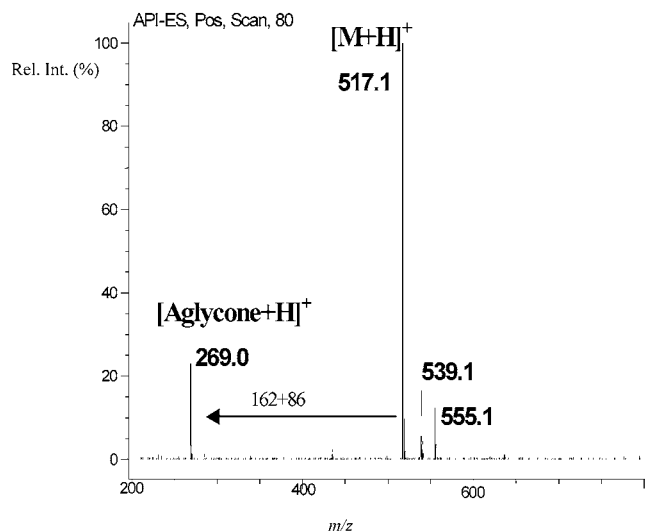
**HPLC/MS Analysis.** HPLC/MS analyses were performed using a HP 1100 MSD API-electrospray (Hewlett-Packard) coupled with a HP 1100L liquid chromatography equipped with a DAD detector (Agilent Technologies, Palo Alto, USA). The HPLC/MS analysis was performed using the same conditions as those of HPLC/DAD with H<sub>2</sub>O adjusted to pH 3.2 by addition of HCOOH. Mass spectrometer operating conditions were nitrogen gas temperature 350 °C at a flow rate of 12 L/min, nebulizer pressure 30 psi, quadrupole temperature 30 °C, and capillary voltage 3500 V. The mass spectrometer was operated in positive and negative mode at 80–180 eV.

## RESULTS

The identity of polyphenols was ascertained using data from HPLC/DAD and HPLC/MS analysis, by comparison and combination of their retention times, mass and UV spectra. The comparison of the first and second derivatives of the spectra was also performed. Applying these techniques, four main chemical classes were found in the analyzed extracts: isoflavones (genistein, daidzein, and glycitein derivatives), flavonols



**Figure 2.** Chromatographic profile acquired by HPLC/DAD (260 and 330 nm) of the hydroalcoholic extract from soy roots at relative maxima of absorbance of isoflavonoids and coumestrol derivatives, respectively. Polyphenolic compounds: (1) daidzein-7-*O*-glucoside; (2) daidzein malonylglucoside; (3) daidzein-malonylglucoside; (4) genistein-7-*O*-glucoside; (5) daidzein-7-*O*-malonylglucoside; (6) coumestrol-7-*O*-glucoside; (7) genistein-7-*O*-malonylglucoside; (8) coumestrol malonylglucoside; (9) daidzein; (10) genistein; (11) coumestrol.



**Figure 3.** Positive ion mass spectrum acquired by API-electrospray HPLC/MS analysis of coumestrol malonylglucoside from the hydroalcoholic extract from soy roots.

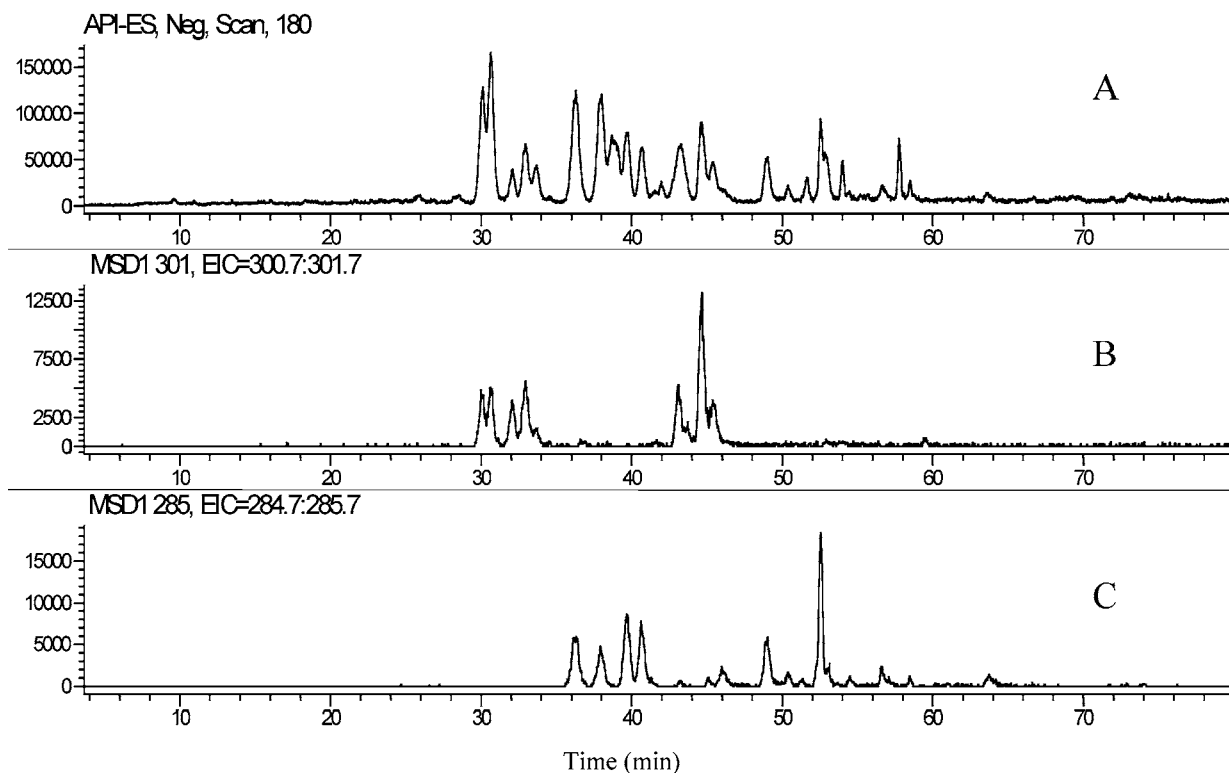
(quercetin and kaempferol derivatives), coumarins (coumestrol derivatives), and phenolic acids (caffeic acids derivatives). **Figure 1** shows the chemical structures of the main classes identified in the soy extracts.

As an example **Figure 2** reports the chromatographic profile obtained from a root extract. The chromatogram was recorded at two different wavelengths (260 and 330 nm) which represent the absorption maxima of isoflavones and coumestrol derivatives, respectively. The figure reveals both the qualitative composition of soy roots and the efficiency of the chromatographic method used. In this extract, we found only isoflavones and coumarins. Among isoflavones, we identified 7-*O*-glucosyl-daidzein, 7-*O*-glucosylmalonyl-daidzein, and the analogous de-

rivatives of genistein found by Graham (8) and Grady et al. (11), while we identified 7-*O*-acetylglucosyl-daidzein and the analogous derivatives of genistein only in seeds. More detailed information about these molecules was obtained by means of the derivative function applied on UV/Vis spectra. In fact, derivative spectra reveal more specific details than original spectra when comparing different compounds. Small differences in the spectra are much more obvious and easier to identify visually. In particular, the minor difference in the spectra derivatives indicated the correlation between the two spectra, and the presence of a genistein glucosyl derivative was thus established. The same mathematical function was applied to the spectra of the polyphenolic compounds present in analyzed extracts. Considering all information that may be obtained from chromatographic techniques and the overlaid derivative spectra, the identity of single compounds can be ascertained. Among the coumarins, we identified coumestrol, coumestrol-7-*O*-glycoside, and coumestrol malonylglucoside. Coumestrol and its 7-*O*-glycoside derivative were previously described in soy root extracts by Porter et al. (12).

For better characterization of analyzed compounds, HPLC/DAD was combined with HPLC/MS operating in the positive mode at 80 eV for isoflavones and coumarins and in the negative mode at 180 eV for flavonols and phenolic acids. **Figure 3** shows the mass spectrum of the coumestrol malonylglucoside, with peaks at *m/z* 517 and 269, corresponding to the quasi molecular ion and to the [aglycone+H]<sup>+</sup> ion, respectively.

The flavonols literature is lacking, since apart from the paper by Graham (8) which deals with seedlings sampled 5 days after germination, flavonols are generally identified as quercetin and kaempferol aglycones after hydrolysis (13). A recent study by Ho et al. (14) compared the flavonol profiles of soybean and soy leaves; they found that soy leaves contain six kaempferol glycosides that are absent in soybean. The six compounds were isolated and identified as kaempferol-3-*O*- $\alpha$ -L-rhamnopyranosyl



**Figure 4.** (A) TIC profile, in negative ion mode, and the extracted ions for (B) quercetin (*m/z* 301) and (C) kaempferol (*m/z* 285) of a hydroalcoholic extract of leaves from cv. Emiliana.

(1→2)-β-D-glucopyranosyl (1→6)-β-D-galactopyranoside, kaempferol-3-O-(2,6-di-O-α-rhamnopyranosyl)-β-galactopyranoside, kaempferol-3-O-α-L-rhamnopyranosyl (1→6)-β-D-galactopyranoside, kaempferol-3-O-digalactopyranoside, kaempferol-3-O-diglucopyranoside, and kaempferol-3-O-rutinoside. During this study, we found flavonols in all the extracts except in those from cotyledons, roots, and seeds. Kaempferol and quercetin glycosides in leaf extracts occurred as major compounds; in particular, we identified two quercetin triglycosides, six quercetin diglycosides, a methyl quercetin triglycoside, three methyl quercetin diglycosides, two kaempferol triglycosides, four kaempferol diglycosides, a kaempferol monoglycoside, and kaempferol aglycone. The two kaempferol triglycosides and the four kaempferol diglycosides we found can be assumed to be the same as those found by Ho et al. (14).

As an example, **Figure 4** shows the TIC profile, in negative ion mode, and the extracted ion chromatograms for quercetin ( $m/z$  301) and kaempferol ( $m/z$  285) obtained from the hydroalcoholic (ethanol/water 70:30, pH 2) extract of soy leaves from cv. Emiliana. In the mass spectrum of a quercetin triglycoside, two fragment ions were recorded, at  $m/z$  771 and 301, corresponding to the quasi-molecular ion  $[M-H]^-$  and to the loss of the triglycoside fragment (162+162+146,  $m/z$  162 = glucose or galactose fragment,  $m/z$  146 = rhamnose fragment). Similarly, the mass spectrum of a kaempferol triglycoside shows the signals at  $m/z$  593 and 285 corresponding to the quasi-molecular ion  $[M-H]^-$  and to the  $[aglycone-H]^-$  ion, respectively.

In **Table 1**, the quantitative data for the "Emiliana" cultivar from sampling III are reported together with the identified compounds. The results show that the highest amount of isoflavones is found in the roots, with a great predominance of daidzein derivatives relative to genistein derivatives, while in the leaves only genistein derivatives were found. The leaves are the part where the most flavonols were found, while smaller quantities were detected in pods and stem. The same evaluation was performed for the three cultivars at the three sampling dates; the polyphenol content of all three cultivars were similar. To our knowledge, this is the first investigation that analyzed both quercetin and kaempferol glycosides from a qualitative and quantitative point of view. In leaves of all three cultivars, irrespective of the sampling age, only genistein and its derivatives were found.

In **Table 2**, the quantitative data for the different parts of the three cultivars from the three sampling periods are summarized; the differences among the three cultivars are not significant, although "Emiliana" exhibited the highest content of isoflavones in leaves, roots, and stem for sampling period III. We also investigated the isoflavone content of soy seeds to study the composition of the edible parts of the plants. **Table 3** shows the composition of the isoflavones and caffeic acid derivatives of dry beans. The amount of isoflavones ranged from 95.7% (cv. Elvir) to 99.1% (cv. Kure) and daidzein derivatives were always the most abundant compounds. The amount of caffeic acid derivatives ranged from 1.9% (cv. Emiliana) to 4.3% (cv. Elvir).

## DISCUSSION

Genistein is the most biologically active isoflavone; it shows antimicrobial activity and is classified as preformed and inducible phytoalexin (15–18). It is interesting to note that genistein is the only isoflavone found in leaves, independent of the cultivar and the sampling period, according to Ho et al. (14). It is known that isoflavonoids play an important role in the establishment of the soybean *Bradyrhizobium japonicum*

**Table 1.** Composition of Different Soy Parts (cv. Emiliana) Collected after 77 Days<sup>a</sup>

compounds (Rt)	stem	leaves	roots	Pods
genistein-glycoside (29.2 min)	86.4 ± 1.7	55.1 ± 1.1	n.d.	n.d.
genistein-7-O-glucoside (44.9 min)	115.3 ± 1.8	1649.3 ± 20.0	traces	n.d.
genistein-malonylglycoside (54.6 min)	17.3 ± 0.5	55.1 ± 1.1	n.d.	n.d.
genistein-7-O-malonyl-glycoside (57.9 min)	691.6 ± 9.6	202.9 ± 3.5	805.7 ± 11.5	n.d.
genistein-acetylglycoside (64.9 min)	17.3 ± 0.5	17.4 ± 0.3	n.d.	n.d.
genistein (74.3 min)	34.6 ± 0.7	n.d.	238.8 ± 4.2	n.d.
daidzein-7-O-glucoside (30.7 min)	n.d.	n.d.	719.7 ± 10.1	n.d.
daidzein-malonylglycoside (40.7 min)	n.d.	n.d.	471.3 ± 7.7	n.d.
daidzein-malonylglycoside (42.9 min)	n.d.	n.d.	592.3 ± 8.4	n.d.
daidzein-7-O-malonyl-glycoside (51.8 min)	890.5 ± 10.1	n.d.	3665.6 ± 36.4	n.d.
daidzein (59.8 min)	n.d.	n.d.	3710.2 ± 32.2	n.d.
coumestrol-7-O-glucoside (52.6 min)	n.d.	n.d.	703.8 ± 9.9	n.d.
coumestrol-malonyl-glycoside (60.5 min)	n.d.	n.d.	2496.8 ± 29.9	n.d.
coumestrol (76.1 min)	n.d.	n.d.	875.8 ± 10.2	n.d.
quercetin-triglycoside (30.0 min)	n.d.	1098.5 ± 14.9	n.d.	1011.6 ± 13.9
quercetin-triglycoside (30.6 min)	n.d.	771.0 ± 9.8	n.d.	n.d.
quercetin-diglycoside (32.0 min)	n.d.	201.0 ± 3.0	n.d.	n.d.
quercetin-diglycoside (32.9 min)	n.d.	371.0 ± 4.05	n.d.	n.d.
quercetin-diglycoside (33.6 min)	n.d.	199.1 ± 2.95	n.d.	n.d.
methyl-quercetin-triglycoside (38.7 min)	n.d.	510.1 ± 7.1	n.d.	n.d.
methyl-quercetin-diglycoside (41.9 min)	n.d.	327.5 ± 4.7	n.d.	n.d.
quercetin-diglycoside (43.1 min)	n.d.	452.2 ± 7.2	n.d.	n.d.
quercetin-diglycoside (44.6 min)	n.d.	501.2 ± 7.9	n.d.	n.d.
quercetin-diglycoside (45.3 min)	n.d.	452.2 ± 7.4	n.d.	n.d.
methyl-quercetin-diglycoside (52.5 min)	n.d.	411.6 ± 5.7	n.d.	n.d.
methyl-quercetin-diglycoside (52.9 min)	n.d.	307.2 ± 4.4	n.d.	n.d.
kaempferol-triglycoside (36.2 min)	n.d.	559.4 ± 8.1	n.d.	n.d.
kaempferol-triglycoside (37.9 min)	n.d.	626.1 ± 9.3	n.d.	n.d.
kaempferol-diglycosides (39.6 min)	n.d.	307.2 ± 4.7	n.d.	n.d.
kaempferol-diglycoside (40.6 min)	89.3 ± 1.7	220.3 ± 3.7	n.d.	110.4 ± 2.3
kaempferol-diglycoside (48.9 min)	106.6 ± 1.8	115.9 ± 2.1	n.d.	866.3 ± 10.9
kaempferol-glycoside (50.3 min)	n.d.	46.4 ± 1.0	n.d.	n.d.
kaempferol-diglycoside (53.0 min)	n.d.	153.6 ± 3.7	n.d.	n.d.
kaempferol (63.6 min)	n.d.	153.6 ± 3.3	n.d.	n.d.
caffeic acid derivatives	54.7 ± 1	1927.5 ± 28	79.6 ± 2	58.1 ± 1

<sup>a</sup> Data are expressed as mg/kg of dry weight. Average value ± SD of three soy samples.

nitrogen fixing symbiosis (19), and it has been shown also that the roots of plants inoculated with *B. japonicum* exhibited a higher concentration of genistein and daidzein with respect to noninoculated plants which were subjected to nitrogen fertilization (20). In seeds, a positive correlation between isoflavone concentration and potassium fertilization on low potassium soils



**Table 2.** Content of the Different Classes of Compounds at the Three Sampling Dates of the Analyzed Cultivars<sup>a</sup>

	cotyledons	stems	leaves	roots	Pods
Emiliana					
isoflavones					
day 21	31804.5 ± 336	387.1 ± 8	1038.6 ± 18	n.s.	n.s.
day 42	12357.8 ± 135	1094.3 ± 17	846.7 ± 14	7396.2 ± 81	n.s.
day 77	n.s.	1853.0 ± 25	1979.8 ± 26	10203.8 ± 110	n.d.
flavonols					
day 21	n.d.	n.d.	4872.9 ± 68	n.s.	n.s.
day 42	n.d.	235.8 ± 5	5598.5 ± 73	n.d.	n.s.
day 77	n.s.	195.9 ± 4	7284.0 ± 107	n.d.	1988.4 ± 27
coumarins					
day 21	n.d.	n.d.	n.d.	n.s.	n.s.
day 42	n.d.	n.d.	n.d.	1122.6 ± 21	n.s.
day 77	n.s.	n.d.	n.d.	4076.4 ± 50	n.d.
phenolic acids					
day 21	425.3 ± 7	268.8 ± 5	513.8 ± 9	n.s.	n.s.
day 42	154.5 ± 3	113.2 ± 2	306.5 ± 7	113.2 ± 2	n.s.
day 77	n.s.	54.7 ± 1	1927.5 ± 28	79.6 ± 2	58.1 ± 1
Kure					
isoflavones					
day 21	13476.2 ± 168	204.5 ± 5	1243.4 ± 23	n.s.	n.s.
day 42	13148.7 ± 158	2090.2 ± 26	803.0 ± 12	4916.0 ± 71	n.s.
day 77	n.s.	488.0 ± 7	398.5 ± 6	1063.3 ± 14	n.d.
flavonols					
day 21	n.d.	n.d.	15868.4 ± 173	n.s.	n.s.
day 42	n.d.	n.d.	1416.6 ± 23	n.d.	n.s.
day 77	n.s.	1259.4 ± 23	6568.2 ± 81	n.d.	4323.5 ± 55
coumarins					
day 21	n.d.	n.d.	n.d.	n.s.	n.s.
day 42	n.d.	n.d.	n.d.	1618.3 ± 29	n.s.
day 77	n.s.	n.d.	n.d.	1646.6 ± 28	n.d.
phenolic acids					
day 21	226.2 ± 4	147.7 ± 3	4210.5 ± 52	n.s.	n.s.
day 42	157.0 ± 3	142.8 ± 4	810.6 ± 10	145.0 ± 3	n.s.
day 77	n.s.	20.5 ± 1	1295.2 ± 20	20.0 ± 1	76.5 ± 2
Elvir					
isoflavones					
day 21	10430.0 ± 123	130.4 ± 3	912.8 ± 12	n.s.	n.s.
day 42	n.s.	1289.6 ± 15	850.3 ± 12	2940.5 ± 32	n.s.
day 77	n.s.	1000.0 ± 14	303.7 ± 6	2541.6 ± 33	n.d.
flavonols					
day 21	n.d.	n.d.	12837.2 ± 144	n.s.	n.s.
day 42	n.s.	n.d.	2605.4 ± 31	n.d.	n.d.
day 77	n.s.	n.d.	7078.5 ± 89	n.d.	2360.4 ± 27
coumarins					
day 21	n.d.	n.d.	n.d.	n.s.	n.s.
day 42	n.s.	n.d.	n.d.	1286.4 ± 16	n.s.
day 77	n.s.	n.d.	n.d.	1291.6 ± 18	n.d.
phenolic acids					
day 21	250 ± 4	65.2 ± 2	3220.9 ± 39	n.s.	n.s.
day 42	n.s.	131.0 ± 3	428.6 ± 6	135.1 ± 2	n.s.
day 77	n.s.	55.1 ± 2	2276.4 ± 26	n.d.	75.6 ± 2

<sup>a</sup> Data are expressed as mg/kg of dry weight. Average value ± SD of three soy samples. n.d. = not detected, n.s. = not sampled.

was previously noted (21). In roots, there are differences between the ratio of genistein and daidzein derivatives in the three cultivars. In cv. "Emiliana" the ratio is 0.12 (samplings II and III); in the case of the cv. "Kure" the ratios are 0.10 (sampling II) and 0.76 (sampling III), while for cv. "Elvir" the ratios are 0.07 (sampling II) and 0.35 (sampling III). In the seeds, the ratios are 0.61 (Emiliana), 0.82 (Elvir), and 0.88 (Kure). This ratio, in particular for the period-III samples, despite the limited number of cultivars examined, could probably be an interesting parameter used to monitor the growing conditions and as a taxonomical marker. Further studies are in progress to verify the effective use of this parameter on statistical basis.

In terms of the isoflavone content in the seeds, our results are higher by 2–10 times than those reported in the pertinent literature (6, 22) and could probably be ascribed to the cultivation conditions of the soy lines. During our studies, we investigated the flavonol content in soy parts and we found both kaempferol and quercetin derivatives; a high content in leaves and minor quantity in pods and stem was noted. The relative

**Table 3.** Polyphenolic Content of Dry Beans from the Three Soy Cultivars<sup>a</sup>

compounds (Rt)	Emiliana	Kure	Elvir
genistein-7-O-glucoside (15.4 min)	1981.1 ± 34.2	1349.7 ± 21.9	845.3 ± 11.1
genistein-glycoside (17.5 min)	80.2 ± 1.3	80.1 ± 1.7	40.2 ± 0.8
genistein-malonylglycoside (18.1 min)	39.3 ± 0.9	39.4 ± 0.7	40.1 ± 0.9
genistein-malonylglycoside (18.6 min)	2380.0 ± 26.3	2393.6 ± 27.9	938.2 ± 9.1
genistein-acetylglycoside (20.4 min)	70.4 ± 1.1	71.2 ± 1.2	71.4 ± 1.3
genistein (22.2 min)	80.2 ± 1.6	39.1 ± 0.8	4.2 ± 0.1
<b>total</b>	<b>4631.2 ± 66</b>	<b>3973.1 ± 54</b>	<b>1939.4 ± 23</b>
daidzein-7-O-glucoside (12.9 min)	3404.1 ± 29.1	2040.9 ± 28.8	1199.3 ± 18.2
daidzein-malonylglycoside (14.7 min)	315.0 ± 3.9	235.8 ± 3.8	159.8 ± 3.3
daidzein-malonylglycoside (15.7 min)	3630.4 ± 33.4	2142.3 ± 31.7	963.7 ± 10.4
daidzein-acetylglycoside (17.3 min)	traces	traces	traces
daidzein (19.7 min)	199.2 ± 3.7	80.5 ± 1.3	80.8 ± 1.6
<b>total</b>	<b>7548.7 ± 70</b>	<b>4499.5 ± 65</b>	<b>2403.6 ± 27</b>
6-OH-daidzein (12.7 min)	40.1 ± 0.8	traces	traces
<b>total</b>	<b>40.1 ± 0.8</b>	<b>traces</b>	<b>traces</b>
glycitin (13.4 min)	198.6 ± 3.9	264.2 ± 5.2	67.1 ± 1.5
glycitein-malonylglycoside (16.1 min)	235.1 ± 4.3	237.4 ± 4.7	79.8 ± 1.5
<b>total</b>	<b>433.7 ± 8</b>	<b>501.6 ± 10</b>	<b>146.9 ± 3</b>
<b>caffeic acid derivatives</b>	<b>239.0 ± 5</b>	<b>80.3 ± 1</b>	<b>203.2 ± 4</b>

<sup>a</sup> Data are expressed as mg/kg of dry weight. Average value ± SD of three soy samples.

distribution and the type of flavonoid compounds in different plant parts may substantially affect the ultraviolet absorption and thus the damaging effect of excess solar radiation on different plant species (23).

In conclusion, isoflavones are present in most of the investigated parts (except pods) and the amounts change with the sampling period and the cultivar. Flavonols of the leaves were characterized and their amount was evaluated so that it may be possible to extract polyphenols from the different parts to exploit the whole plant also for its food use.

## ACKNOWLEDGMENT

The authors gratefully acknowledge the skilled technical assistance of Dr. Sandra Gallori.

## LITERATURE CITED

- Choi, Y. S.; Lee, B. H.; Kim, J. H.; Kim, N. S. Concentration of phytoestrogens in soybeans and soybean products in Korea. *J. Sci. Food Agric.* **2000**, *80*, 1709–1712.
- Pandjaitan, N.; Hettiarachchy, N.; Ju, Z. Y.; Crandall, P.; Sneller, C.; Dombek, D. Evaluation of genistin and genistein contents in soybean varieties and soy protein concentrate prepared with three basic methods. *J. Food Sci.* **2000**, *65*, 399–402.
- Hoeck, J. A.; Fehr, W. R.; Murphy, P. A.; Welke, G. A. Influence of genotype and environment on isoflavone contents of soybean. *Crop Sci.* **2000**, *40*, 48–51.
- Nurmi, T.; Mazur, W.; Heinonen, S.; Kokkonen, J.; Adlercreutz, H. Isoflavone content of the soy based supplements. *J. Pharm. Biomed. Anal.* **2002**, *28*, 1–11.
- Cassidy, A.; Hanley, B.; Lamuela-Raventos, R. M. Isoflavones, lignans and stilbenes: Origins, metabolism and potential importance to human health. *J. Sci. Food Agric.* **2000**, *80*, 1044–1062.
- Mazur, W. M.; Duke, J. A.; Wahala, K.; Rasku, S.; Adlercreutz, H. Isoflavonoids and lignans in legumes: Nutritional and health aspects in humans. *Nutr. Biochem.* **1998**, *9*, 193–200.
- Nijveldt, R. J.; Van Nood, E.; Van Horn, D. E. C.; Boelens, P. G.; Van Norren, K.; Van Leeuwen, P. A. M. Flavonoids: A review of probable mechanism of action and potential applications. *Clin. Nutr.* **2001**, *74*, 418–425.

- (8) Graham, T. L. Flavonoid and isoflavonoids distribution in developing soybean seedling tissues and in seed root exudates. *Plant Physiol.* **1991**, *95*, 594–603.
- (9) Middleton, E. M.; Teramura, A. H. The role of flavonol glycosides and carotenoids in protecting soybean from ultraviolet-B damage. *Plant Physiol.* **1993**, *103*, 741–752.
- (10) Romani, A.; Mulinacci, N.; Pinelli, P.; Vincieri, F. F.; Tattini, M. Identification and quantitation of polyphenols in *Myrtus communis* L. leaves. *Chromatographia* **1999**, *49*, 17–20.
- (11) Grady, H.; Palmer, R. G.; Imsande, J. Isoflavonoids in root and hypocotyl of soybean seedlings. *Am. J. Bot.* **1995**, *82*, 964–968.
- (12) Porter, P. M.; Banwart, W. L.; Hassett, J. J. HPLC isolation and GC-MS identification of genistein, daidzein and coumestrol from unhydrolyzed soybean root extracts. *Environ. Exp. Bot.* **1985**, *25*, 229–232.
- (13) Porter, P. M.; Banwart, W. L.; Hassett, J. J. Phenolic acids and flavonoids in soybean root and leaf extract. *Environ. Exp. Bot.* **1986**, *26*, 65–73.
- (14) Ho, H. M.; Chen, R. Y.; Leung, L. K.; Chan, F. L.; Huang, Y.; Chen, Z.-Y. Difference in flavonoid and isoflavone profile between soybean and soy leaf. *Biomed. Pharmacother.* **2002**, *56*, 289–295.
- (15) Fukutake, M.; Takahashi, M.; Ishida, K.; Kawamura, H.; Sugimura, T.; Wakadayashi, K. Quantification of genistein and genistin in soybeans and soybean products. *Food Chem. Toxicol.* **1996**, *34*, 457–461.
- (16) Messina, M. J. Legumes and soybeans: Overview of their nutritional profiles and health effects. *Am. J. Clin. Nutr.* **1999**, *70*, 439S–450S.
- (17) Ratna, W. N. Inhibition of estrogenic stimulation of gene expression by genistein. *Life Sci.* **2002**, *71*, 865–877.
- (18) Dixon, R. A.; Ferreira, D. Genistein. *Phytochemistry* **2002**, *60*, 205–211.
- (19) Kosslak, R. M.; Bookland, R.; Barkei, J.; Paaren, H.; Appelbaum, E. R. Induction of *Bradyrhizobium japonicum* common *nod* genes by isoflavones isolated from *Glycine max*. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 266–271.
- (20) Zhang, F.; Mace, F.; Smith, D. L. Mineral nitrogen availability and isoflavonoid accumulation in the root systems of soybean. *J. Agron. Crop Sci.* **2000**, *184*, 197–204.
- (21) Vyn, T. J.; Yin, X. H.; Bruulsema, T. W.; Jackson, C. J. C.; Rajcan, I.; Brouder, S. M. Potassium fertilization effect on isoflavones concentrations in soybean. *J. Agric. Food Chem.* **2002**, *50*, 3501–3506.
- (22) Franke, A. A.; Custer, L. J.; Cerna, C. M.; Narala, K. K. Quantitation of phytoestrogens in Lp legumes by HPLC. *J. Agric. Food Chem.* **1994**, *42*, 1905–1913.
- (23) Day, T. A.; Howells, B. W.; Rice, W. J. Ultraviolet absorption and epidermal-transmittance spectra in foliage. *Physiol. Plant.* **1994**, *92*, 207–218.

---

Received for review December 13, 2002. Revised manuscript received May 9, 2003. Accepted May 12, 2003. This research was performed thanks to CNR Grant No. C000BCC003.

JF0212136