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BASIC AREAS
Article

Isoflavonoid composition and antioxidant activity on elicited and non-elicited sprouts of six soy cultivars grown in Colombia

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ABSTRACT: Isoflavonoid composition of freshly harvested soy sprouts of six cultivars grown in Colombia was analyzed. Evaluations on the effect of storage time at 12 °C and exogenous application of the elicitor 2,6-dichloro isonicotinic acid (INA) were carried out to establish their influence in the ability of soy sprouts for synthesizing isoflavonoids. In addition, elicited and non-elicited sprouts of soy cultivar Soy SK-7 was assessed for total phenolic content and antioxidant activity according to the growth stage (VE, VC, and V1) and tissues (cotyledon-epicotyl and hypocotyl-root). Isoflavonoid content and antioxidant capacity were dependent on the cultivar, growth stage, tissue, and storage time at 12 °C. Growth stages VE and V1 and the hypocotyl-root exhibited the highest total phenolic content and antioxidant activity. In general, 6"-*O*-malonylgenistin, daidzin and genistin were the major constituents in the cultivars. Soy SK-7 and Panorama 358 displayed the highest amounts of 6"-*O*-malonylgenistin (155.9 µg/g) and daidzin (83.5 µg/g), respectively. Results showed that the isoflavonoids and phenolic contents and antioxidant activity were significantly increased by application of INA. This information can be useful to produce soybean sprouts with increased amounts of bioactive compounds and improved response to abiotic and biotic stresses.

Key words: soy sprouts, *Glycine max* L., elicitor, isoflavones.

INTRODUCTION

Consumption of soy sprouts exhibits beneficial effects for health, reducing the risk of suffering cardiovascular diseases and cancer (Applegate et al. 2018). Soy sprouts provide high nutrient content to human diet because they are a natural source of minerals, amino acids, carbohydrates, fatty acids, vitamins, and isoflavones, among others (Ghani et al. 2016). Soy isoflavones (Fig. 1) are bioactive compounds that have been reported for exhibiting phytoestrogenic activity and preventing some diseases such as osteoporosis, and ovarian, cervix, prostate, and mama cancer (Cho et al. 2010). These compounds are present in the plant as glucosides (daidzin, 6"-*O*-malonyldaidzin, genistin, 6"-*O*-malonylgenistin) and their correspondent aglycones (daidzein and genistein); the last ones being recognized as the most bioactive metabolites of these group of compounds. Other substances, like coumestrol (a coumestan) and glyceollins (prenylated pterocarpan-type compounds) are also present in the soybean plants. Antimicrobial, antioxidant, estrogenic, and anti-oestrogenic activities, as well as anticancer properties, have been described for these compounds (Lim et al. 2017).

It has been reported that the isoflavone content in soy is influenced by different abiotic and biotic factors, including soil moisture, organ tissue, pest pressure, temperature, among others (Teekachunhatean et al. 2013). In addition, the isoflavonoid

levels can be modulated by elicitors (Zhang et al. 2006), which are compounds capable of stimulating the mechanisms of defense in plants, promoting the biosynthesis and accumulation of this secondary metabolites (Zhang et al. 2006, Durango et al. 2013). Exogenous application of elicitors such as salicylic acid, 2,6-dichloroisonicotinic acid (INA), and benzo(1,2,3) thiadiazole-7-carbothioic acid S-methyl ester has been shown increasing the isoflavonoids concentration in soybean seedlings (Durango et al. 2018). Currently, there is great interest in improving the content of bioactive substances in fruit and vegetables through elicitors (Zhang et al. 2006, Durango et al. 2013, Durango et al. 2018).

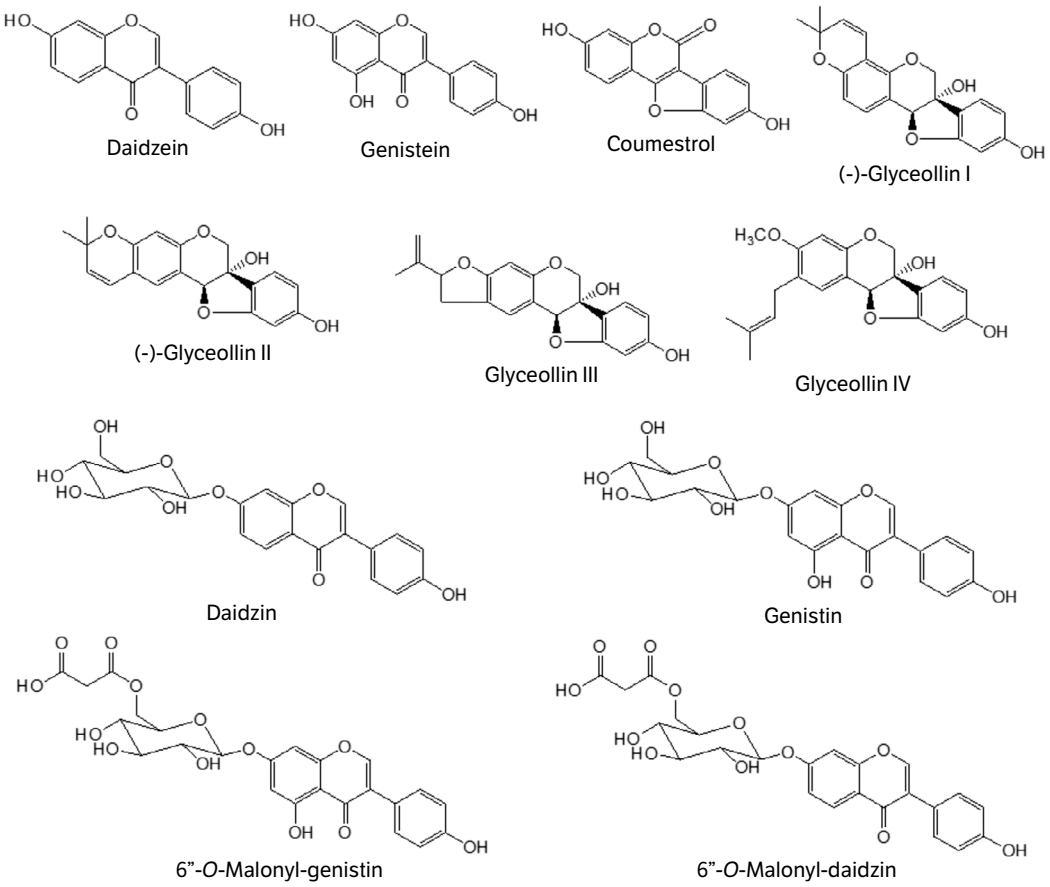


Figure 1. Structure of isoflavonoid compounds found in soy sprouts.

On the other hand, despite having suitable soil and weather conditions to grow soy, Colombia is more a soybean importer than a producer (Rojas et al. 2018). The national soybean production, cultivated in Eastern plains regions and Valle del Cauca department, is mainly used as a crop in rotation systems and to produce animal concentrates (Valencia and Ligarreto 2010). At present, this panorama has changed with the introduction of new soybean cultivars, but it is essential to characterize them in order to obtain high quality soybean sprouts.

Studies about characterization of isoflavonoid content are frequently carried out in Asian countries like Japan, China, and Korea. According to a recent review, there are no reports on isoflavone levels from American countries, despite the main soybean producers being from this region (Wang et al. 2022). The nutritional value of Colombian soy sprouts is still lacking.

In the present study, we provide pertinent information to fill this knowledge gap and report for the first time the isoflavonoid content (daidzin, 6"-*O*-malonyldaidzin, daidzein, genistin, 6"-*O*-malonylgenistin, genistein, coumestrol, and glyceollins) present on sprouts of six soybean cultivars grown in Colombia. In the same way, the influence of the storage time and the elicitation with INA in the isoflavonoid levels was also assessed. Furthermore, the total phenolic content and antioxidant capacity of elicited and non-elicited sprouts of soy cultivar SK-7 were evaluated, and the effect of growth stage and tissue was estimated.

MATERIALS AND METHODS

Reagents

Daidzein, genistein, gallic acid, INA, the Folin-Ciocalteau's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were acquired from Sigma-Aldrich Co. (St. Louis, MO, USA). Coumestrol was obtained from a preceding work and characterized as described by Durango et al. (2002). All other chemicals were purchased from Merck KGaA (Darmstadt, Germany).

Plant material

Five of the six soybean (*Glycine max* L.) seeds cultivars (Soyica P34, Panorama 358, Panorama 27, Panorama 357, and Panorama 29) employed in this work were supplied by Monsanto Colombia. The cultivar Soy SK-7 was purchased to Semillas Kamerún S.A.S. (Cartago, Valle, Colombia). Cultivars seeds were sterilized with NaClO (2%) for 5 min, rinsed with water, and sown in sterile sand. Seedling germination was carried out under environmental conditions (20 °C and average relative humidity of 80%) in the dark. Seedlings were obtained 12 days after sowing.

Sample preparation

The cut sprouts were extracted with 95% ethanol (20 mL) and filtered through cotton. The filtrate obtained was taken to dryness at 40 °C under reduced pressure using a Rotavapor Buchi R-210. Then, the residue was extracted with ethyl acetate (3 × 20 mL), and the solvent was evaporated to dryness under vacuum. The remnant obtained was dissolved in methanol (5 mL), and filtered through 0.45-mm pore-size membrane. The solution obtained was kept at 4°C in an amber glass vial until HPLC chromatographic analysis.

Isoflavonoid composition in freshly harvested soy sprouts of different cultivars

All soybean cultivars were selected in vegetative stage of growth VE (Emergence: cotyledons are through the soil surface), as described by Purcell et al. (2013). Soy sprouts were carefully harvested, washed with water, extracted with solvent, and analyzed as described in the HPLC analysis section.

Treatments

Effect of storage time at 12 °C on soy sprouts isoflavonoid composition

Soy sprouts (20 g) of each cultivar were placed on a sterile petri dish containing a moistened filter paper. After that, the petri dishes were wrapped with parafilm and kept in the darkness at 12 °C for 24, 48, and 72 hours. All assays were carried out twice. Isoflavonoids composition in the extracts was obtained and analyzed as previously described.

Effect of the exogenous application of INA on soy sprouts isoflavonoid composition

The elicitor capacity of INA was evaluated employing 20 g of sprouts from each cultivar; all assays were carried out twice. The sprouts were treated by immersion during 4 h with a solution of INA (1.60 mM). Then, they were kept in the dark at environmental conditions (20 °C and average relative humidity of 80%). After post-induction times (24, 48 and 72 h); the samples were extracted with ethanol (40 mL) followed by filtration. The ethanol extract was concentrated under reduced pressure and extracted with ethyl acetate (3 × 15 mL). The extracts obtained were analyzed as described

in the HPLC analysis section. Water-treated sprouts in vegetative stage of growth VE and 72-h post-induction were used as the negative control.

Effect of growth stage and organ tissue on isoflavonoid composition

Seeds of Soy SK-7 were germinated during five days under environmental conditions (20 °C and average relative humidity of 80%) in the dark. Thereafter, samples were collected and cleaned with tap water. Soy sprouts (20 g) of different growth stages described by Purcell et al. (2013) (vegetative stages of growth VE: once the cotyledons are through the soil surface; VC: once cotyledons and the leaf unrolled are not touching; and V1: once the unifoliate leaves are fully expanded) were analyzed by HPLC. In addition, soybean seedlings in the VC growth stage were carefully divided into hypocotyl/roots (20 g) and cotyledons/epicotyls (20 g). The extracts were obtained and analyzed as described in the HPLC analysis section.

HPLC analysis

The isoflavonoid composition was done by HPLC chromatography under the same conditions used by Durango et al. (2013). Briefly, a Gilson chromatograph equipped with a diode array detector (Gilson model 170) was used. The injection volume was 20 µL; methanol (solvent A) and 0.5% acetic acid (solvent B) were used as the mobile phase with the following gradient: increase from 10 to 70% of solvent A in 40 min, followed by 70 to 90% of solvent A in 20 min; subsequently, the proportion of solvent A was maintained for 8 min, and the column was equilibrated again to the initial conditions. A Phenomenex Luna 5 µ C18 (2) reverse-phase column (150 mm × 4.6 mm id, 5 µm) (Phenomenex, Torrance, CA, USA), fitted with a Phenomenex Security column guard C18 (4 × 3 mm), was used as the stationary phase. Isoflavonoid compounds were examined at 248, 254, 270, 286, and 310 nm. All samples from six cultivars were analyzed by duplicate.

Identification and quantification of bioactive compounds

Isoflavonoid compounds genistein, daidzein and coumestrol were characterized by comparison of the retention times (Rt) and co-elution with standards. Additionally, Rt of these isoflavonoids, besides with glyceollins, 6-*O*"-malonyldaidzin, daidzin, and 6-*O*"-malonylgenistin, was corroborated by LC-MSD as described in Durango et al. (2013). The isoflavonoids were identified in an HP 1100 series HPLC equipment (Agilent Technologies, Waldbronn, Germany) equipped with a mass selective detector (HP 1100), API-ES chamber operated in positive ion mode, and the chromatographic conditions described previously. MSD conditions were as follows: dry gas 12 L/min, gas pressure 60 psi; spray capillary voltage 3 kV; dry gas temperature 350 °C. Retention times (min) of isoflavonoids were 20.5 (daidzin), 24.5 (genistin), 27.4 (6-*O*"-malonyl-daidzin), 30.7 (6-*O*"-malonyl-genistin), 32.9 (dadzein), 36.6 (genistein), 37.4 (coumestrol), and 43.2, 43.5 and 44.0 min corresponds to glyceollins. Quantification of isoflavonoids was carried out according to the procedure reported by Durango et al. (2013). The results were expressed as µg isoflavonoids/g fresh material and reported as mean values ± standard deviation (SD).

Determination of the total phenolic content

The quantification of total phenolic content (TPC) on the extracts obtained from Soy SK-7 sprouts was done using the Folin-Ciocalteu reagent (Aryal et al. 2019). Briefly, to achieve complete dissolution, 2 mL of methanol were added to each extract (10-25 mg). Then, the solution obtained (100 µL) was mixed with Na_2CO_3 (400 µL, 0.7 M) and the Folin-Ciocalteu testing agent (500 µL, 0.2 M). The sample was preserved 2 h in dark and past that time, and the absorption at 760 nm was recorded by means of a Shimadzu, UV-1800 Spectrophotometer. The TPC was reported as mg gallic acid equivalents (GAE)/g extract.

Antioxidant activity

DPPH antioxidant activity assay

The ability of the soy sprout extract (cv. Soy SK-7) to scavenge the DPPH$^{\bullet}$ was determined through a modification of the protocol description given by Brand-Williams et al. (1995) (Durango et al. 2018). Extracts (10-25 mg) were dissolved in 2 mL of methanol. Then, the DPPH$^{\bullet}$ stock solution (950 µL) was mixed with the sample (50 µL). Next, the mixture was left reacting for 30 min in the dark before reading the absorbance at 517 nm. The data was expressed in µmol Trolox equivalent/100 g extract.

ABTS radical cation scavenging activity

The ability of soy sprouts extracts (cv. Soy SK-7) to scavenge the ABTS$^{\bullet+}$ free radical was determined as stated by Re et al. (1999). Extracts (10-25 mg) were dissolved in 2 mL of methanol. Afterwards, 10 µL of this solution and 1,000 µL of ABTS solution were mixed in a cell and left for 7 minutes in the dark. Absorption was taken at 734 nm against a blank. The ABTS results were expressed in µmol Trolox equivalent/100 g extract.

Statistical analysis

All tests are expressed as mean ± SD. Data analysis was conducted by one-way analysis of variance (ANOVA) followed by the Fisher's least significant difference with the confidence interval of 95% ($p < 0.05$). Correlation coefficients (Pearson's correlation coefficient, *r*), as well as all the other analyses, were carried out making use of Microsoft Excel software, 2010 version. All the treatments with the seedlings were carried out in duplicate, and the analyses of antioxidant activity were done in triplicate.

RESULTS AND DISCUSSION

Isoflavonoid composition on freshly harvested soy sprouts: growth stage VE

A characteristic chromatographic profile of soy sprouts showing the isoflavonoid constituents is shown in Fig. 2. The HPLC method employed permitted to separate and identify all the isoflavonoids analyzed. The glucosides, genistin and daidzin, showed Rt of 20.5 and 24.5 min. The malonyl-glycosides compounds, 6-*O*"-malonyldaidzin and 6-*O*"-malonylgenistin, were detected at 27.4 and 30.7 min, respectively. Aglycones, genistein and daidzein, were found at Rt of 32.9 and 36.6 min. Coumestrol was found at 37.3, and glyceollins were found between 43 and 45 min. Some peaks could not be identified in this study. Daidzein, genistein, daidzin, genistin, malonyldadzein, and malonylgenistein have been reported as the major soybean flavonoids (Król-Grzymała and Amarowicz 2020), but other isoflavonoids such as glycitein, formononetin, biochanin A, glycitin, ononin, ambocin, malonyl glycitin, and acetyl derivatives of daidzin, glycitin and genistin have been reported in soybean sprouts and were no detected in this study (Wang et al. 2022). A similar chromatographic profile can be found in other studies, even when they were not carried out under the same HPLC conditions (Farrell et al. 2017).

Isoflavonoid content in sprouts of the different soy cultivars in vegetative stage of growth VE is shown in Fig. 3. In general, the isoflavonoid content varied according to the cultivar, which agrees with previous studies (Carrão-Panizzi et al. 2009, Shohag et al. 2012). The highest amount of isoflavonoids was exhibited by the cultivar Panorama 358, followed by SK-7. Genistein and glyceollins did not present significant differences between cultivars. In addition, both compounds were detected at concentrations below 20 µg/g.

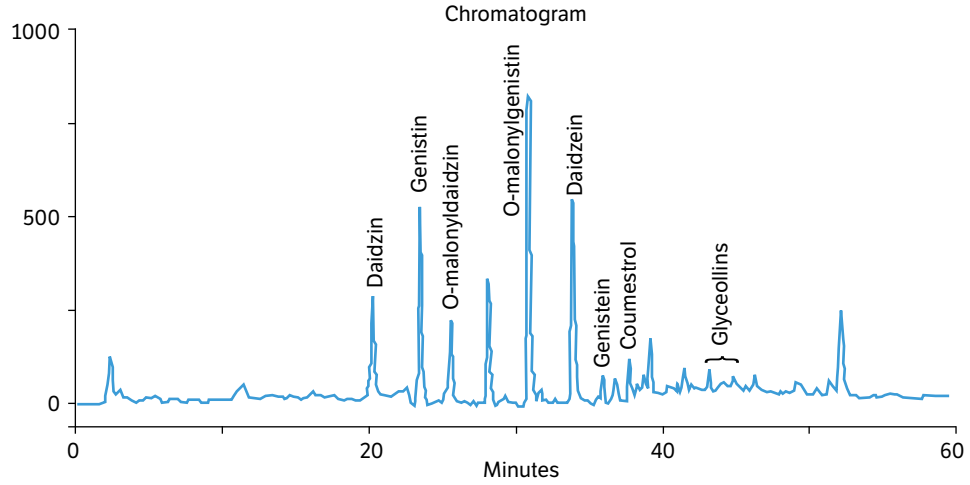
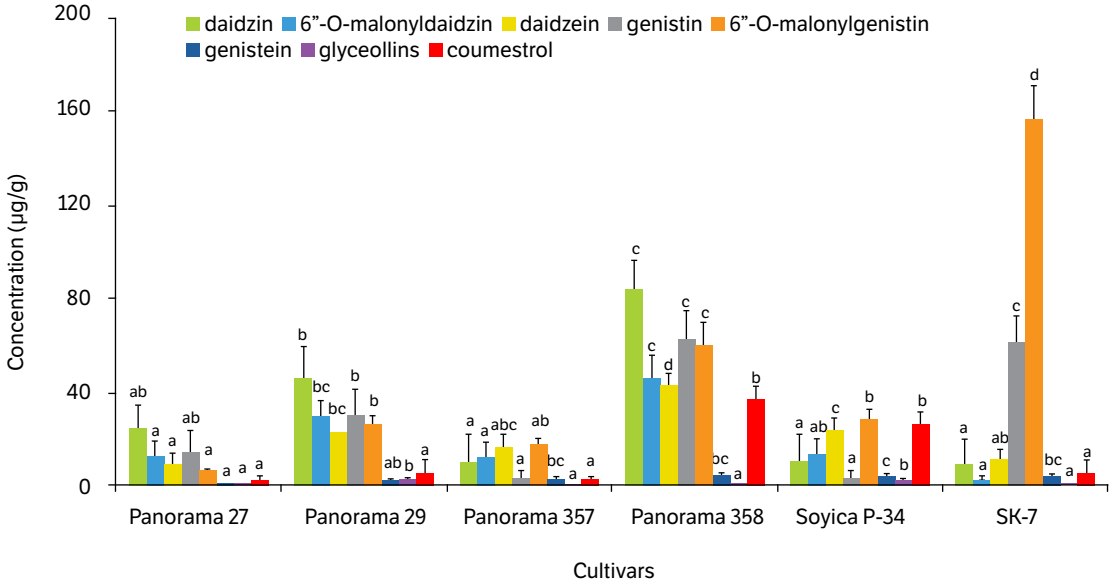


Figure 2. Chromatographic profile of freshly harvested soy sprouts (cv. Panorama 29). Analysis wavelength: 254 nm.



*values are presented as means ± standard deviation. Means with different letters in the same column indicate significant differences ($p < 0.05$) between treatments (Fisher's least significant difference test).

Figure 3. Isoflavonoid composition in freshly harvested soy sprouts (vegetative state VE) of the different cultivars evaluated*.

As can be seen, the major isoflavonoids detected in the cultivar SK-7 were 6"-*O*-malonyl-genistin (155.9 µg/g) and genistin (60.7 µg/g). On the other hand, daidzin reached the highest levels in cultivars Panorama 358 (83.5 µg/g), Panorama 29 (45.7 µg/g), and Panorama 27 (23.5 µg/g). Cultivars Panorama 358 and Panorama 29 also exhibited higher levels of 6"-*O*-malonyldaidzin and daidzein than Panorama 27, Soyica P34, and Panorama 357. Coumestrol reached the highest amounts in Panorama 358 (36.3 µg/g) and Soyica P34 (25.7 µg/g). In soybean seedlings evaluated, 6"-*O*-malonyl-glucosides represents between 27 (Panorama 27) to 64% (SK-7) of the total isoflavonoids, while glucosides represent 12.8% in Soyica P34 cultivar up to 57.1% in Panorama 27 cultivar. Genistein and glyceollins were absent or present in low concentrations.

A similar behavior has been described by different authors, which have seen the malonyl-glucoside group displayed the highest content, followed by glucoside derivatives daidzin and genistin, then their correspondent aglycones daidzein and genistein, ending with negligible amounts of the pterocarpan-type compounds, glyceollins, and the coumestan-type isoflavonoid, coumestrol (Park et al. 2018, Guo et al. 2019).

Total isoflavonoid contents were 332.3 (Panorama 358), 242.8 (SK-7), 160.9 (Panorama 29), 79.5 (Soyica P34), 64.9 (Panorama 27), and 60.8 μg/g (Panorama 357). In general, the concentration of isoflavonoids in soy sprouts of cultivars grown in Colombia can be considered low when compared with other reports from soy sprouts from different countries. In the main, the isoflavone content values in soybean sprouts vary from 1×10^{-2} to 1×10^{1} g/kg fresh weight (Wang et al. 2022). For example, Kim et al. (2006) and Lee et al. (2007) have reported amounts of isoflavonoids in soy sprouts from Korea above 2,000 μg/g. In addition, Devi et al. (2009) reported for soy seedlings from India a total isoflavonoid content between 602-794 μg/g, and Park et al. (2018) found values between 425.59-1,198.46 and 350.61-1,318.67 μg/g for soy sprouts cultivars from China and Japan, respectively.

Effect of storage time at 12 ˚C on isoflavonoid composition

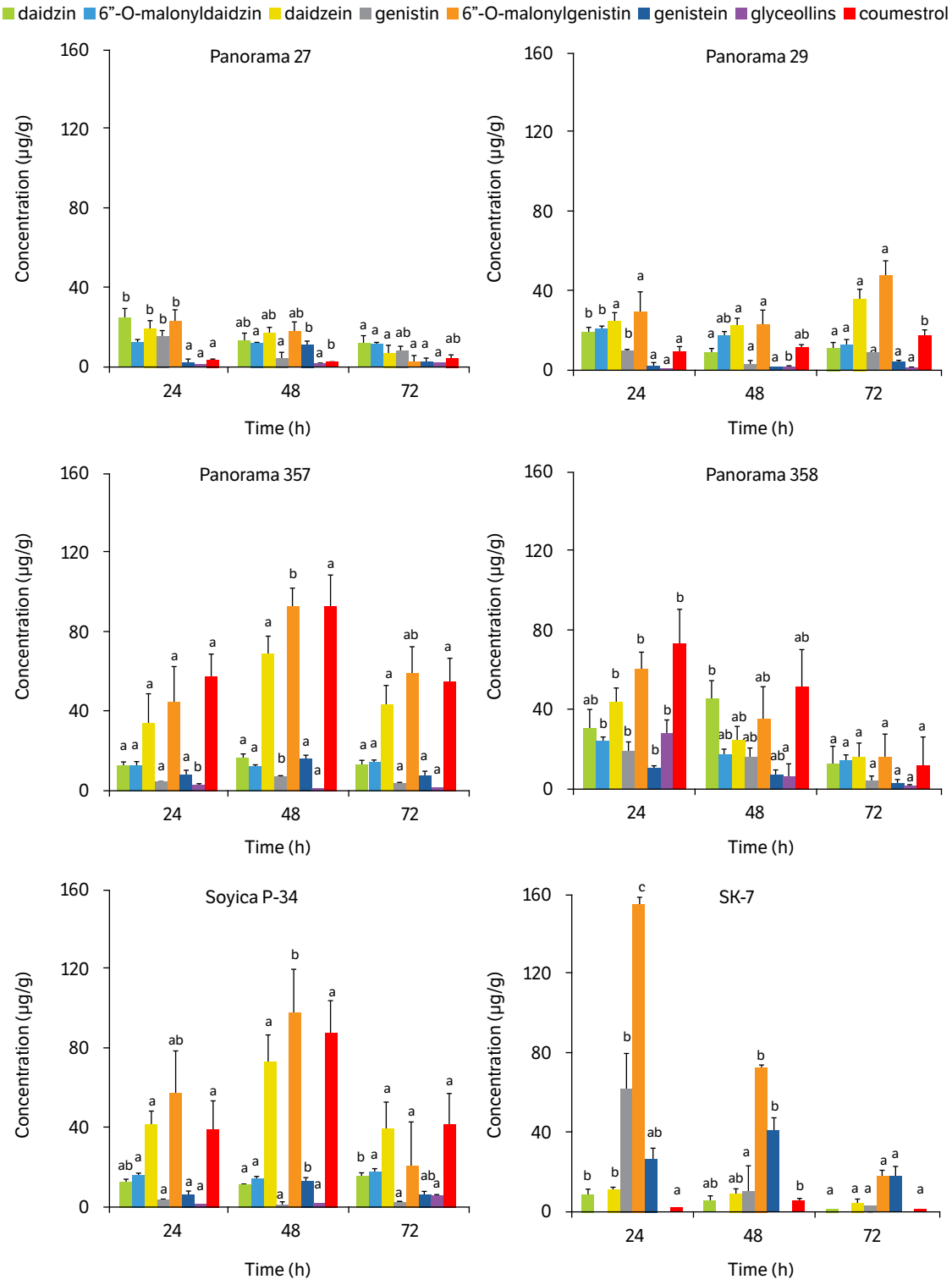
It is well known the negative impact of light and high temperatures during the storage in the total content of isoflavonoids (Zaheer and Akhtar 2017). Hence, the concentration of isoflavonoids was analyzed every 24 h during three days for soy sprouts stored at 12 °C in the darkness. The accumulation of isoflavonoids over time for each cultivar is shown in Fig. 4. The greatest accumulation of isoflavonoids was 48 h for cultivars Panorama 357 and Soyica P34. For cultivars Panorama 27, Panorama 358, and SK-7, there was a decrease in the concentration of isoflavonoids while the time increased. The highest accumulation of 6”-*O*-malonylgenistin (155.6 μg/g) and genistin (61.2 μg/g) was found for cultivar SK-7 after 24 h storage. Daidzein and coumestrol reached the maximum levels in cultivars Panorama 357 and Soyica P34 after 48 h storage. Glyceollins and daidzin showed the highest amounts in cultivar Panorama 358 after 24 and 48 h, respectively. In general, the isoflavonoid concentration was dependent on the storage time; when the storage period progresses, and consequently the growth stage, the content of isoflavonoids decreases. Similar results have been obtained by Kim et al. (2004a) and Eum et al. (2020).

In this study, however, an increasing in the isoflavonoid content was observed during the first 48 h of storage at 12 °C for cultivars Panorama 357 and Soyica P34, and during 72 h for Panorama 29. After that, the synthesis of these bioactive metabolites declined. The increases in the isoflavonoid content may be a response to cold stress, one of the most potent abiotic stressors (Swigonska et al. 2014). It has been reported that the phenolic and isoflavones in soy sprouts roots is considerably increased when it is submitted to cold conditions (Swigonska et al. 2014). Several factors, such as seed quality, seed size, temperature, humidity, sprout harvesting time, light and storage, among others, have been described to influence the growth, yield, and quality of soybean seedlings (Ghani et al. 2016). Our findings clearly indicate notable changes in isoflavonoid content depending on the cultivar evaluated. These results are consistent with previous studies, in which variations in isoflavonoid concentration depending on the germination period and cultivar were observed (Shohag et al. 2012, Lee et al. 2018, Eum et al. 2020, Wang et al. 2022).

Inducer effect of exogenous application of INA

Synthetic elicitors are molecules which have been recognized for their ability to modulate biochemical ways and activate transcription factors of genes involved in plant defense (Bektas and Eulgem 2015, Liu et al. 2019). This kind of compounds is also recognized by its capacity to enhance the accumulation of bioactive compounds in sprouts of economic importance (Liu et al. 2019). Particularly, INA is a widely used elicitor in research because it is a potent systemic acquired resistance (SAR) inducer providing high degree of protection (Bektas and Eulgem 2015). Moreover, Durango et al. (2018) reported the capacity of INA (1.6 mM) to significantly increase the amount of isoflavonoids in soybean seedlings of cv. Soyica P34.

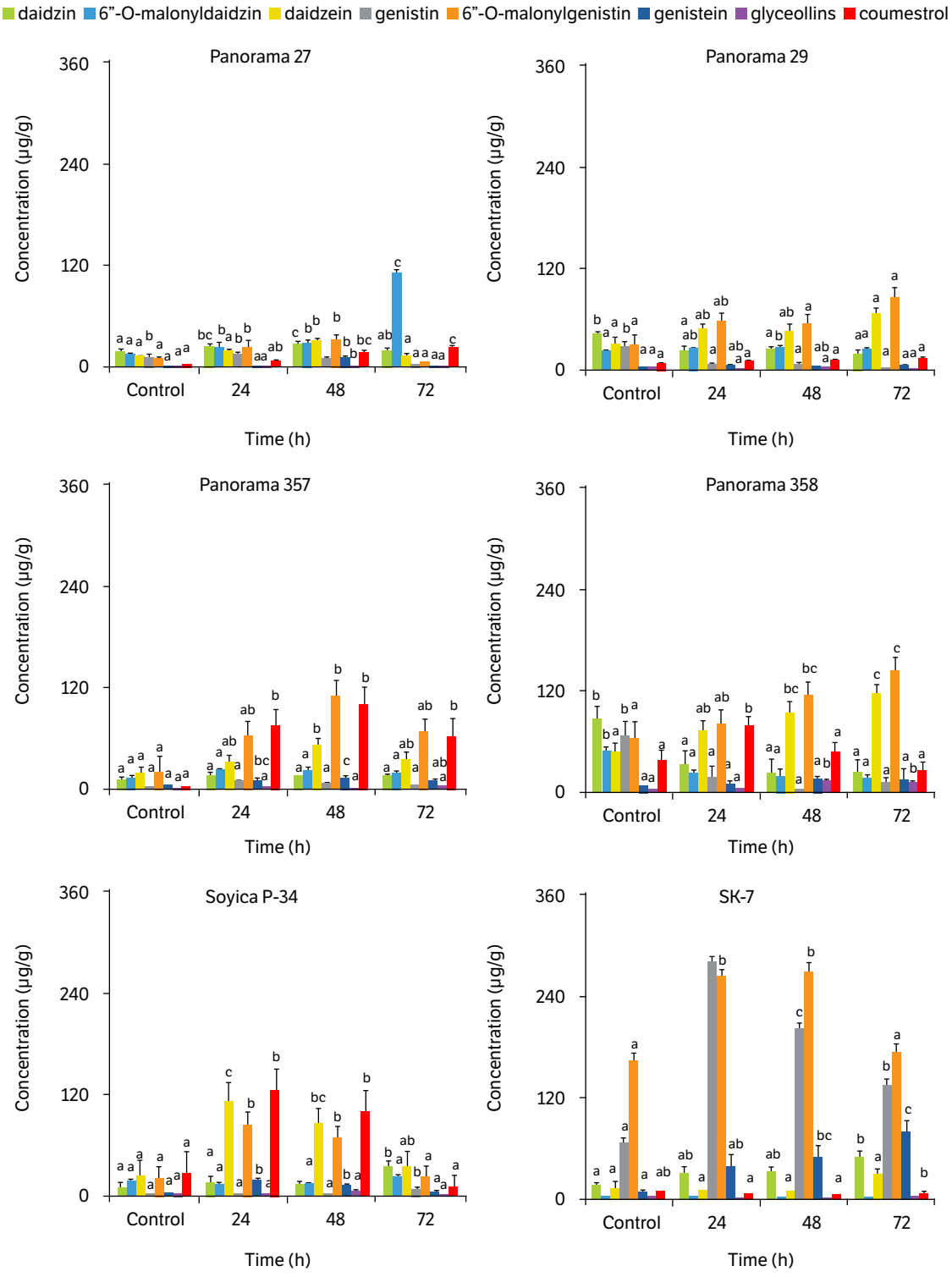
In the present study, six Colombian soybean cultivars were treated with the exogenous application of INA, and changes in the total isoflavones content were recorded each 24 hours during three days (Fig. 5). Our findings, after INA application, showed a rise in the accumulation of isoflavones in the cultivars Panorama 27, Panorama 29, Panorama 357, and Soy SK-7 in all post-induction times when they were compared to the control (soy sprout treated with water). Inversely, Panorama 358 showed a decrease after INA application in all times compared to control.



*values are presented as means ± standard deviation. Means with different letters in the same column indicate significant differences ($p < 0.05$) between treatments (Fisher's least significant difference test).

Figure 4. Isoflavonoid composition in sprouts of different soy cultivars stored at 12 °C in the darkness during 24, 48 and 72 h*.

It can be seen that alterations in the content of isoflavonoids in Colombian soybean sprouts depend on the cultivar. In cultivar Panorama 27, 6"-*O*-malonyldaidzin had the highest level at 72-h post-induction (109.5 µg/g); in Panorama 29, daidzein (65.4 µg/g), and 6"-*O*-malonylgenistin (85.1 µg/g) concentration increased with time and reached the maximum levels at 72 h.



*values are presented as means ± standard deviation. Means with different letters in the same column indicate significant differences ($p < 0.05$) between treatments (Fisher's least significant difference test).

Figure 5. Isoflavonoid composition in sprouts of different soy cultivars elicited by 1.6 mM 2,6-dichloro isonicotinic acid*.

On the other hand, Panorama 357, Panorama 358 and Soyica P34 cultivars mainly presented variations in the amounts of daidzein, 6"-*O*-malonylgenistin and coumestrol. The maximum levels of these metabolites were detected at 48-h post-induction for Panorama 357 and at 24-h post-induction for Soyica P34. Finally, SK-7 soybean cultivar showed a constant

elevation on content of 6"-*O*-malonylgenistin during the three days of analysis. Likewise, great variations in content were observed for the glucosylisoflavone, genistin, whose highest amount was found at 24-h post-induction (266.1 µg/g). In reference to glyceollins, the rise in the levels of this kind of metabolites was low. Although glyceollins are widely known as soybean phytoalexins, their content can depend on the soybean cultivar and elicitor used, and it is non-related to the total isoflavones or daidzein levels (Park et al. 2018).

There are numerous studies that have reported the increasing of isoflavones in soy after exposure to different biotic and abiotic elicitors (Aisyah et al. 2013, Saini et al. 2013). These findings are consistent with our results, that showed most of the cultivars analyzed displayed a rise in the amount of bioactive compounds. Only one cultivar, Panorama 358, presented a decrease in daidzin, 6"-*O*-malonyldaidzin and genistin content as consequence of INA application. This behavior has also been reported by several authors (Liu et al. 2019) and can be explained, in part, by the phytotoxicity effect produced by the elicitor employed (Oostendorp et al. 2001, Durango et al. 2013). In summary, elicitors emerge as an attractive alternative for enhancing the accumulation of bioactive metabolites and biological/pharmacological activities of soybean sprouts.

The changes observed can be understood considering that when the plant is stressed it develops a resistance mechanism, and as result, the production of secondary metabolites is activated. Some researchers such as Saini et al. (2013) have reported the variation in the antioxidant activity of soybean cultivars induced by different elicitors.

Taking into account that the cultivar Soy SK-7 displayed the highest concentration of isoflavonoids after induction with INA, the TPC and antioxidant activity of the extracts from this cultivar at different post-induction times were evaluated (Table 1). The highest and the lowest levels of TPC were found at 48- and 24-h post-induction, 31.8 and 12.9 mg GAE/g extract, respectively. Chen and Chang (2015) and Guzmán et al. (2017) reported phenolic content values for soybean sprouts germinated for six days of 11.59 ± 0.75 and 9.50 mg GAE/g dry base, respectively. Attempts were conducted to correlate the isoflavonoid and phenolic content and the antioxidant activities using the Pearson's correlation coefficients (r).

Table 1. Phenolic content and antioxidant activity of soybean cv. Soy SK-7 elicited by 1.60 mM 2,6-dichloro isonicotinic acid*.

Treatments	TPC mg GAE/g of extract	DPPH$^{\bullet}$ µmol Trolox/100 g of extract	ABTS$^{\bullet+}$ µmol Trolox/100 g of extract
Control	13.9 ± 0.2a	984.8 ± 156.5a	4,566.8 ± 115.6b
24 h	12.9 ± 0.7a	871.1 ± 40.0a	3,377.9 ± 266.0a
48 h	31.8 ± 0.7c	4,984.6 ± 37.2b	13,298.6 ± 562.0c
72 h	19.5 ± 0.2b	963.5 ± 19.3a	5,946.8 ± 524.8b

*Values are presented as means ± standard deviation (n = 3). Means with different letters in the same column indicate significant differences ($p < 0.05$) between treatments (Fisher's least significant difference test); TPC: total phenolic content; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid.

According to our results, the correlation between isoflavonoid and phenolic content was low, 30% ($r = 0.30$). A weak correlation between flavonoid and phenolic content in soybeans was also reported by Kuswantoro et al. (2014)[1]. The low correlation between isoflavonoid and phenolic contents may be due to the fact that other isoflavonoids (glycitein, glycitin, malonyl- and acetyl-glucoside) and phenolic compounds (quercetin, and chlorogenic, gallic, *p*-coumaric, protocatechuic and syringic acids), not quantified in this study, are present during the germination of soybean (Guzmán et al. 2017).

According to Kim et al. (2002), the ABTS$^{\bullet+}$ assay can be used in both lipophilic and hydrophilic antioxidant systems, but the DPPH$^{\bullet}$ assay is only applicable to hydrophobic systems. The analysis of radical scavenging activity showed that the highest antioxidant capacity was exhibited by the extract from the 48-h extract (4,984.6 and 13,298.6 µmol Trolox/100 g extract for the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays, respectively), followed by the 72-h one (963.5 and 5,946.8 µmol Trolox/100 g extract for the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays), although no significant differences ($p = 0.05$) were found between radical scavenging activity of the extracts from control, 24- and 72-h post-induction for the DDPH$^{\bullet}$ assay.

[1] Kuswantoro, H., Yusnawan, E. and Inayati, A. (2014). Total phenolic contents and antioxidant activities of ten soybean promising lines tolerant to acid soil. International Congress on Challenges of Biotechnological Research in Food and Health, Surakarta, Indonesia, November 2014. Slamet Riyadi University.

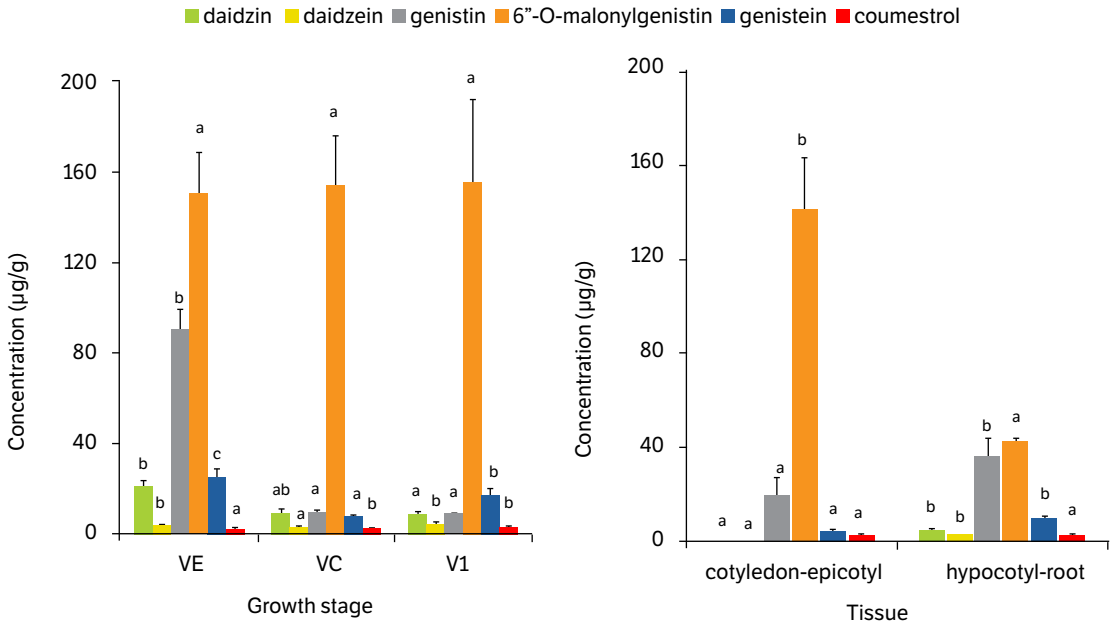
The high antioxidant activity in the extract from soybean seedlings elicited with INA and 48-h post-induction was concordant with high amounts of genistin, 6”-*O*-malonyl-genistin and genistein. In contrast, the extracts obtained in 24 and 72 h were less effective as antioxidants. In general, the correlation between isoflavonoid content and radical scavenging activity was low: 39 ($r = 0.39$) and 27% ($r = 0.27$) for DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays, respectively.

Again, the low correlation between isoflavonoids content and radical scavenging activity (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$) is due to the fact that compounds other than isoflavonoids can exhibit antioxidant activity (such as vitamin C, flavonoids, anthocyanins and phenolic acids) (Guzmán et al. 2017, Król-Grzymała and Amarowicz 2020). In fact, a high correlation was found between the total phenolic content and DPPH values, 95% ($r = 0.95$), as well as the total phenolic content and ABTS values, 99% ($r = 0.99$). Similar results were published by Devi et al. (2009) and Guzmán et al. (2017), who noticed antioxidant activity of soybean extracts also correlated well with total phenolic contents.

Effect of growth stage and tissue

Several studies have reported variations in total isoflavonoid concentration depending on the germination time (Kim et al. 2004a, Kim et al. 2004b) and the part of the germinated soybean examined (Júnior and Ida 2015, Eum et al. 2020). Therefore, and taking into account availability and high isoflavonoid production of the SK-7 cultivar, we decided to use sprouts from this variety in order to determine its isoflavonoid content in three vegetative stages (VE, VC and V1) and in different tissues (cotyledon-epicotyl and hypocotyl-root).

As can be seen in Fig. 6, the major compounds detected were 6”-*O*-malonylgenistin and genistin. The compound 6”-*O*-malonylgenistin was the major metabolite, representing more than 50% of the total isoflavonoids in all vegetative stages evaluated. Moreover, significant differences in the amount of this compound were not found in the samples analyzed.



*values are presented as means ± standard deviation. Means with different letters for the same isoflavonoid indicate significant differences ($p < 0.05$) between treatments (Fisher’s least significant difference test).

Figure 6. Isoflavonoid composition in soy sprouts (cultivar SK-7) of different growth stage and organ tissue*.

For the glycosyl-isoflavone genistin, significant differences were observed between the three growth stages. The highest amount of genistin was present in the vegetative stage VE (89.6 µg/g). The other compounds displayed low levels with concentration values below 40 µg/g. In general, the highest total isoflavonoid content was observed in the VE (289.6 µg/g) stage, followed by V1 (194.1 µg/g) and VC (183.1 µg/g). Most of the isoflavones increased their levels during the initial period of germination

and then they decreased, which is consistent with the results described by Wang et al. (2022). Differences in the total isoflavone content for various germination periods have been reported by Júnior and Ida (2015), Lee et al. (2019), and Eum et al. (2020).

In general, the amounts of glycosyl-isoflavones (genistin and daidzin) decreased with increasing germination time. According to Simons (2011a), the changes in isoflavones profile are characterized by a reduction in glycosides, except malonyl-glycosides; and a rise in aglycones such as genistein and daidzein. The deglucosylation of daidzin can cause an increase in the amount of daidzein in the soybean sprouts, in addition to the activation of the flavonoids biosynthetic route, which can promote the production of glyceollins. (Simons et al. 2011a). In fact, a significant increase in the concentration of glyceollins with the advance of germination time was found in the present study. Similarly, the decreasing in the level of genistin and genistein may afford the production of a hydroxylated genistein derivative (such as 2'-hydroxygenistein) or a prenylated genistein derivative (Simons et al. 2011b).

Regarding the tissue, the analysis displayed an isoflavonoid content value of 165.8 μg/g for the tissues cotyledon-epicotyl and 95.9 μg/g for hypocotyl-root. In general, significant differences were found between tissues for all isoflavonoids. Hypocotyl-root exhibited the highest content of isoflavonoids, excepting for 6"-*O*-malonylgenistin. These results are in agreement with those reported by Yoshiara et al. (2018) and Kim and Kim (2020), who indicated that the levels of isoflavones were higher in hypocotyl and roots than cotyledons. However, opposite results can also be found in the literature, in which authors report higher total isoflavone content values in cotyledons rather than roots and hypocotyls (Lee et al. 2007, Phommalth et al. 2008). These differences have been attributed to several causes, being the genotype of the cultivars a crucial factor (Lee et al. 2003).

In addition, the antioxidant capacity of extracts from different growth stages and some tissues of SK-7 soybean cultivar is shown in Table 2. Growth stage V1 and VC had the highest and the lowest levels of phenolic contents, which were 14.5 and 8.1 mg GAE/g dry weight of extract, respectively. The content of total phenols for hypocotyl-root and cotyledon-epicotyl were 21.1 and 17.7 mg GAE/g dry weight of extract. The cultivar analyzed showed a lower phenolic content compared to the cultivars Kalitur (81.7 mg GAE/g extract) and Alankar (65.3 mg GAE/g extract), but higher than cultivars Palam Soya (6.4 mg GAE/g extract), JS-335 (7.6 mg GAE/g extract), Macs-330 (8.4 mg GAE/g extract), JS-355 (9.8 mg GAE/g extract), among others (Prakash et al. 2007). Also, TPC for sprouts of soy SK-7 cultivar was comparable (near 25 mg GAE/g extract) to those reported by Devi et al. (2009).

Table 2. Antioxidant capacity at different growth stage and tissues in soy SK-7 cultivar*.

Group	Description	TPC mg GAE/g of extract	DPPH$^{\bullet}$ μmol Trolox/100 g of extract	ABTS$^{\bullet+}$ μmol Trolox/100 g of extract
Growth stage	VE	10.8 ± 0.1^{b}	1,017.4 ± 98.1^{b}	3,633.4 ± 202.6^{b}
	VC	8.1 ± 0.1^{a}	456.5 ± 29.7^{a}	1,828.6 ± 38.7^{a}
	V1	14.5 ± 0.1^{c}	542.1 ± 120.7^{a}	3,656.1 ± 124.9^{b}
Tissue	Cotyledon-epicotyl	17.7 ± 0.2^{A}	940.3 ± 110.7^{A}	4,040.2 ± 52.4^{A}
	Hypocotyl-root	21.1 ± 0.1^{B}	767.5 ± 343.9^{A}	6,083.4 ± 372.1^{B}

*Values are presented as means ± standard deviation (n = 3). Means with different lower-case letters or capital letters in the same column indicate significant differences ($p < 0.05$) between treatments (Fisher's least significant difference test); TPC: total phenolic content; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid.

Interestingly, the analysis of radical scavenging activity (Table 2) showed that the highest antioxidant capacity evaluated by the DPPH$^{\bullet}$ method was exhibited by the extract from the VE stage (1,017.4 μmol Trolox/100 g extract), followed by the V1 extract (542.1 μmol Trolox/100 g extract). No significant differences were found between radical scavenging activity of the extracts from VE and V1 for the ABTS$^{\bullet+}$ assay.

The lowest antioxidant activity was displayed by the extract from VC, with values of 456.5 and 1,828.6 μmol Trolox/100 g extract for the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays, respectively. It is important to notice that the VC growth stage was also the vegetative stage with the lowest TPC (8.1 mg GAE/g of extract) and isoflavonoid (183.1 μg/g) content.

ABTS$^{\bullet+}$ assay (Table 2) showed that extract from hypocotyl-root (6,083.4 μmol Trolox/100 g extract) exhibits higher activity than cotyledon-epicotyl (767.5 μmol Trolox/100 g extract). No significant differences were found in the DPPH$^{\bullet}$

assay for the extracts from cotyledon-epicotyl and hypocotyl-root. Our results contrast with those of Yoshiara et al. (2018) and Kim and Kim (2020), who reported that radical scavenging activities (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$) were significantly higher in the cotyledons and epicotyls of soybean sprouts than in the hypocotyl and root tissues. This apparent contradiction may be associated with the soybean cultivar analyzed.

Taking into account both the growth stage and tissue, the correlations between TPC and radical scavenging activity were 32% (r = 0.32) for DPPH$^{\bullet}$ and 92% (r = 0.92) for ABTS$^{\bullet+}$. Both DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays are based on the ability to donate electrons or hydrogen atoms. The solubility of compounds with antioxidant capacity may be a factor that contributes to obtaining higher values in the ABTS$^{\bullet+}$ test compared to the DPPH$^{\bullet}$ test. The radical cation ABTS can be quenched by both lipophilic and hydrophilic compounds, that are soluble in the reaction medium, which in soybeans can be represented by phenols, polysaccharides, uronic acids, and soluble proteins (Lee et al. 2018). In addition, the lower DPPH$^{\bullet}$ radical scavenging values in comparison to ABTS$^{\bullet+}$ values are associated with the steric hindrance of the DPPH$^{\bullet}$ radical.

CONCLUSION

Soybean sprouts are a desirable source of nutrients and bioactive metabolites. Due to the scarce information available about composition and antioxidant activity of Colombian soy cultivars, in the present work we analyzed the isoflavonoid and phenolic content and radical scavenging activity (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays) on sprouts of six soy cultivars. Likewise, isoflavonoid content variation depending on storage time, growth stage and tissue was determined, as well as its variation before and after elicitation with INA. In general, the isoflavone content can be considered low and it is dependent on the cultivar and the storage time. 6"-*O*-malonylgenistin and the glucosyl derivatives were the major constituents in the cultivars. The highest levels of bioactive compounds were found in V1 stage and hypocotyl-root tissue and presented positive correlation with their antioxidant activity. Results also showed that the concentration of isoflavonoids and antioxidant activity were significantly increased by the elicitation with INA.

To the best of our knowledge, this is the first report about characterization of isoflavonoid constituents in sprouts from soybean cultivars grown in Colombia. Our findings provide useful information, which might be applied to maximize therapeutic, nutritional, and agronomical benefits of soybean sprouts.

AUTHORS' CONTRIBUTION

Conceptualization: Durango, D. L.; **Funding acquisition:** Durango, D. L.; **Investigation:** Gómez, K. D., Durango, D. L. and Parra-González, V.; **Supervision:** Marín-Loaiza, J. C. and Gil, J.; **Writing – original draft:** Gómez, K. D. and Durango, D. L.; **Writing – review & editing:** Marín-Loaiza, J. C., Durango, D. L. and Gil, J.; **Formal analysis:** Parra-González, V.; **Resources:** Marín-Loaiza, J. C. and Gil, J.

DATA AVAILABILITY STATEMENT

Data will be available from the corresponding author upon request.

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