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Effect of germination and elicitation on phenolic composition and bioactivity of kidney beans

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ABSTRACT

Ready-to-eat sprouts are becoming popular healthy fresh foods. Germination and elicitation may be used to improve phytochemical and functional quality of sprouts. The aim of this study was to unveil the efficacy of germination and the use of elicitors (500 μM ascorbic, 50 μM folic and 5 mM glutamic acid) to enhance the phenolic composition and antioxidant and angiotensin I converting enzyme (ACE) inhibitory activities of 8 day-old *Phaseolus vulgaris* L. var. Pinto sprouts. Sprouting produced a general decrease in flavan-3-ols and anthocyanins of sprouts that was compensated with a higher content of flavanones and flavonols. Although elicitor treatments reduced total phenolic content of sprouts, they promoted the synthesis of specific flavanones and flavonol *O*-glycosides, effect that was dependent on the type of elicitor. Antioxidant activity was not affected by ascorbic and folic acid treatments whereas it was slightly reduced in glutamic acid-treated sprouts. Folic acid was the only treatment that caused an outstanding increase in the ACE inhibitory activity of sprouts. In conclusion, elicitation may produce positive changes in the phenolic profile and improve health-promoting potential of sprouts, although selection of elicitor is crucial to deliver marketplace ready to eat sprouts enriched in specific bioactive phytochemicals.

Keywords: kidney beans, germination, elicitors, phenolic compounds, flavonoids, ACE inhibition activity, antioxidant activity.

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1. Introduction

Nowadays, legumes are gaining considerable interest in developed countries because there is a positive association between their consumption and the reduction of the risk of cardiovascular diseases, obesity, diabetes type 2 and cancer (Rondini, Barrett, & Bennink, 2013; Bouchenak, & Lamri-Senhadji, 2013; Rebello, Greenway, & Finley, 2014). Health benefits of legumes are attributed to their nutritional composition. Nutritionally, legumes are rich in protein, carbohydrates, dietary fibre, minerals and vitamins. In addition, legumes also contain a rich variety of phytochemicals, such as phenolic compounds, which are closely associated to beneficial effects in human health (Duranti, 2006). Phenolic compounds are the major antioxidants in legumes that play a key role in limiting the effects of cellular and molecular damages by reducing reactive oxygen species (Theriault, Caillet, Kermasha, & Lacroix, 2006) involved in the development of many diseases and aging. Besides antioxidant activity, *in vitro* and animal studies have shown that phenolic compounds may exhibit other positive effects such as anti-inflammatory, anti-hypertensive, anti-atherosclerotic and cytotoxic antitumor activities (del Rio *et al*., 2013).

Legume sprouts are extensively accepted since they are considered fresh and healthy products from natural origin which provide a source of bioactive compounds (Donkor, Stojanovska, Ginn, Ashton, & Vasiljevic, 2012). Germination is a cost-effective process that improves the nutritional quality of legumes by increasing protein digestibility and free amino acids content (Alonso, Aguirre & Marzo, 2000; Gravidel & Prakash, 2007). Legume sprouting has also been suggested as a powerful strategy to increase total antioxidant activity (Fernández-Orozco et al., 2006). However, phenolic content and composition, often related to their antioxidant properties, depend on the type of legume and germination conditions (López-Amorós, Hernández, & Estrella, 2006). There are no

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reports concerning the influence of elicitation on the protein quality of sprouts. Recent studies have shown that abiotic elicitation of lentil decrease protein content while increasing free amino acids and peptides (Swieca & Baraniak, 2014; Swieca, Baraniak & Gawlik-Dziki, 2014). Elicitation is a good strategy to induce the synthesis of different classes of bioactive secondary metabolites. Efficacy of this treatment depends mainly on plant genetics, elicitor nature and dose (Baenas, García-Viguera, & Moreno 2014). Several studies (reviewed by Baenas, García-Viguera, & Moreno 2014) have used pre-elicitor treatments to affect the phenolic composition of different sprouts and only few of them have focused on legume seeds (Burguieres, McCue, Kwon, & Shetty, 2007, 2008; Limon, Peñas, Martínez-Villaluenga, & Frias, 2014; Liu, Cao, Huang, Guo, & Kang, 2013). Burguieres et al. (2007) demonstrated that soaking pea seeds for 12-48 h in ascorbic (500 μM) and folic (50 μM) acids solutions results in increased total phenolic content and antioxidant activity in 8-day old sprouts. These changes in the phenolic composition were also related to an increased bioactivity of the phenolic extracts including inhibition of angiotensin I converting enzyme (ACE) and gastrointestinal α-amylase and α-glucosidase enzymes, which are molecular targets for the management of hypertension and diabetes, respectively (Burguieres, McCue, Kwon, & Shetty, 2008). Similarly, Liu *et al*. (2013) reported that ethylene (100 mg/L) improved total phenolic content and free radical scavenging-linked activity in mung bean sprouts. More recently, our research group found that kidney bean seeds treated with glutamic (5 mM), folic (50 μM) or ascorbic (500 μM) acid during germination for 8 days showed an enhanced content of total phenolic compounds and γ-aminobutyric acid as well as antioxidant and ACE inhibitory activities (Limon, Peñas, Martínez-Villaluenga, & Frias, 2014). There is still a knowledge gap concerning the effect of elicitation during kidney bean germination on the concentration of individual phenolic

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compounds of kidney bean sprouts and how these changes in the phenolic composition affect different bioactivities of the phenolic fraction. Therefore, the aim of this study was to evaluate the effect of germination and elicitation of kidney bean seeds on the phenolic composition and the antioxidant and ACE-inhibitory activities of phenolic extracts. This study will bring new insights to fresh produce industry to obtain healthier products by improving phenolic composition with specific active compounds that have functional properties.

2. Materials and methods

2.1. Chemicals

Acetonitrile, formic acid and ultrapure water were of HPLC grade from Carlo Erba (Rodano, Italy) and Panreac (Barcelona, Spain). Phenolic compounds standards were purchased from Sigma-Aldrich (Madrid, Spain) and Extrasynthese (Barcelona, Spain). Tripeptide Abz-Gly-Phe(NO_2)-Pro was purchased from Cymit-Quimica (Barcelona, Spain). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified.

2.2. Plant material

Light speckled kidney beans (*Phaseolus vulgaris* var. Pinto) were purchased from Semillas Iglesias S.A. (Salamanca, Spain). Seeds were stored in polyethylene containers at 4 ºC and cleaned before germination process.

2.3. Elicitor solutions

Elicitor solutions of 500 μM ascorbic acid, 50 μM folic acid and 5 mM glutamic acid were selected according to previous studies (Limón et al., 2014). Elicitor solutions were freshly prepared before daily application.

2.4. Germination conditions

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Kidney beans were germinated as described by Limon et al. (2014). Germination was performed at 20 ºC, 95% of relative humidity in darkness for 8 days. Seeds were irrigated daily with fresh solutions of elicitors or distilled water (control). Each treatment was performed in triplicate and sprouts were collected at day 8 of germination for analysis. Sprouts were freeze-dried, milled, and stored at -20 ºC in vacuum bags until further analysis.

2.5. Extraction of phenolic compounds

Freeze-dried samples (5 g) were macerated in methanol:TFA ($1^0/_{000}$)-water 80:20 (v/v) at 4ºC for 16 h. Subsequently, they were centrifuged at 4000 *g* and 5ºC for 20 min in a super-speed centrifuge (Sorval RC 5B). The extraction process was repeated twice. The extracts were combined and concentrated at 30 ºC under vacuum for methanol evaporation.

For phenolic analysis, the dry extracts were dissolved in 10 mL of water. For purification, an aliquot (4 mL) was passed through a C18 Sep-Pak cartridge (Waters, Milford, MA, USA), previously activated with methanol followed by water. Sugars and polar substances were removed by passing 10 mL of ultrapure water and phenolic compounds were eluted with methanol. Afterwards, extract was concentrated under vacuum in a rotary evaporator (30 ºC) and then dissolved in aqueous 0.1% TFA:acetonitrile (90:10 v/v) for phenolic compounds analysis.

2.6. HPLC-DAD-ESI/MS^n qualitative and quantitative analyses of phenolic compounds

2.6.1. Non-anthocyanin compounds. Samples were analyzed using Hewlett–Packard 1100MS (Agilent Technologies, Palo Alto, CA) chromatograph equipped with a quaternary pump, diode array detector (DAD) coupled to an HP Chem Station (rev.A.0504) data-processing station. Solvents used were 0.1% formic acid in water

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(solvent A), and 100% acetonitrile (solvent B). The elution gradient established was 15% B for 5 min, 15-20% B for 5 min, 20-25% B for 10 min, 25-35% B for 10 min, 35-50% B for 10 min, and re-equilibration of the column. The separation of phenolic compounds was performed in a Spherisorb S3 ODS-2 C8 column (Waters, Millford, USA) (3 μm, 150 mm×4.6 mm i.d.) operating at 35 °C and a flow rate of 0.5 mL min^{-1}. Double online detection was carried out in the DAD using 280 nm and 370 nm as preferred wavelengths. Mass spectrometer (MS) connected to the HPLC system via the DAD cell outlet was used and detection was performed in an API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) equipped with an ESI source, triple quadrupole-ion trap mass analyzer and controlled by the Analyst 5.1 software. The setting parameters were: zero grade air as the nebulizer gas (30 psi), turbo gas for solvent drying (400 ºC, 40 psi), nitrogen served as the curtain (20 psi) and collision gas (medium). The quadrupoles were set at unit resolution. The ion spray voltage was set at -4500 V in the negative mode. The MS detector was programmed to perform a series of two consecutive modes: enhanced MS (EMS) and enhanced product ion (EPI) analysis. EMS was employed to show full scan spectra, to give an overview of all the ions in sample. Settings used were: declustering potential (DP) -45 V, entrance potential (EP) -6V, collision energy (CE) -10 V. Spectra were recorded in negative ion mode between m/z 100 and 1000. EPI mode was further performed with the aim to obtain the fragmentation pattern of the parent ion(s) of the previous experiment using the following parameters: DP: -50 V, EP: -6 V, CE: -25 V, and collision energy spread (CES) 0 V.

The non-anthocyanin phenolic compounds were characterized according to their UV and mass spectra and retention times, and comparison with authentic standards when available. For quantitative analysis, calibration curves were prepared by injection of

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known concentrations of different standard compounds. Aldaric, quinic and hexosides derivatives of *p*-coumaric, ferulic and sinapic acids were quantified by the curves of the corresponding free acids; (+)-catechin-*O*-hexoside and (+)-catechin-*O*-acylhexoside based on the curve of (+)-catechin; flavonols, derivatives of kaempferol, quercetin and isorhamnetin by the curves of kaempferol-3-*O*-glucoside, quercetin-3-*O*-glucoside and isorhamnetin 3-*O*-rutinoside, respectively. Flavanones, eriodictyol and naringenin derivatives were quantified by the curve of naringenin; and hesperetin derivatives as hesperitin. Results were expressed as μg/g of sprout d.m.

2.6.2. Anthocyanins. Samples were analyzed in an AQUA® HPLC system (Phenomenex) equipped with a reverse phase C18 column (5 μm, 150 mm × 4.6 mm i.d) at 35 ºC according to Garcia-Marino, Hernández-Hierro, Rivas-Gonzalo, & Escribano-Bailón, (2010). Detection was carried out at 520 nm. MS was performed in the same equipment described above. Zero grade air served as the nebulizer gas (40 psi) and turbo gas (600 ºC) for solvent drying (50 psi). Nitrogen served as the curtain (100 psi) and collision gas (high). Both quadrupole units were set at unit resolution. The ion spray voltage was set at 5000 V in the positive mode. EMS and ESI methods were used for acquisition of full scan spectra and fragmentation patterns of the precursor ions, respectively. Setting parameters used for EMS mode were: declustering potential (DP) 41 V, entrance potential (EP) 7.5 V and collision energy (CE) 10 V. Parameters for EPI mode were: DP 41 V, EP 7.5 V, CE 10 V, and collision energy spread (CES) 0 V. For the quantitative analysis of anthocyanins a calibration curve was obtained by injection of different concentrations of cyanidin 3-*O*-glucoside, malvidin 3-*O*-glucoside and pelargonidin 3-*O*-glucoside. The results were expressed in μg/g of sprout.

2.7. Antioxidant activity

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Oxygen Radical Absorbance Capacity (ORAC-FL) was determined by fluorescence in methanolic extracts as described by Torino et al. (2013). Fluorescence was measured at λ_{exc} 485 nm and λ_{emi} 485 nm every minute for 150 min using a microplate reader (Synergy HT, BioTek Instruments, Inc Vermont, USA). The equipment was controlled by Gen 5^{TM} software (version1.1.). Trolox was used as standard. Results were expressed as μg Trolox equivalents (TE)/g extract.

2.8. Angiotensin-I converting enzyme (ACE) inhibitory activity

ACE inhibitory activity was determined in methanolic extracts according to the method described by Martínez-Villaluenga et al. (2012). IC_{50} values (concentration of sample that inhibits 50% of the ACE activity) were determined by dose-response curves in which the range of concentrations was distributed in a logarithmic scale and using a sigmoidal curve fit in GraphPad Prism 4.00 (Graphpad Software Inc., San Diego, CA, USA).

2.9. Statistical analysis

Data were expressed as mean ± standard deviation of three independent germination experiments. Data were subjected to one-way analysis of variance (ANOVA) and significant differences between samples were determined by the least significant differences (LSD) test at $p \leq 0.05$ probability level, using PASW Statistics 18.0 software (IBM, Armonk, NY, USA). Principal Components Analysis (PCA) was performed with covariance matrix obtained by data transformation using also PASW Statistics 18.0 software.

3. Results

3.1 Identification of phenolic compounds in kidney bean seeds and sprouts. Identification and quantification of individual phenolic compounds of kidney bean

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(*Phaseolus vulgaris* L. var. pinto) seeds and sprouts were performed by HPLC-DAD-MS^n analysis. The retention time, λ_{max}, molecular ion and their fragment ion pattern allowed the identification of 38 phenolic compounds belonging to hydroxybenzoic and hydroxycinnamic, flavan-3-ol, flavanone, flavonol and anthocyanin phenolic classes (Table 1).

3.1.1. Hydroxybenzoic and hydroxycinnamic compounds. A large group of hydroxycinnamic acid derivatives was identified (Table 1). Nine compounds (peaks 3-6, 8-10, 12 and 17) were detected as aldaric acid (glucaric or galactaric acid) derivatives according to their UV-vis spectra (λ_{max} at 312-328 nm) and precursor ions$[M-H]^-$ at *m/z* 355, 385 and 415, all of them with a fragment ion at *m/z* 209, corresponding to an aldaric acid residue. No information about the identity of aldaric acid moieties and location onto the acid was obtained; therefore, these compounds were assigned to isomers of *trans*-*p*-coumaroylaldaric, *trans*-feruloylaldaric and sinapoylaldaric acid.

Other compounds were identified as feruloyl hexoside acid and *p*-coumaroyl acetylhexoside acid (peaks 1 and 16) based on their UV-vis spectra with maximum at 328-330 nm, precursor ions $[M-H]^-$ (at *m/z* 355 and 367, respectively) and fragment ions at *m/z* 193 and 163 corresponding to the loss of an hexose unit and acetyl hexoside unit respectively. Three compounds (peaks 18, 24 and 27) showed an UV-vis spectrum similar to ferulic acid with λ_{max} at 326-330 nm, but eluted at different retention times. They presented precursor ions $[M-H]^-$ at *m/z* 367 and fragments at *m/z* 191 and 193, due to the deprotonated quinic acid and [ferulic acid –H]$^-$, respectively. *p*-Coumaroyl (peak 15) and sinapoyl acid derivatives (peaks 19 and 21) were also detected by their typical UV-vis spectra and fragments at *m/z* 163 and 223, ([*p*-coumaric acid –H]$^-$ and [sinapic acid-H]$^-$, respectively). These compounds could not be fully identified. *p*-

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Hydroxybenzoic (peak 13) and ferulic acids (peak 25) were identified by comparison of their UV-vis spectra and retention times with commercial standards.

3.1.2. Flavan-3-ols. (+)-Catechin (peak 11) was identified by comparison of their UV spectra and retention time with a commercial standard. (+)-catechin-*O*-hexoside (peak 2) and (+)-catechin-*O*-acetylhexoside (peak 7) were detected on the basis of their UV spectrum similar to (+)-catechin and $[M-H]^-$ at *m/z* 451 and 493, respectively. Fragment ions corresponded to the loss of hexosyl and the acetylhexoxyl moieties. In none of them the identity of the sugar and location of the substituents could be established.

3.1.3. Flavanones. Peaks 14, 20, 22, 23, and 33 presented a typical UV-vis spectra of flavanones. Peak 20 was identified as eridictyol-7-*O*-glucoside by comparison with a commercial standard. Peaks 14 and 33 were assigned to eriodictyol-*O*-hexoside and eriodictyol-*O*-acetylhexoside, respectively, according to their $[M-H]^-$ at 449 and 491, respectively, and fragments corresponding to the losses of hexoxyl moieties (-162 uma) in peak 14, and the acetylhexoside residue (-162+42 uma), in the peak 33. In none of them the identity of the sugar and location of the substituents could be established. Other detected flavanone was hesperetin-*O*-glucuronyl-hexoside (peak 22) on the basis of their mass spectra with $[M-H]^-$ at *m/z* 639, and fragment ion at *m/z* 301 due to the loss of glucuroyl-hexoside moiety. Peak 23 presented UV-vis spectra similar to naringenin, but it eluted at a different retention time. No clear signal associated to its molecular ion could be obtained, although ion at *m/z* 271 (possibly naringenin) was observed using ESI detection. Since this compound eluted earlier than the naringenin standard, it was tentatively identified as naringenin derivative.

3.1.4. Flavonols. Quercetin derivatives (peaks 26, 28, 29 and 32) were found in seeds and sprouts, which presented similar UV spectra (λ_{max} at 356 nm) to quercetin. Peak 26 (quercetin 3-*O*-rutinoside) and peak 28 (quercetin 3-*O*-glucoside) were identified

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according to their retention time, mass and UV-vis characteristics by comparison with commercial standards. Quercetin-*O*-acetylhexoside (peak 29) and quercetin-*O*-hexoside (peak 32) were identified according to $[M-H]^-$ at *m/z* 505 and 463, respectively. Fragment ion at *m/z* 301 corresponded to quercetin. Kaempferol derivatives were also observed according to their UV-vis and mass spectra with fragment ions at *m/z* 285. Peaks 30 and 31 were identified as kaempferol-3-*O*-rutinoside and kaempferol-3-*O*-glucoside, respectively, by comparison with commercial standards. Isorhamnetin derivative (peak 34) showed a UV-vis spectra with maximum at 355 nm, a precursor ion $[M-H]^-$ at *m/z* 519, an unique fragment ions at *m/z* 315 (corresponding to isorhamnetin), due to the loss of -hexosyl+acetyl residues, which allowed its identification as isorhamnetin-*O*-acylhexoside.

3.1.5. Anthocyanins. Several anthocyanins were detected in kidney bean seeds and sprouts. Peaks 35 and 36 were identified as cyanidin-3-*O*-glucoside and pelargonidin-3-*O*-glucoside on the basis of their retention time, mass and UV-vis features by comparison with commercial standards. Peak 37 showed a characteristic UV-vis spectra with maximum at 504 nm of pelargonidin derivative, $[M+H]^+$ at *m/z* 519, and fragment at *m/z* 271 ($[M-248]^+$), due to the loss of a malonylglycoside moiety. This information allowed its identification as pelargonidin-3-*O*-malonylglucoside. Peak 38 showed λ_{max} at 534 nm characteristic of malvidin derivative. The presence of malvidin as anthocyanidins in this peak was confirmed by its mass spectrum, which showed MS^2 signal at *m/z* $[M]^+$ 331. This peak was tentatively identified as malvidin derivative.

3.2. Effect of germination and elicitation of kidney bean on phenolic profile

Results showed significant differences in the phenolic profile between kidney bean seeds and sprouts (Table 2). Hydroxycinnamic compounds were the most abundant phenolic class detected in kidney bean seeds and sprouts accounting for approximately

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50% of the total phenolic content (Figure 1). Kidney beans included hydroxycinnamic compounds in free forms (ferulic acid) and those linked to hydroxyacids and sugars, being synapoyl aldaric acid the main compound of this group in seeds and sprouts. Elicitation treatments decreased the concentration of a large number of these compounds on the sprouts ($P<0.05$). However, ascorbic acid treatment enhanced by 72% the content of synapoyl acid derivative compared to control ($P<0.05$).

The hydroxybenzoic compounds in the form of *p*-hydroxybenzoic acid were found at the same concentration in seeds and sprouts (Table 2). Elicitation caused a significant reduction of the content of this compound, which was not detected in elicited sprouts

The most outstanding variations found as consequence of germination were those occurred in the flavonoid group. Flavan-3-ols, the main group of flavonoids in kidney bean seeds, decreased significantly in 8-day old sprouts ($P<0.05$). (+)-catechin-*O*-acylhexoside significantly increased in comparison to seeds being the main flavonoid in kidney bean sprouts. (+)-Catechin and catechin glycosides contents decreased significantly in elicited sprouts ($P<0.05$) with the exception of (+)-catechin-*O*-acylhexoside in those samples elicited with glutamic acid ($P>0.05$). Similarly to control, (+)-catechin-*O*-acylhexoside was the most abundant flavan-3-ol in all elicited sprouts.

Total flavanones and flavonol groups increased considerably in sprouts ($P<0.05$) (Figure 1). Control sprouts were richer in hesperetin-*O*-glucuronide hexoside, naringenin derivative, and eridictyol-*O*-hexoside 2 as well as in kaempferol and quercetin glycosides than ungerminated seeds (Table 2). Regarding flavanones, hesperitin-*O*-glucuronide hexoside was the most abundant compound in elicited sprouts, regardless the elicitor type. Elicitation reduced eriodictyol-*O*-hexoside 1, hesperitin and naringenin glucosides compared to control ($P<0.05$). On the contrary, elicited sprouts were higher in eriodictyiol-*O*-hexoside 2 (94%-118% increase) than control ($P<0.05$).

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Interestingly, eriodictyol-*O*-acylhexoside increased by 65% and 112% in sprouts elicited with folic and glutamic acids. Total flavonol content was also reduced by 67%, 51% and 35% in sprouts elicited with ascorbic, folic and glutamic acids, respectively, in comparison to control ($P<0.05$). Although total flavonol content was lower in elicited sprouts, it must be emphasized that folic and glutamic acids increased several flavonol compounds compared to control (Table 2). After folic acid treatment, kaempferol-*O*-acylhexoside was increased by 29.2% in sprouts ($P<0.05$). Kidney bean sprouts elicited with glutamic acid improved quercetin 3-*O*-glucoside, kaempferol-*O*-acylhexoside and isorhamnetin-*O*-acylhexoside contents that increased by 136%, 100% and 58%, respectively ($P<0.05$).

Total and individual anthocyanin content were reduced as a result of germination with the exception of pelargonidin-3-*O*-malonylglucoside that increased in control sprouts ($P<0.05$; Table 2). Germination in the presence of elicitors brought about a more extensive decrease of total and individual anthocyanin content to levels below the quantification limit.

Considerable variation in the total phenolic content was observed among elicited sprouts (Table 2). Seed treatment with ascorbic, folic and glutamic acids caused a significant decrease (64%, 57% and 44%, respectively) of total phenolic content in sprouts compared to control ($P<0.05$; Table 2).

Changes in the total and individual phenolic content of sprouts due to germination and elicitation resulted in a considerable variation in the percentage of distribution of phenolic groups. Figure 1 shows the percentage of distribution of different phenolic groups to the total phenolic content. All elicited sprouts showed an absence of hydroxybenzoic acids and anthocyanins. Sprouts treated with ascorbic acid showed a higher percentage of hydroxycinnamic acids (59.1% of the total content) and lower of

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catechins (17.4 % of the total content) than control ($P<0.05$). Folic acid produced sprouts with lower percentage of hydroxycinnamic acids (44% of the total phenolic content) and higher of flavonoids from catechin, flavanone and flavonol groups (29%, 10% and 16.9% of the total phenolic content) compared to the control. Finally, elicitation with glutamic acid increased the proportion of total flavonols (17.4% of the total phenolic content) compared to control.

3.3. Effect of germination and elicitation on antioxidant activity of phenolic extracts from kidney bean sprouts

Antioxidant activity of phenolic extracts obtained from kidney bean seeds and sprouts is presented in Figure 2. Antioxidant activity of phenolic extracts from control sprouts was significantly similar to seeds ($P>0.05$). Phenolic extracts from sprouts elicited with ascorbic and folic acids showed similar ORAC values than control extracts ($P>0.05$). In contrast, a slight reduction (14%) of the antioxidant activity was observed in phenolic extracts from sprouts treated by glutamic acid ($P<0.05$).

3.4. Effect of germination and elicitation on angiotensin converting enzyme inhibitory activity of phenolic extracts from kidney bean.

ACE inhibitory activity of phenolic extracts from seeds, non-elicited (control) and elicited sprouts is shown in Figure 2. Considerable variation was observed in the ACE inhibition of phenolic extracts among samples. Phenolic extracts from control sprouts showed the lowest ACE inhibition ($P<0.05$). Lower ACE inhibitory activity of the phenolic extract from control sprouts compared to that from the seeds indicates that germination reduced the ACE inhibitory activity of the phenolic fraction. ACE inhibition values of extracts from elicited sprouts treated with ascorbic acid were higher than control and similar to seeds. Interestingly, phenolic extracts from sprouts treated by

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folic and glutamic acids were more effective inhibiting ACE (88% and 62% inhibition, respectively) than extracts from sprouts elicited with ascorbic acid ($P<0.05$).

3.5. Principal Component Analyses (PCA).

PCA was carried out to determine whether there were patterns of bioactivity and total content of phenolic groups which could be associated with an elicitor treatment (Figure 3A). Seven components were obtained of which the first two ones accounted for 80.3 % of the total variance. PC1 primarily separates sprouts treated with elicitors from seeds and control sprouts. Moreover, PC2 separates ascorbic and folic acid treated sprouts from glutamic acid sprouts. The variable projection was useful in determining which phenolic groups or bioactivities are most differentiating. For PC1, ACE inhibitory activity and hydroxycinnamic, hydroxybenzoic, anthocyanin and catechin contents were more dominant in separating all elicited sprouts from control sprouts and seeds. PC2 was positively correlated with flavonols and flavanones. It is worth to emphasize that total content of catechins, hydroxycinnamic and hydroxybenzoic compounds were positively related to ORAC.

A second PCA analysis was performed taking into consideration the percentage of distribution of different phenolic groups and bioactivity of the phenolic fraction normalized to total phenolic content (Figure 3B). Two principal components (PC1 and PC2) explaining 89.53% of data variations were obtained. As it can be seen in the graph, PC1 was mainly involved in the separation of seeds and germinated samples. Among germinated samples, there was a clear separation between elicited and non-elicited samples. Elicited sprouts showed negative values for PC1 whereas control sprouts and seeds showed positive values for this component. PC1 was positively related to hydroxybenzoic and anthocyanins and negatively related to flavanones, flavonols, ORAC and ACE inhibitory activities. On the contrary, PC2 separated elicited

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samples as function of elicitor type. Ascorbic acid-elicited sprouts exhibited negative values for this component, whereas folic and glutamic acid-elicited sprouts appeared on the half top of the graph showing positive values for PC2. PC1 was positively related to catechins and negatively to hydroxycinnamic compounds. As seen in the variable projection, percentage of flavanones and flavonols in the phenolic fraction was positively correlated to ORAC and ACE inhibitory activities.

These results emphasized the influence of germination and elicitation on phenolic composition and bioactivity of kidney bean extracts. In addition, the observed effects were dependent on the type of elicitor used.

4. Discussion

Optimizing the phenolic composition of foods has attracted interest as a cost-effective strategy to improve nutrition and disease prevention that would not represent any added costs for the health sector. Specific treatments such as germination alone or in combination with precursor feeding and elicitor application can be used to increase metabolite production in the sprouts and enhance its nutritional value (Smetanska, 2008; Poulev et al., 2003).

Beans are good sources of phenolic compounds (Hayat, Ahmand, Masud, Ahmed, & Bashir, 2014). The present study showed that total phenolic content of kidney bean var. Pinto was in the range of that reported for other bean varieties (19.1-48.3 mg/100g) (López-Amorós, Hernández, & Estrella, 2006; Luthira & Pastor Corrales, 2006; Lin, Harnly, Pastor-Corrales, & Luthria, 2008). Phenolic fraction was composed mostly of synapoyl acid derivatives (hydroxycinnamic acids) unlike other bean varieties in which ferulic aldaric acid derivatives are the most abundant phenolic compounds (Lin, Harnly, Pastor-Corrales, & Luthria, 2008; López et al., 2013). Flavonoids were also detected mainly as catechin aglycone and *O*-glycoside. Flavanones (eriodictyol, hesperitin and

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naringenin glycosides), flavonols (kaempferol and quercetin glycosides) and anthocyanins (pelargonidin, cyanidin and malvidin glycosides) were also present as minor compounds. All these flavonoids were previously reported in different bean varieties (Lin, Harnly, Pastor-Corrales, & Luthria, 2008; López et al., 2013; Macz-Pop, Rivas-Gonzalo, Pérez-Alonso, & González-Paramás, 2006). Proanthocyanidins were not detected in kidney bean var. Pinto although they were described in other bean varieties (López et al., 2013; Aguilera, Estrella, Benítez, Esteban, & Martín-Cabrejas, 2011). Our results indicated that total phenolic content of kidney bean remained stable after 8 days of germination, contrary to earlier studies showing an increase in the total phenolic content of legumes after germination (Dueñas, Hernández, Estrella, Fernández, 2009; Shohag, Wei, & Yang, 2012; Tajodding, Manohar, & Lalitha, 2014).

Regarding flavonoid groups, germination brought about a general decrease in flavan-3-ols and anthocyanins that was compensated with a higher content of flavanones and flavonols. These changes in the phenolic composition could be explained by the activation of phenolic metabolism (Khandelwal, Upidi, & Ghugre, 2010) being dependent on legume type and germination conditions (López-Amorós, Hernández, & Estrella, 2006; Świeca, Gawlik-Dziki, Kowalczyk, & Złotek, 2012). New phenolic compounds (eriodictyol-*O*-hexoside 2, quercetin glucuronide, kaempferol glucuronide and acylhexoside) were identified in kidney bean sprouts indicating their *de novo* synthesis. which indicates the activation of the phenylpropanoid pathway. Biosynthesis of phenolic compounds can be drastically induced by elicitation in plant derived-foods. Our study showed that elicitor treatments during 8 days of germination promote the synthesis and accumulation of different flavonoid compounds (flavonones and flavonol *O*-glycosides), depending on the elicitor type. However, most of phenolic compounds decreased in kidney bean sprouts as consequence of elicitation. This effect could be

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attributed to the activation of hydroxylases and polyphenoloxydases (Świeca, Gawlik-Dziki, Kowalczyk, & Złotek, 2012). Randhir & Shetty (2007) reported that ascorbic acid treatment enhances in a time-dependent manner guaiacol peroxidase activity in fava bean sprouts. This enzyme is involved in cell wall modifications (lignification and suberinization). This finding suggests that longer times of germination in the presence of elicitors could result in an increased partition of soluble phenolics to lignification lowering the total content of free phenolic compounds. In contrast with our results, other studies have reported that abiotic elicitation increased total free phenolic content of legume sprouts (Shetty, Atallah, & Shetty, 2003; Randhir, Lin, & Shetty, 2004; Burguieres, McCue, Kwon, & Shetty, 2007; Limón, Peñas, Martínez-Villaluenga, & Frias, 2014; Swieca, Seczyk, & Gawlik-Dziki, 2014). These differences could be attributed to elicitor nature, doses and time of treatment (Baenas, García-Viguera, & Moreno, 2014) being these effects more dependent on plant genetics than on the elicitor nature. For instance, ascorbic acid (500 μM), folic acid (50 μM) or proline are able to increase the total phenolic content in pea (Burguieres, McCue, Kwon, & Shetty, 2007) and fava bean (Rhandir & Shetty, 2007) sprouts due to the up-regulation of the pentose phosphate pathway. Lentil supplementation with 0.1 mM phenylalanine or tyrosine increased slightly the total phenolic and flavonoid content of 4-day old sprouts (Swieca et al. 2014). Differences observed between our study and previous literature data could be also attributed to the methodology used to determine total phenolic content. Earlier studies have used the colorimetric Folin-Ciocalteu method to quantify total phenolic content in seeds and elicited legume sprouts. This method is less reliable for total phenolic quantification as it interferes with nitric compounds and reducing sugars and does not correlates well with the sum of individual phenolic compounds evaluated chromatographically (Siger et al., 2012).

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Kidney bean germination and elicitation did not affect radical scavenging activity of kidney bean sprouts with the exception of glutamic acid treatment, which induced a significant decrease of ORAC values. Previous literature data showed controversial results on the effect of germination on the antioxidant activity of sprouts (Aguilera et al. 2015, Guajardo-Flores, Serna-Saldívar, & Gutiérrez-Uribe 2013) probably due to plant genetics and germination conditions. Previous research showed that elicitors increase total phenolic content and, consequently, antioxidant activity of sprouts (Swieca, Seczyk, & Gawlik-Dziki, 2014). In the present study no correlation was found between total phenolic content and antioxidant activity which could be due to differences in phenolic compositional and structural complexity of extracts from seeds and sprouts. Different studies have revealed the important role that phenolic structure plays in its radical scavenging activity; the position and number of hydroxyl groups in the basic structure significantly affects antioxidant activity of such molecules (Jing et al., 2012). Structure-activity analysis showed that *p*-coumaric acid exhibit the largest oxygen radical absorbance capacity, followed by ferulic acid, and hydroxybenzoic acids (Koroleva et al., 2014), which are mostly present in seeds and sprouts. Furthermore, esters of *p*-coumaric and ferulic acids are good inhibitors of oxidative stress in biochemical and cell culture models (Meyer, Donovan, Pearson, Waterhouse, & Frankel, 1998; Kanski, Aksenova, Stoyanova, & Butterfield, 2002). Besides phenolic compounds, other additional antioxidants such as melatonin and vitamins could be contributing to overall radical scavenging activity of legume sprouts (Aguilera et al. 2015; Fernández-Orozco et al., 2008).

Sprouts exhibited a lower ACE inhibition activity than kidney bean seeds. By contrast, elicitor treatment produced an increase in ACE inhibitory activity of sprouts compared to control germination, being this effect outstanding in elicited sprouts with folic acid.

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No reports have been found about the effect of germination and elicitation on the antihypertensive potential of sprouts. Previous *in vitro* and *in vivo* studies have demonstrated that certain flavonoids (anthocyanins, flavones, flavanones, flavanols and flavones) have an inhibitory effect on ACE activity, which plays a key role in the regulation of arterial blood pressure (Guerrero et al., 2012; Aviram & Dornfeld; 2001). Quercetin and kaempferol are among the most potent ACE inhibitors out of 39 phenolic compounds belonging to different structural subtypes (Guerrero et al., 2012; Al Shukor et al. 2013). Higher ACE inhibition activity of flavonols has been reported to be due to the combination of the following elements in the flavonoid structure: the catechol group in the B-ring, the double bond between C2 and C3 at the C-ring, and the cetone group in C4 at the C-ring (Guerrero et al., 2012). A significant linear correlation between ACE inhibitory activity and eridictyol-*O*-acylhexoside ($r = 0.789$, $p=0.02$) was found that could explain the highest ACE inhibition observed in elicited sprouts with folic acid. **5.**

Conclusions

Germination in the presence of elicitors gave rise to important changes in the phenolic profile and bioactivity of sprouts. A general decrease in the concentration of total and individual phenolic compounds was observed in elicited sprouts, although an enhancement in the content of specific flavanones (eriodictyol derivatives) and flavonols (quercetin and kaempferol derivatives) was found. Elicitor treatment positively affected the ACE inhibitory activity of sprouts while no improvements were found for antioxidant activity. Folic acid was the most suitable elicitor to improve phytochemical quality and maximize the health-promoting properties of kidney bean sprouts.

Notes

The authors declare no conflict of interests.

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Figure captions

Figure 1. Percentage of distribution of different phenolic groups to the total phenolic content.

Figure 2. ORAC (mg Trolox equivalent/g dry extract) and ACEI (% inhibition) activities of phenolic extracts from kidney bean seeds and sprouts. ACEI activity was assayed using an extract concentration of 0.1 mg/mL. Different lowercase and uppercase letters indicate statistical differences for antioxidant and ACEI activity, respectively ($P<0.05$, LSD test). ORAC: antioxidant activity; ACEI: ACE inhibition. Error bars represent the standard deviation of the mean.

Figure 3. Principal component analysis (PCA) biplot (score and loading values) for (A) total concentration of phenolic groups and bioactivity (ACEI and ORAC) in seeds, non-elicited (control) and elicited kidney bean sprouts; (B) percentage of distribution of phenolic groups to the total phenolic content and bioactivity (ACEI and ORAC) of phenolic fraction in seeds, non-elicited (control) and elicited kidney bean sprouts. ORAC: antioxidant activity; ACEI: ACE inhibition.

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Table 1. Identification of phenolic compounds from kidney bean (*Phaseolus vulgaris* L.) seeds by using high performance liquid chromatography (HPLC) coupled to ultraviolet–visible diode array detector (DAD) and mass spectrometry detectors (triple quadrupole ion trap).

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	MS^2(*m/z*)	Tentative identification
			Non-Anthocyanin		
1	4.63	330	355	193*	Feruloyl hexoside acid
2	4.82	278	451	289(100), 245(33)	Catechin hexoside
3	5.22	312	355	209(64),191(100),163(21)	*trans*-*p*-Coumaroyl aldaric acid 1
4	5.81	324	385	209(24),193(21),191(100)	*trans*-Feruloyl aldaric acid 1
5	6.04	316	355	209(75),191(100),163(37)	*trans*-*p*-Coumaroyl aldaric acid 2
6	6.37	314	385	209(64),193(8),191(100)	*trans*-Feruloyl aldaric acid 2
7	6.53	278	493	451(12),289(100)	Catechin acylhexoside
8	6.89	326	385	209(49),193(31),191(100)	*trans*-Feruloyl aldaric acid 3
9	7.23	328	385	209(79),193(7),191(100)	*trans*-Feruloyl aldaric acid 4
10	7.58	326	415	223(16),209(45),191(100)	Sinapoyl aldaric acid
11	7.90	278	289	245(67), 205(40), 151(26), 137(21)	(+)-catechin
12	9.02	320	385	209(41),193(23),191(100)	*trans*-Feruloyl aldaric acid 5
13	9.33	256	137	-	*p*-Hydroxybenzoic acid
14	10.2	294	449	287(41)	Eridictyol-*O*-hexoside 1
15	10.83	330	387	163 (28)	*p*-Coumaroyl derivative acid
16	12.03	328	367	163(100)	*p*-Coumaroyl acetylhexoside acid
17	13.02	322	415	223(11)	Sinapoyl aldaric acid
18	13.75	330	367	193(51), 191(100), 175(78), 175(5)	Feruloyl quinic acid
19	14.30	314	429	223(100)	Sinapoyl derivative
20	15.30	292, 330sh	449	287(20)	Eridictyol-7-*O*-glucoside
21	15.80	324	429	223(95)	Sinapoyl derivative
22	16.68	284, 334sh	639	301(100)	Hesperetin-*O*-glucuronyl-hexoside
23	17.11	288, 310sh	-	271(100)	Naringenin derivative
24	18.10	326	367	193(100),191(79),175(65),149(26),134(47)	Feruloyl quinic acid
25	19.22	322	193	149(6)	Ferulic acid
26	19.3	352	609	301(3)	Quercetin-3-*O*-rutinoside
27	19.9	330	367	193(100),191(7),175(71),149(35),134(49)	Feruloyl quinic acid
28	20.89	360	463	301(72)	Quercetin 3-*O*-glucoside
29	22.38	356	505	463(75),301(5)	Quercetin-*O*-acetyhexoside

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30	22.8	346	593	285(6)	Kaempferol-3-*O*-rutinoside
31	24.51	344	447	285(41)	Kaempferol-3-*O*-glucoside
32	24.68	354	463	301(100)	Quercetin-*O*-hexoside
33	27.15	287,330sh	491	449*,287(59)	Eridictyol-*O*-acetylhexoside
34	28.13	342	519	315(82)	Isorhamnetin-*O*-acetyhexoside
			Anthocyanin		
35	17.77	516	449	287(100)	Cyanidin 3-*O*-glucoside
36	20.53	500	433	271(100)	Pelargonidin 3-*O*-glucoside
37	21.28	504	519	271(100)	Pelargonidin 3-*O*-malonylglucoside
38	31.63	534	-	331(100)	Malvidin derivative

* Relative abundance < 2%

Note that different chromatographic conditions were used in each case.

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Table 2. Individual and total phenolic compounds (μg/g sprout d.m.) of seeds and sprouts under elicitor treatment

Compounds	Seeds	Sprouts			
		Control	Ascorbic acid	Folic acid	Gutamic acid
Non-anthocyanins					
Hydroxybenzoic and hydroxycinnamic compounds					
trans-Feruloyl hexoside acid	4.03 ± 0.43^{c}	3.32 ± 0.01^{b}	1.11 ± 0.00^{a}	1.17 ± 0.12^{a}	2.88 ± 0.03^{b}
trans-p-Coumaroyl aldaric acid 1	1.07 ± 0.20^{a}	3.77 ± 0.32^{b}	0.63 ± 0.00^{a}	1.86 ± 0.68^{a}	3.92 ± 0.81^{b}
trans-Feruloyl aldaric acid 1	3.72 ± 0.32^{b}	5.56 ± 0.59^{c}	0.65 ± 0.06^{a}	1.50 ± 0.41^{a}	1.69 ± 0.38^{a}
trans-p-Coumaroyl aldaric acid 2	6.43 ± 0.61^{b}	6.93 ± 0.56^{b}	0.93 ± 0.10^{a}	2.63 ± 1.49^{a}	6.35 ± 0.88^{b}
trans-Feruloyl aldaric acid 2	5.20 ± 1.07^{b}	nd^{a}	nd^{a}	nd^{a}	nd^{a}
trans-Feruloyl aldaric acid 3	12.76 ± 0.73^{b}	17.03 ± 0.55^{c}	6.80 ± 0.27^{a}	5.34 ± 0.48^{a}	5.54 ± 0.30^{a}
trans-Feruloyl aldaric acid 4	15.45 ± 0.35^{b}	21.76 ± 0.54^{d}	7.79 ± 0.05^{a}	7.86 ± 0.56^{a}	18.20 ± 0.72^{c}
Sinapoyl aldaric acid	39.14 ± 5.54^{c}	8.65 ± 0.85^{b}	nd^{a}	nd^{a}	nd^{a}
trans-Feruloyl aldaric acid 5	13.32 ± 0.97^{c}	10.90 ± 0.25^{b}	6.06 ± 0.21^{a}	6.56 ± 0.06^{a}	10.85 ± 0.64^{b}
p-hydroxybenzoic acid	10.33 ± 0.64^{b}	10.00 ± 0.85^{b}	nd^{a}	nd^{a}	nd^{a}
trans-p-Coumaroyl derivative acid	0.41 ± 0.05^{a}	1.24 ± 0.02^{b}	0.33 ± 0.10^{a}	0.68 ± 0.18^{a}	0.56 ± 0.03^{a}
trans-p-Coumaroyl acylhexoside acid	2.35 ± 0.26^{b}	3.60 ± 0.11^{c}	1.00 ± 0.02^{a}	1.59 ± 0.42^{b}	2.11 ± 0.05^{b}
Sinapoyl aldaric acid	12.44 ± 0.52^{b}	29.36 ± 1.27^{c}	4.96 ± 0.62^{a}	3.49 ± 0.46^{a}	3.63 ± 0.67^{a}
Feruloyl quinic acid	1.39 ± 0.08^{a}	5.32 ± 0.63^{b}	3.84 ± 0.16^{b}	3.79 ± 0.53^{b}	4.23 ± 0.32^{b}
Sinapoyl derivative acid	13.66 ± 1.86^{b}	4.29 ± 0.73^{a}	2.98 ± 0.75^{a}	2.66 ± 0.05^{a}	3.12 ± 0.12^{a}
Sinapoyl derivative acid	11.30 ± 1.84^{a}	11.23 ± 0.59^{a}	19.27 ± 1.49^{b}	10.97 ± 1.25^{a}	10.53 ± 0.40^{a}
Feruloyl quinic acid	0.83 ± 0.03^{b}	1.60 ± 0.11^{c}	0.59 ± 0.02^{a}	0.45 ± 0.02^{a}	0.51 ± 0.10^{a}
Ferulic acid	2.71 ± 0.06^{c}	0.66 ± 0.03^{b}	0.40 ± 0.01^{a}	0.36 ± 0.02^{a}	0.45 ± 0.07^{a}
Feruloyl quinic acid	1.21 ± 0.06a^{b}	1.78 ± 0.34^{b}	0.73 ± 0.11^{a}	0.45 ± 0.05^{a}	0.94 ± 0.26^{a}
Flavan-3-ols					
(+)-Catechin *O*-hexoside	55.11 ± 5.20^{c}	27.11 ± 0.80^{b}	4.65 ± 0.12^{a}	8.27 ± 0.94^{a}	3.88 ± 0.19^{a}
(+)-Catechin *O*-acylhexoside	16.51 ± 0.70^{b}	33.21 ± 1.43^{d}	12.39 ± 0.03^{a}	25.64 ± 1.32^{c}	33.26 ± 1.04^{d}
(+)-Catechin	32.15 ±3.94^{b}	3.76 ± 0.27^{a}	nd^{a}	nd^{a}	nd^{a}
Flavanones					
Eridictyol *O*-hexoside 1	1.98 ± 0.09^{c}	1.94 ± 0.17^{c}	0.37 ± 0.00^{a}	0.22 ± 0.01^{a}	0.89 ± 0.09^{b}
Eridictyol 7-*O*-glucoside	0.77 ± 0.09^{b}	0.31 ± 0.10^{a}	0.16 ± 0.00^{a}	0.29 ± 0.17^{a}	0.35 ± 0.07^{a}
Hesperetin *O*-glucuronide-hexoside	6.17 ± 0.04^{a}	16.81 ± 0.66^{b}	7.10 ± 1.17^{a}	8.54 ± 1.23^{a}	9.01 ± 1.58^{a}
Naringenin derivative	2.57 ± 0.09^{a}	3.89 ± 0.39^{b}	1.74 ± 0.05^{a}	2.28 ± 0.16^{a}	2.43 ± 0.39^{a}

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Eridictyol-*O*-hexoside 2	nd[a]	0.16 ± 0.05[b]	0.31 ± 0.05[c]	0.35 ± 0.05[c]	0.32 ± 0.03[c]
Eridictyol-*O*-acylhexoside	0.21 ± 0.05[a]	0.40 ± 0.08[a]	0.39 ± 0.09[a]	0.66 ± 0.03[cb]	0.85 ± 0.09[b]
Flavonols					
Quercetin 3-*O*-rutinoside	0.54 ± 0.05[a]	9.83 ± 0.12[e]	3.91 ± 0.06[b]	5.88 ± 0.05[c]	6.92 ± 0.16[d]
Quercetin 3-*O*-glucuronide	nd[a]	9.21 ± 0.28[e]	2.35 ± 0.05[b]	3.67 ± 0.80[c]	5.01 ± 0.17[d]
Quercetin 3-*O*-glucoside	0.79 ± 0.07[v]	0.86 ± 0.02[v]	0.27 ± 0.01[a]	0.61 ± 0.08[b]	2.03 ± 0.11[c]
Quercetin *O*-acylhexoside	0.96 ± 0.07[a]	2.60 ± 0.32[c]	1.79 ± 0.01[b]	1.98 ± 0.05[b]	2.80 ± 0.16[c]
Kaempferol 3-*O*-rutinoside	0.23 ± 0.01[a]	9.72 ± 0.16[e]	2.83 ± 0.01[b]	4.36 ± 0.29[c]	5.31 ± 0.05[d]
Kaempferol *O*-glucuronide	nd[a]	6.28 ± 0.00[e]	1.01 ± 0.02[b]	1.78 ± 0.05[c]	2.38 ± 0.14[d]
Kaempferol 3-*O*-glucoside	0.61 ± 0.03[c]	1.13 ± 0.05[d]	0.37 ± 0.00[a]	0.51 ± 0.01[b]	0.67 ± 0.00[c]
Quercetin *O*-hexoside	0.45 ± 0.10[b]	0.56 ± 0.02[b]	nd[a]	nd[a]	nd[a]
Kaempferol *O*-acylhexoside	nd[a]	0.48 ± 0.00[b]	0.54 ± 0.02[bc]	0.62 ± 0.04[c]	0.96 ± 0.07[d]
Isorhamnetin *O*-acylhexoside	0.26 ± 0.02[a]	0.31 ± 0.00[a]	0.33 ± 0.01[a]	0.36 ± 0.07[a]	0.49 ± 0.05[b]
Anthocyanins					
Cyanidin 3-*O*-glucoside	0.31 ± 0.00[b]	0.01 ± 0.00[a]	nd	nd	nd
Pelargonidin 3-*O*-glucoside	1.90± 0.02[b]	0.06 ± 0.00[a]	nd	nd	nd
Pelargonidin 3-*O*-malonylglucoside	0.02± 0.00[a]	0.08 ± 0.00[b]	nd	nd	nd
Malvidin derivative	0.06± 0.00[a]	0.06 ± 0.01[a]	nd	nd	nd
TOTAL	**279.68 ± 7.20[c]**	**275.71 ± 9.09[c]**	**98.62 ± 3.69[a]**	**117.42 ± 4.37[a]**	**153.06 ± 3.44[b]**

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Figure 1

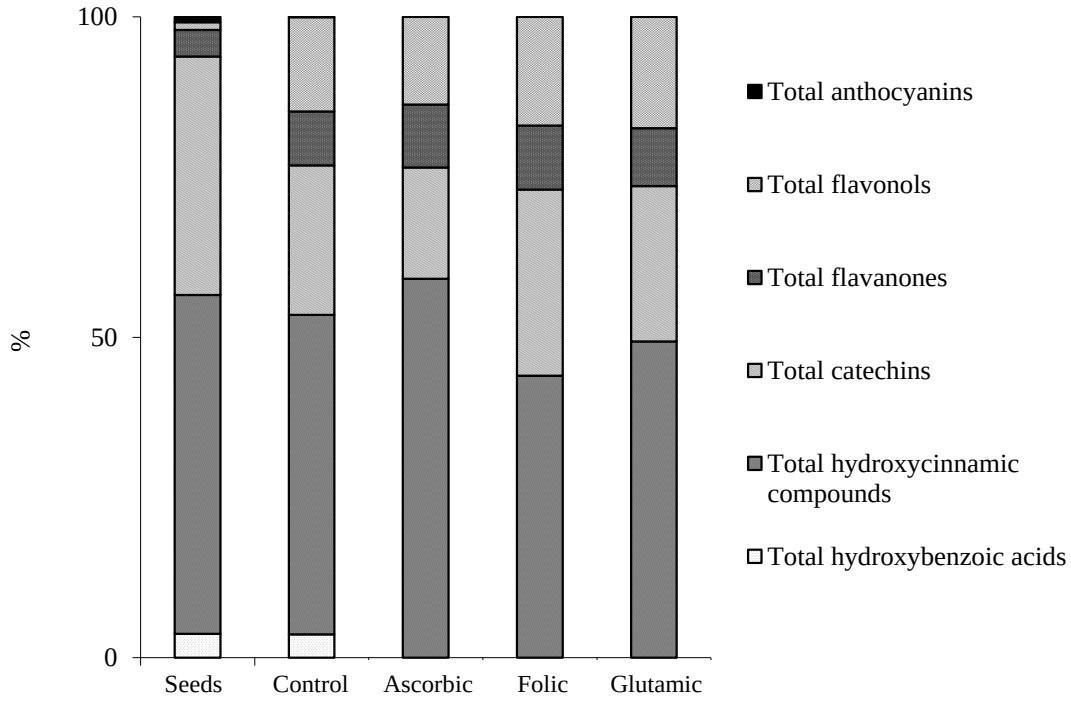

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Figure 2

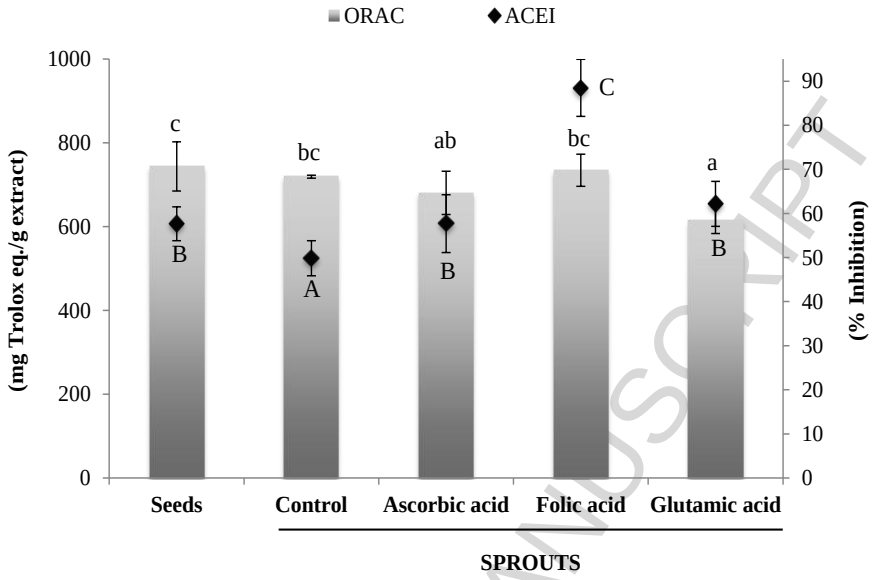

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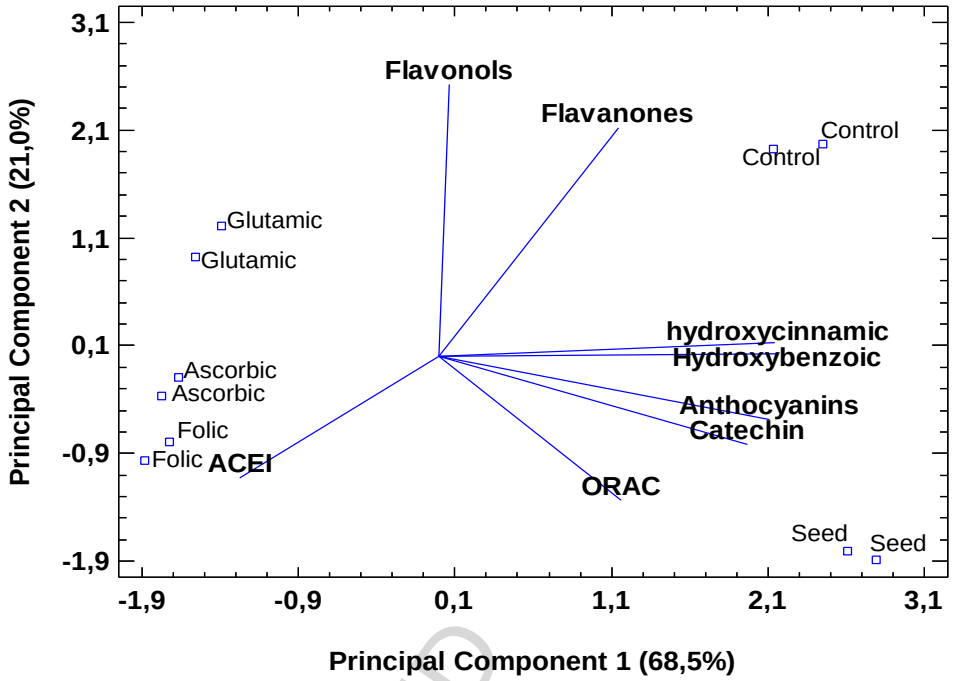
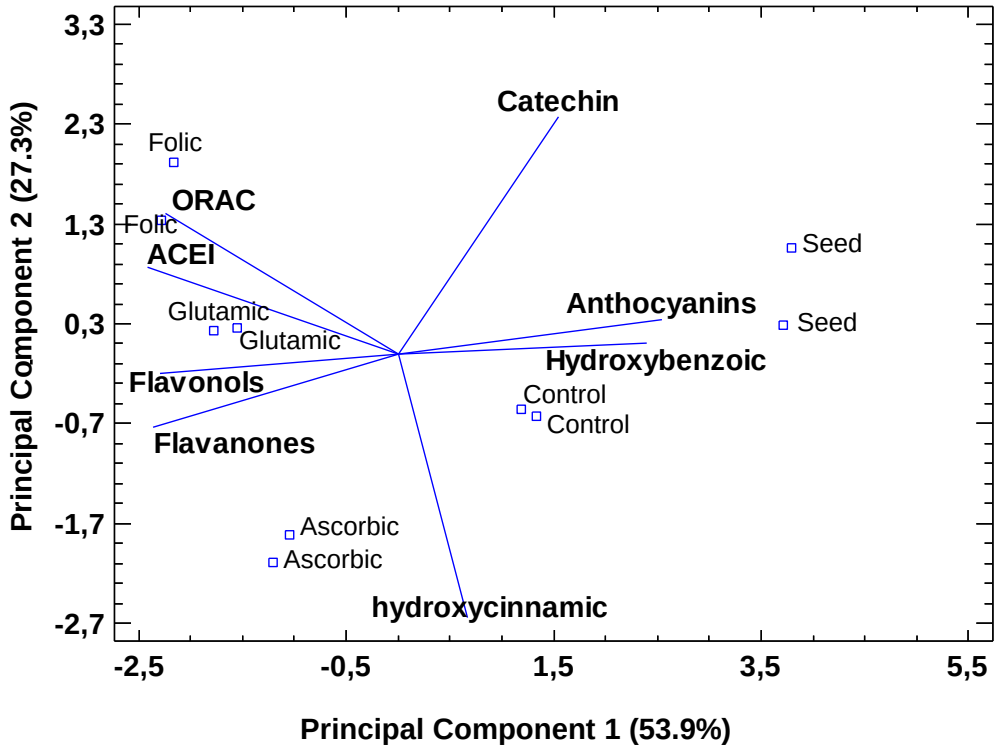
Figure 3

A



B



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Highlights

- ✓ Kidney beans were germinated for 8 days in water and in different elicitor solutions.
- ✓ Elicitation promoted the synthesis of specific phenolic compounds of sprouts.
- ✓ Folic acid caused an outstanding increase in the ACE inhibitory activity of sprouts.
- ✓ Selection of elicitor is crucial to deliver ready to eat sprouts with bioactive phytochemicals.

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