Role of elicitation on the health-promoting properties of kidney bean sprouts

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ABSTRACT

The influence of germination of kidney beans for 4, 6 and 8 days in different elicitors (ascorbic acid, folic acid, glutamic acid, glutamic acid/chitosan and lactic acid/chitosan) on the production of health-promoting ingredients was studied. Sprouts were characterised according to their protein profile, soluble phenolic compounds (SPC) and γ-aminobutyric acid (GABA) content. Additionally, the antioxidant and angiotensin-converting enzyme (ACE) inhibition activities of aqueous-extracts from kidney bean elicited sprouts were determined. Elicitation by glutamic acid for 8 days led to the highest SPC and GABA accumulation in kidney bean sprouts and their extracts were selected based on their highest phytochemical content. These extracts also showed remarkable antioxidant and ACE-inhibitory activities. The IC\textsubscript{50} for ACE inhibition of glutamic acid-extracts increases 89-fold after the in-vitro gastrointestinal digestion. These results will contribute not only to improving the health-promoting potential of kidney bean sprouts but also to obtaining ingredients which might reduce oxidative stress and hypertension.

Keywords: kidney beans, germination, ACE-inhibition, antioxidant capacity, γ-aminobutyric acid, soluble phenolic compounds
1. Introduction

Legumes have an excellent nutritional profile and provide phytochemicals that are involved in cardiometabolic risk prevention (Bouchenak & Lamri-Senhadji, 2013). A recent evidence has suggested that complex mixture of bioactives compounds in foods may be more healthful than individual isolated components (Liu, 2004).

Germination is an effective technology that involves physiological changes that improve the digestibility and nutritive value of legumes (Urbano et al., 2005). Germination of legumes has been shown to be a successful strategy to increase the content of antioxidant compounds (Fernandez-Orozco, Frias, Zielinski, Piskula, Kozlowska, & Vidal-Valverde, 2008) such as phenolic compounds that present free-radical scavenging activity and therefore can exert cardiovascular protective effects (Andriantsitohaina et al., 2012). γ-Aminobutyric acid (GABA) also increases during seed germination (Oh, 2003) and it contributes to a reduction in high blood pressure (Hayakawa, Kimura, Kasaha, Matsumoto, Sansawa, & Yamori, 2004). In addition, the release of bioactive peptides with ACE-inhibitory activity may also occur during seed germination mitigating the risk of CVD (Bamdad et al, 2009). Thus, germinated legumes could constitute an excellent food choice for the prevention of CVD.

Several authors have applied exogenous elicitors during germination of seeds to stimulate seedling vigour and, in response, the biosynthesis of bioactive compounds, thereby contributing to a significant step toward disease prevention. Burguiere, McCue, Kwon, & Shetty (2007) found that the addition of vitamin C and folic acid during pea germination stimulated pea seedling vigour and increase its phenolic content and antioxidant activity. Similarly, soybean seeds soaked in chitosan solutions presented high sprout growth (Lee, Kim, & Kim, 2005). Moreover, glutamic and lactic acids solutions alone or combined with chitosan enhanced the synthesis of GABA in brown rice sprouts (Oh, 2003).

Production of kidney beans is receiving a great attention as a sustainable agriculture crop throughout Europe. Germination is a current strategy to diversify the kidney bean market and the production of sprouts with bioactive compounds enhancement by elicitation will provide health benefits. However, to our knowledge, there is no information about the effect of elicitors during kidney bean germination and resulting health-promoting properties.
Therefore, the primary goal of this research was to evaluate the effectiveness of different elicitors on the sprout growth, proteolysis, soluble phenolic compounds and GABA. The second goal of this study was to evaluate the antioxidant and ACE-inhibitory activity of aqueous extracts from kidney bean elicited sprouts. Finally, those extracts with higher phytochemical content and bioactive properties were submitted to simulated gastrointestinal digestion to assess the effect of in vitro digestion on ACE-inhibitory activity. Results from this study will contribute to support the health-promoting potential of kidney bean sprouts and also to obtain functional ingredients from this legume.

2. Materials and methods

2.1. Seeds

Light speckled kidney beans (*Phaseolus vulgaris* var. Pinto) were provided by Semillas Iglesias S. A. (Salamanca, Spain). Seeds were stored in polyethylene containers at 4 °C.

2.2 Elicitors and treatment of sprouts

Elicitor solutions and their concentration were selected according to previous studies (Burgieres et al., 2007; Oh, 2003). Elicitors were dissolved in distilled water at the following concentrations: 500 µM ascorbic acid; 50 µM folic acid; 5 mM glutamic acid; 50 ppm low-molecular weight (LMW) chitosan in 5 mM glutamic acid; 50 ppm LMW chitosan in 5 mM lactic acid. Elicitors were provided by Sigma-Aldrich (St. Louis, MO, USA). All solutions were freshly prepared before each daily application.

2.3 Germination of seeds

50 g of seeds were soaked in 0.07% sodium hypochlorite solution (1:6 w/v) for 30 min at room temperature. Then, seeds were drained and washed with distilled water until they reached a neutral pH. Afterwards, seeds were soaked in distilled water (1:6 w/v) for an additional 5.5 h and shaken every 30 min. Finally, hydrated seeds were placed in trays where a wet filter paper was extended, and were then covered. The trays were introduced into a pilot scale germinator G-120 model (ASL Snijders International S.L., The Netherlands) and filter paper was watered daily with the different elicitor solutions. Seeds were maintained wet by capillarity. Germination was performed in the darkness for 4, 6 and 8 days at 20 °C. A negative control experiment of kidney seeds germinated in distilled water was also performed. Every germination experiment was performed in triplicate. The germination percentage
was determined based on the total number of seedlings that fully emerged. Sprouts were freeze-dried, milled, packed in vacuum bags and stored at -20 °C.

2.4. Chemical analysis of kidney bean sprouts

2.4.1. Total protein determination

Nitrogen content was analyzed by the Kjeldahl method, as described in the AOAC method 992.15 (AOAC, 1992), using a nitrogen analyzer (LECO Corp., St. Joseph, MI). A factor of 6.25 was used for protein content conversion.

2.4.2. SDS-PAGE protein profile

The protein profile of germinated kidney beans was analysed by SDS-PAGE. Freeze-dried kidney bean sprouts were suspended in a sample buffer (containing 0.125 M TRIS-HCl pH 6.8, 3.75% glycerol, 1% SDS and 5% β-mercaptoethanol) diluted in water (1:1, v:v) at a final concentration of 5 mg/mL. 10 μL of each sample were loaded in a gel having the following composition:

Gradient running gel: 9-19 % acrylamide, 0.08-0.17% bis-acrylamide, 0.36 M TRIS-HCl pH 8.8, 35% glycerol, 0.1% SDS, 0.02% ammonium persulfate and 0.15% N,N,N',N'-tetramethylenediamine (TEMED).

Stacking gel: 3.5% acrylamide, 0.09% bis-acrylamide, 0.125 M TRIS-HCl pH 6.8, 0.1% SDS, 0.02% ammonium persulfate, and 0.15% TEMED.

Running buffer: 25 mM TRIS, 0.19 M glycine and 0.1% SDS, pH 8.8.

Gels were dyed with Coomassie Brilliant Blue G-250. A pre-stained molecular weight marker solution (broad range, Bio-Rad. Hercules, CA, USA) was used.

2.4.3. Soluble phenolic compounds

The content of soluble phenolic compounds (SPC) was determined following the protocol of Singleton, Orthofer, & Lamuela-Raventos (1999), adapted to a microplate reader, after their extraction in water adjusted to pH 8 with 0.1 M NaOH. Results were expressed as mg of gallic acid equivalents (GAE) per g of freeze-dried sprout.

2.4.4. GABA content

The quantification of GABA in kidney bean sprouts was performed using a high-performance liquid chromatography (HPLC) as described in Torino et al. (2013).
2.5. Preparation of water soluble extracts

Soluble extracts from kidney bean sprouts were obtained at pH 8 by suspending 2 g of freeze-dried sample in 20 mL of water (adjusted to pH 8.0 with 0.1N NaOH) and stirring at room temperature for 1 h. The supernatant was collected by centrifugation (15300 x g at 10ºC for 15 min) and subsequent filtration through a Whatman no. 1 filter paper. A single extract was obtained from each germination assay. Finally, soluble extracts were freeze-dried and stored under vacuum at -20 ºC until further analysis.

2.6. Functional characterisation of water-soluble extracts

2.6.1. Soluble protein content

Protein concentration of soluble extracts was determined using the DC Microplate Assay (Bio-Rad Laboratories, Hercules, CA) following the manufacturer instructions.

2.6.2. Antioxidant capacity

Oxygen Radical Absorbance Capacity was determined in the germinated kidney bean extracts by fluorescence measurement (ORAC-FL), as recently described by Torino et al. (2013). Results were expressed as mg of Trolox equivalents per g of extract.

2.6.3. ACE inhibitory activity

ACE inhibitory peptides are generally short sequences since the active site of ACE cannot accommodate large peptide molecules (Sirtori, Galli, Anderson, & Arnoldi, 2009). Therefore, soluble extracts were ultra-filtrated through a hydrophilic 3 kDa cutoff membrane (Amicon® Ultra-0.5, Millipore Corporation, Billerica, MA, USA). ACE-inhibitory activity of 3-kDa permeates was determined according to Martinez-Villaluenga et al. (2012). IC50 values, corresponding to the amount of extract that inhibits by 50% the activity of the ACE, were determined by dose-response curves in which the range of concentrations was distributed in a logarithmic scale and calculated using a sigmoidal curve fit function in GraphPad Prism 4.00 software (Graphpad Software Inc., San Diego, CA, USA).

2.6.4. In vitro gastrointestinal digestion

The in vitro gastrointestinal digestion studies were performed in two different phases:

a) **In vitro** gastric digestion (phase 1): 30 mg of freeze-dried extracts were dissolved in 3200 µL of simulated gastric fluid (SGF, 0.15 M NaCl, pH 2.5). Then 400 µL of a solution containing 0.59 %
(w/v) porcine pepsin (EC 3.4.23.1) (Sigma-Aldrich) in SGF were added and the pH was adjusted to 2.5 with 0.01 M HCl. Digestion was performed at 37 °C for 2 h and subsequently stopped by increasing the pH to 7.5 with 0.1 M NaOH.

b) *In vitro* duodenal digestion (phase 2): For the intestinal digestion step, the pH of the gastric digest was adjusted to 6.5 with 0.01 M HCl. In order to simulate a duodenal environment the following solutions were added: 150.8 µL of a bile salt mixture containing equimolar quantities (0.125 M) of sodium taurocholate (Sigma-Aldrich) and glycochenoic acid (Sigma-Aldrich), 46.08 µL of 1 M CaCl₂ (Merck, Darmstadt, Germany), 500 µL of 0.25 M Bis-Tris (pH 6.5) (Sigma-Aldrich), and 100 µL of pancreatin from porcine pancreas (EC number 232-468-9) (Sigma-Aldrich) in SFG at pH 7.0. Digestion was carried out at 37 °C for 2h. Finally, pancreatin was inactivated by heating at 80 °C for 15 min.

2.6.5. *SDS-PAGE* protein profile of digested samples

The NuPAGE® Electrophoresis System was used for the analysis of the digested extracts under non-reducing conditions. Samples were dissolved in sample buffer (4X NuPAGE LDS buffer, Invitrogen, CA, USA) at a final protein concentration of 2 mg/mL and they were heated at 70°C for 10 min. Samples (20 µl) were loaded onto a 4-12% polyacrylamide NuPAGE® Bis Tris pre-cast gel and were run by using the NuPAGE MES-SDS running buffer (Invitrogen) at 150 mV for 1 h. A molecular weight marker solution (NuPAGE mark 12™ solution, Invitrogen) was run in parallel to the samples. Gels were dyed with the Simply Blue safe stain solution (Invitrogen).

2.7. Statistical methods

Data were expressed as mean ± standard deviation of three independent germination experiments. Three determinations of every experiment were carried out. One-way analysis of variance (ANOVA) using the least significant difference test was conducted to determine differences between samples. Statistical analysis was performed by using Statgraphics 5.0 software (Statistical Graphics Corp, Rockville, MD, USA).

3. Results and Discussion.

3.1. Percentage of germination
Figure 1 shows the effect of elicitors on germination percentage of kidney bean seeds. Up to the fourth day, the germination percentage reached values between 60% for folic acid to 81% for ascorbic acid. Germination for 6 days led to a noticeable increase in germination rate and values in the range of 80 %-94 %, depending on the elicitor used, were observed. At the 8th day, germination percentages around 90 % were achieved, irrespective of the elicitor used.

Folic and ascorbic acids have been suggested as exogenous inducers of seedling growth since they enhance seed vigour and seedling performance (Burguières et al., 2007). Our results showed that ascorbic acid produced the highest level of germination at the fourth day and a slight increase was observed from the fourth to the eighth day. The application of folic and glutamic acids produced the lowest level of germination at the fourth day and no significant (P≤0.05) differences between both solutions were found. However, the sprouts obtained with folic acid at 6 and 8 days showed the highest largest germination rates (P≤0.05), results not observed for glutamic acid. Glutamic acid is usually added during germination to promote GABA synthesis. To our knowledge, there is no information on the effect of glutamic acid on seed germination. In fact, our results show that glutamic-sprouts presented similar germination percentage (P≥0.05) than those obtained with distilled water. Lee et al. (2005) reported that chitosan stimulates the growth and yield of soybean sprouts. In the present work, chitosan in combination with glutamic or lactic acids also led to a gradual increase of the germination percentage for 6 days, values that did not change significantly (P≥0.05) up to 8 days (Figure 1).

3.2. Total protein content and SDS-PAGE profile of kidney bean sprouts

The total protein content increased from 23% d.m. in kidney bean seeds to 26-27% d.m. in kidney bean sprouts, regardless of the assayed elicitor. It is well known that germination increases the total and soluble protein content due to the synthesis of novel peptides. To better understand these changes, SDS-PAGE of germinated kidney beans was studied (Figure 2). Raw seeds (lane RS) yielded a complex protein pattern characterized by proteins with molecular weight ranging from 6 to 150 kDa. The protein band of ~115 kDa likely corresponded to a large protein or to a protein aggregate stabilised by forces other than disulfide bonds (such as hydrogen bonds) since β-mercaptoethanol was unable to disrupt all the disulfide bonds. Two other low-intensity bands (90 and 74 kDa) were observed in RS, but it was not possible to identify them. Other three major bands of about 57, 47 and
45 kDa, were also present and correspond to individual vicilins subunits, as reported by other authors (Rui, Boye, Ribereau, Simpson, & Prasher, 2011; Savelkoul, Tamminga, Leenaars, Schering, & Ter Maat, 1994). Another predominant band present in kidney bean seeds (31 kDa) could correspond to phytohemagglutinin, a protein largely present in beans with a molecular weight ranging from 27 to 37 kDa, results that are in agreement with those reported recently in kidney beans var. Pinto (Rui et al., 2011).

Figure 2 also illustrates the changes in protein profile during kidney bean germination in the presence of different elicitors. The germination process led to a gradual hydrolysis of kidney bean proteins, which was reflected in the disappearance or loss of density of some protein bands, together with the appearance of a smear of lower molecular weight polypeptides in most of the extracts.

The hydrolysis was particularly evident after 8 days of germination, regardless of the elicitor used. Water-germination (lanes W4-8) caused a significant hydrolysis of bands of about 90 and 74 kDa that were almost hydrolysed after 8 days (lane W8). The density of bands corresponding to vicilin subunits and hemagglutinin (lectin) was gradually reduced during germination. Addition of folic acid (lanes F4-8), ascorbic acid (lanes A4-8), glutamic acid (lanes G4-8), glutamic acid/quitosan (lanes GQ4-8) and lactic acid/quitosan (lanes LQ4-8) did not affect the proteolysis pattern during germination. Germination is a catabolic process characterised by an extensive hydrolysis of seed storage proteins, (Urbano et al., 2005). Our results are in agreement with those found by Savelkoul et al (1994), who observed the degradation of lectin and phaseolin during germination of kidney beans up to 10 days.

3.3. Content of soluble phenolic compounds (SPC) in kidney bean sprouts

*Phaseolus vulgaris* can be considered a good source of polyphenols, compounds related to antioxidant and antihypertensive activity of plant-based foods (Shahidi & Naczk, 2004). Table 1 shows the content of SPC in kidney bean sprouts produced with different elicitor solutions. The germination for 6 days with folic and glutamic acids led to bean-sprouts with similar SPC content to water-sprouts; these were significantly higher (P≤0.05) than those observed with ascorbic acid, glutamic acid/chitosan and lactic acid/chitosan. At the end of the germination, beans treated with glutamic acid showed the highest SPC content (6.0 mg/g sprout d.m.), followed by folic acid (5.7
mg/g d.m.). Phenolic compounds are essential for the physiology, morphology, growth, defense and stress response in plants and they are affected by many factors such as growth-regulating substances (Randhir, Shetty, & Shetty, 2002). Our results indicate that the largest increase in SPC content was observed after the application of glutamic acid for 8 days, effect that has not been reported so far. No information has been found on the role of glutamic acid on the biosynthesis of polyphenols during germination. The present results suggest that glutamic acid might stimulate the phenylpropanoid pathway triggering the accumulation of phenolic compounds in kidney bean sprouts.

Ascorbic and folic acids have been applied as exogenous antioxidant elicitors to enhance the phenolic content in pea sprouts due to their inherent antioxidant potential (Burguieres et al., 2007). Our results showed that folic acid increased SPC content only after 8 days compared to control, results in agreement with those of Burguieres et al., (2007). Shetty & Wahlqvist (2004) suggested that folic acid could potentially serve as indirect stimulator for proline biosynthesis, linked to the pentose phosphate pathway activity during stress, which itself is the source of phenolic synthesis precursors.

3.4. GABA content in sprouts

The GABA content of beans germinated with different elicitor solutions is collected in Table 1. The addition of ascorbic acid and glutamic acid + chitosan during germination for 4 days led to higher GABA content than that observed in water, while significantly (P≥0.05) similar amounts were found between folic and glutamic acids and water. After 6 days, germination with water produced slight, but significant (P≤0.05) differences than those observed with ascorbic and glutamic acids and glutamic acid + chitosan solutions. In contrast, at the end of the germination period the highest GABA concentration was found when glutamic acid solution was applied (0.95 mg/g d.m.), whilst the rest of elicitors led to lower GABA accumulation than water. As it is well known, glutamic acid acts as substrate of glutamate decarboxylase (GAD) for producing GABA during germination. Oh (2003) and Oh and Oh (2003) observed an enhancement in GABA levels in brown rice germinated for 72 hours in glutamic acid and glutamic acid/chitosan. However, results presented here showed an increase in GABA content with glutamic acid solution only after 8 days of germination.
The potential antihypertensive effect of GABA in mammals is well known and GABA-enriched products have been found to lower the blood pressure in spontaneously hypertensive rats (Hayakawa et al., 2004). In addition, brown rice extracts enriched in GABA stimulate immune cells by the production of γ-interferon and interleukin 4 in mice (Oh & Oh, 2003).

3.5. Effect of pH on the content of proteins, peptides and phenolic compounds of extracts from kidney bean sprouts.

To obtain water-soluble extracts enriched in proteins and peptides, protein solubility was measured at different pH values. Results are shown in Figure 3. Proteins showed the highest solubility at pH 8-12, which led to recoveries ~90% of the total protein content. No remarkable differences were observed in the protein solubility at pH values from 8 to 12.

Polyphenols solubility is also pH-dependent. Therefore, their extractability at different pH was assayed to enrich bean extracts in these compounds. The largest amount of SPC was extracted at basic pH value (Figure 3). The increase in SPC concentration at pH in the range 8-12 could be attributable to the alkaline hydrolysis of some insoluble phenolic compounds, as has been previously reported in germinated brown rice by Tian, Nakamura, & Kayahara (2004).

Our results confirm that pH 8 enhances the solubility of protein, peptides and SPC. Consequently, water at pH 8 was used as solvent to prepare bean extracts in which antioxidant and ACE-inhibitory activity was evaluated.

3.6. Antioxidant activity of extracts from kidney bean sprouts

Elicitor solutions used in the present study did not improve the antioxidant activity, measured by ORAC-FL assay, compared to the control during the germination period. The antioxidant activity of extracts was affected in different manner by the solutions applied and germination time. Extracts from 4-day sprouts elicited with ascorbic, folic and glutamic acids presented higher antioxidant activity (P≤0.05) than glutamic/chitosan and lactic acid/chitosan extracts. Similarly, antioxidant activity of extracts from 6-days sprouts treated with ascorbic and folic acids was higher compared to other elicitors studied. At the end of the germination period, the antioxidant activity remained almost constant (P≥0.05) except for folic acid treatment that was reduced significantly (P≤0.05) (Table 2).
It is well known that phenolic compounds display peroxyl-scavenging activity and, therefore, they can contribute to the antioxidant activity of legume sprouts (Burguieres et al., 2007). The concentration of phenolic compounds was influenced by the type of elicitor; however, SPC content in sprouts was not significantly correlated with the antioxidant activity of extracts. Differences in the antioxidant activity of bean extracts could be due to different phenolic composition. The number of hydroxyl groups and the hydroxylation pattern has been linked to polyphenol antioxidant activity (Kim & Lee, 2004). In addition, Randhir et al. (2002) and Burguieres et al. (2007) suggested that some polyphenols do not act as antioxidants since some of them are involved in lignification and structural development during seedling growth.

3.7. ACE-inhibitory activity of extracts from kidney bean sprouts

Table 3 shows the ACE-inhibitory activity of 3 kDa fraction obtained from germinated bean extracts. Large ACE inhibition activity was observed in all the sprout extracts, irrespective of the germination treatment applied (85-91%). Our results after 4 days of germination match with those found by Bamdad et al. (2009) in lentil sprouts who reported 80-85% after 3-5 days of germination. The results indicate that ACE-inhibitory activity suffered slight changes during germination, but we can consider that, in general, the application of elicitor solutions during germination did not affect the ACE-inhibitory activity. To our knowledge, this is the first time that ACE-inhibitory activity has been determined in sprouting legume extracts enriched in proteins and polyphenols. These results suggest that the soluble bean-sprout extracts could provide advantageous health-promoting properties.

Previous studies conducted by our group showed that ACE activity was not inhibited by pure GABA solutions (data not shown). Therefore, ACE-inhibitory activity might be attributed to bioactive peptides released during germination, as has been reported for lentils (Bamdad et al., 2009). Two types of cysteine proteases are generally considered to be the major endopeptidases responsible for the degradation of seed storage protein during early seedling growth (Zakharov, Carchilan, Stepurina, Rotari, Wilson, & Vaintraub, 2004). As it was shown in Figure 2, no changes in SDS-PAGE profile of bean sprouts obtained with different elicitors were found, suggesting that elicitor treatment did not affect endopeptidases activity during germination and similar ACE-inhibitory peptides were formed, regardless of the elicitor employed.
3.8. Selection of soluble extracts from kidney bean sprouts with potential antioxidant and antihypertensive properties.

On the basis of our results, the extract from seeds germinated with glutamic acid for 8 days were selected as potential antihypertensive ingredients based on their higher GABA and SPC contents, as well as their potent antioxidant and ACE-inhibitory activities. Antioxidant activity of this extract was ~50 times higher than those reported by Lopez et al. (2013), who found 0.94 µmol TE/mg of raw dark beans (Phaseolus vulgaris L. cv. Tolosana). Moreover, ACE-inhibitory activity of selected extracts was similar than that of fermented lentil ingredients (90-92%) recently reported (Torino et al., 2013).

3.9. Effect of gastrointestinal digestion of soluble extracts from kidney bean sprouts on their ACE-inhibitory activity

In-vitro digestion of bioactive peptides may modify their activity as a consequence of subsequent proteolysis by gastrointestinal enzymes. To assess the effective ACE-inhibitory activity of extracts from bean sprouts obtained with glutamic acid, they were subjected to a simulated in-vitro gastrointestinal digestion. Figure 4 shows that in-vitro digestion led to the hydrolysis of storage proteins and, consequently, peptides lower than 6 kDa were formed. IC$_{50}$ value of non-digested sample was 16 mg/mL and after in-vitro digestion this value was markedly lower (0.18 mg/mL), which indicates that the ACE-inhibitory potency increased 89 fold. These results suggest that new ACE-inhibitory peptides are released by gastrointestinal proteases, results that are in accordance with those observed by SDS-PAGE (Figure 4). Antihypertensive peptides generally contain from 2 to 20 amino acid residues (Sirtori, et al., 2009) and thus, small peptides present in the 3 kDa permeate after in-vitro digestion could be the responsibles of the ACE-inhibitory activity observed in glutamic acid-extracts.

4. Conclusions

Our results reveal that the application of some elicitors during kidney bean germination can increase the sprouts productivity, proteolysis, SPC and GABA. Moreover, extracts from kidney bean seeds germinated with elicitor solutions also showed high antioxidant and ACE-inhibitory activity. Glutamic...
acid-extracts presented the lowest ACE IC₅₀, value which sharply decreased after simulated gastrointestinal digestion, indicating that ACE inhibitory potency was 89-fold higher after in-vitro digestion. Due to the growing consumer interest for functional foods, the identification of health-promoting ingredients may provide further insights into novel nutraceutical applications for kidney bean sprouts.

Acknowledgements

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References


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hydrophilic antioxidants from these foods. *Journal of Food Composition and Analysis*, 29, 25-31.


Figure captions

**Figure 1.** Germination rate of kidney bean seeds in water and in different elicitors solutions.

**Legend**

- 4 days
- 6 days
- 8 days

*) Data expressed as: (Germinated seeds/total number of seeds) x100

In the statistical analysis, the same superscript letter between germination times for the same elicitor solution means no significant difference (P≤0.05); the same subscript number between elicitors and the same germination time means no significant difference (P≤0.05).

**Figure 2.** SDS-PAGE profiles of kidney beans germinated in water and in different elicitors solutions.

**Legend**

- RS: Raw kidney bean seeds
- W4, 6, 8: Kidney bean germinated in water for 4, 6 or 8 days
- F4, 6, 8: Kidney bean germinated in folic acid for 4, 6 or 8 days
- G4, 6, 8: Kidney bean germinated in glutamic acid for 4, 6 or 8 days
- GQ4, 6, 8: Kidney bean germinated in glutamic acid/chitosan for 4, 6 or 8 days
- LQ4, 6, 8: Kidney bean germinated in lactic acid/chitosan for 4, 6 or 8 days
- Mk: Prestained molecular weight marker

**Figure 3.** Protein and soluble phenolic compounds (SPC) extracted from kidney beans at different pH values

**Figure 4.** SDS-PAGE profile of extracts from 8-day sprouted kidney beans obtained in glutamic acid before (BD) and after (AD) *in vitro* gastrointestinal digestion.
Table 1. Content of soluble phenolic compounds (SPC) and γ-aminobutyric acid (GABA) in kidney beans germinated with water and different elicitor solutions and water for 4, 6 and 8 days

<table>
<thead>
<tr>
<th>Germination solutions</th>
<th>SPC (mg GAE/g)</th>
<th>GABA (mg/g)</th>
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<tr>
<td></td>
<td>4 days</td>
<td>6 days</td>
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<tr>
<td>Ascorbic acid</td>
<td>4.24 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.43 ± 0.15&lt;sup&gt;1&lt;/sup&gt;&lt;sub&gt;1,2&lt;/sub&gt;</td>
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<tr>
<td></td>
<td></td>
<td>0.70 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>0.65 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Folic acid</td>
<td>5.43 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.48 ± 0.34&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td>0.62 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>0.47 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Glutamic acid</td>
<td>5.41 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.64 ± 0.51&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0.59 ± 0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>0.95 ± 0.08</td>
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<td>Glutamic acid + chitosan</td>
<td>4.21 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.77 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.65 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>0.54 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Lactic acid + chitosan</td>
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<td>4.58 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>0.44 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Distilled water</td>
<td>5.16 ± 0.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.27 ± 0.56&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

Results are the mean of three independent experiments ± SD. For each compound, the same superscript letter in the same column means no significant difference (P≤0.05), and the same subscript number in the same row means no significant difference (P≤0.05).
Table 2. Antioxidant activity (µmol TE/g extract) in water-soluble extracts of kidney beans germinated with water and different elicitors solutions for 4, 6 and 8 days

<table>
<thead>
<tr>
<th>Germination solutions</th>
<th>4 days</th>
<th>6 days</th>
<th>8 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>55.54 ±3.54&lt;sup&gt;b&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</td>
<td>54.61 ±4.15&lt;sup&gt;bc&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</td>
<td>53.72 ±1.20&lt;sup&gt;b&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td>Folic acid</td>
<td>54.40 ±3.26&lt;sup&gt;b&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</td>
<td>56.78 ±3.16&lt;sup&gt;c&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</td>
<td>48.64 ±2.90&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>56.47 ± 2.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.73 ± 3.80&lt;sup&gt;a&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</td>
<td>50.80 ± 4.11&lt;sup&gt;ab&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td>Glutamic acid + chitosan</td>
<td>49.75 ±1.63&lt;sup&gt;a&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</td>
<td>49.92 ±3.23&lt;sup&gt;ab&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</td>
<td>50.64 ±2.02&lt;sup&gt;ab&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td>Lactic acid + chitosan</td>
<td>48.59 ±2.67&lt;sup&gt;a&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</td>
<td>48.94 ±2.23&lt;sup&gt;a&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</td>
<td>50.39 ±1.22&lt;sup&gt;ab&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td>Distilled water</td>
<td>62.27 ± 2.68&lt;sub&gt;1&lt;/sub&gt;</td>
<td>62.29 ± 3.19&lt;sub&gt;1&lt;/sub&gt;</td>
<td>58.63 ± 4.13</td>
</tr>
</tbody>
</table>

Results are shown in mg Trolox/g extract and are the mean of three independent experiments ± SD. The same superscript letter in the same column means no significant difference (P≤0.05). The same subscript number in the same row means no significant difference (P≤0.05).
Table 3. ACE activity inhibition (%) in soluble extracts of kidney beans germinated with water and different elicitors solutions for 4, 6 and 8 days

<table>
<thead>
<tr>
<th>Germination solutions</th>
<th>4 days</th>
<th>6 days</th>
<th>8 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>88.40 ± 0.68a1</td>
<td>88.17 ± 1.39ab1</td>
<td>87.44 ± 1.67b1</td>
</tr>
<tr>
<td>Folic acid</td>
<td>89.82 ± 0.45bc1</td>
<td>89.91 ± 0.67c1</td>
<td>89.24 ± 1.49bc1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>88.56 ± 1.16ab1</td>
<td>89.65 ± 1.54bc1</td>
<td>88.53 ± 1.07bc1</td>
</tr>
<tr>
<td>Glutamic acid + chitosan</td>
<td>88.50 ± 1.63ab1</td>
<td>90.41 ± 1.40c2</td>
<td>89.65 ± 1.06c12</td>
</tr>
<tr>
<td>Lactic acid + chitosan</td>
<td>87.73 ± 0.69a1</td>
<td>86.53 ± 0.55a1</td>
<td>89.35 ± 1.24bc2</td>
</tr>
<tr>
<td>Distilled water</td>
<td>91.09 ± 0.48c</td>
<td>87.24 ± 0.81a</td>
<td>84.72 ± 2.87a</td>
</tr>
</tbody>
</table>

Results are expressed as the mean of three independent experiments ± SD. The same superscript letter in the same column means no significant difference (P≤0.05). The same subscript number in the same row means no significant difference (P≤0.05).
Figure 1

Germination rate

- Water
- Ascorbic acid
- Folic acid
- Glutamic acid
- Glutamic acid + chitosan
- Lactic acid + chitosan

Legends:
- a
- b
- ab
- c
- bc
- a
- b
- a b
- a b
- a b
- a b
Figure 2
Figure 3