Effect of soaking and fermentation on content of phenolic compounds of soybean (*Glycine max* cv. Merit) and mungbeans (*Vigna radiata* [L] Wilczek)

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Abstract

"Green soybeans" obtained from a Spanish company clearly showed by HPLC-PAD-ESI/MS analysis a different phenolic composition than soybeans and they were identified as mungbeans (*Vigna radiata* [L] Wilczek). In spite that isoflavones were predominant in yellow soybeans (*Glycine max* cv. Merit), they were completely absent in the green seeds; contrarily, flavanones were predominant in the green beans. In order to enhance their health benefits, both beans were subjected to technological processes such as soaking and fermentation. Soaking at pH 4.5 increases malonyl glucoside isoflavone extraction in yellow beans. In relation to the green bean, the soaking treatment at pH 6.5 produced an increase in the phenolic compounds extracted (875.20 versus 503.18 μg/g) and the most apparent changes are the decrease in the apigenin 8-*C*-glucoside (vitexin) and the increase of apigenin derivatives. Fermentation was carried out with *Lactobacillus plantarum* CECT 748 strain, a species present in the human gastrointestinal tract which possess β-glycosidase activity. *L. plantarum* fermentation produced an increase in the bioactivity of both beans since, on yellow soybeans a conversion of glycosylated isoflavones into more bioactive aglycones was detected, and on green beans the concentration of bioactive vitexin was increased. In spite of the potential consumer confusion since soybean and "green soybean" are different legumes, the health benefits of both bean seeds could be enhanced by lactic fermentation.

Keywords: Consumer confusion; Isoflavones; Flavonoids; Bioactive compounds; Vitexin

Introduction

There is a growing evidence that consumption of soy-derived foods has potential health benefits related to cardiovascular diseases, menopausal symptoms, osteoporosis, breast and prostate cancers, mainly due as they are sources of bioactive phenolic compounds (Devi et al., 2009).Whereas numerous studies support the positive effects of consuming soy, concerns regarding a potential fraud to the consumers have surfaced. The use of the word "soybean" for the designation of different legumes can result in misuse generating some consumer confusion. Soybean (*Glycine max* cv. Merit) is commercialized in a wide variety of colors and tones: from yellow to black passing through green, brown or red (García-Ruiz et al., 2007). The most commercial and abundant soybean is the yellow pigmented one. Although, in principle, the other colored seeds are also considered as soybean, it is not very clear whether they are different varieties of soybean or different legumes. In Spanish markets, "soja verde" (green soybean) is widely found, and, in addition, commercial advertisements representing soybeans generally showed green beans, leading the consumer to associate green beans with soybean. However, it have been described that "soja verde" purchased from a local market in Spain is not a true soybean, being a different legume originally from China called mungbean (*Vigna radiata* [L] Wilczek) (García-Ruiz et al., 2007). Taking into account that soybean is a very valuable bean due to its nutritional and functional properties, these properties could be different in "green bean" leading at the absence of their specific health benefits claimed by the consumers. In fact, yellow soybean presented a protein profile different from that obtained from beans marketed as "green soybean" in Spain (García-Ruiz et al., 2007). However, no studies comparing the specific phenolic composition of yellow soybean and seeds sold as "green beans" have been reported so far.

The soybean health benefits are mainly related to its isoflavone content. Isoflavones are secondary plant metabolites, which constitute a group of natural bioflavonoids synthesized almost exclusively by plants of *Leguminaceae* family (Frische and Steinhart, 1999). Isoflavones occur in large amounts in soybean and soy products, and other legumes. However, the isoflavone content of the so-called "green soybean" remains unknown. In soybean, isoflavones are present both as aglycones and as glycosides (β-glycosides, acetyl and malonyl glycosides). The concentration and distribution of isoflavone forms in soybeans is influenced by the genotype, location and crop year, whereas in processed soy products it depends on the sort of soybean used as on the type of processing. Depending on the pH and temperature conditions, a fermentation process may modify the content and compositions of these bioactive compounds (Nufer et al., 2009).

In relation to the metabolism of isoflavone in humans, the isoflavone aglycones are absorbed faster and in greater amounts than their glycosides counterpart (Setchell et al., 2002). Aglycones are absorbed directly through the gut wall, while isoflavone glycosides are very poorly absorbed from the gut due to their higher hydrophilicity and larger molecular weight. It is generally though, that isoflavone glycosides are converted to their corresponding aglycones by gut microbiota or gut glycosidases and then absorbed from the small intestine (Tsangalis et al., 2002; Lee et al., 2006; Otieno et al., 2007). *Lactobacillus plantarum* is an important member of the normal intestinal microbiota in humans possessing β-glycosidase activity (Aguirre et al., 2008). Thus, soybean fermentation by *L. plantarum* strains proceeded in the premise that both, the enhancement of isoflavone aglycones before consumption of soy foods and the modulation of intestinal microbiota through the ingestion of viable lactic acid bacteria could improve the bioavailability of isoflavones from soy food products (Choi et al., 2002). In addition, it

had been described that *L. plantarum* fermentation of soybean is an adequate process for the development of hypoallergenic soy food products (Frias et al., 2008).

The aim of our work was to investigate the composition and the chemical modifications occurring in the phenolic profile of yellow soybean and "green soybean" during soaking and fermentation experiment carried out with the lactic acid bacterium *Lactobacillus plantarum*. The comparative results obtained would provide data about the healthy phenolic compounds present in both seeds and will bring relevant data regarding the potential fraud to the consumer when the so-called "green soybeans" are sold as soybeans.

Materials and methods

Chemicals and solvents

Solvents used, methanol, ethanol and acetonitrile were of HPLC grade. The HPLC grade standard compounds, *p*-hydroxyphenylacetic, *p*-hydroxybenzoic, *trans p*-coumaric acids; *p*-hydroxybenzaldehyde; tryptophan; taxifolin; catechin; naringenin 7-*O*-glucoside, naringenin 7-*O*-rutinoside; naringenin 7-*O*-neohesperidoside, naringenin; eriodictyol 7-galactoside, eriodictyol 7-glucoside; the identified apigenin and luteolin derivatives; kaempferol 3-*O*-glucoside, kaempferol 3-*O*-galactoside; genistein 7-*O*-glucoside, daidzein 7-*O*-glucoside, all of them purchased from Extransynthèse (France). Glycitein 7-glucoside was purchased from Phytolab (Vestenbergsgreuth, Germany).

Preparation of cultures

L. plantarum CECT 748 (ATCC 14917) was purchased from the Spanish Type Culture Collection (CECT) to be used as inocula. Stock cultures were grown and maintained on

MRS agar (Difco Laboratories, Detroit, Michigan). The culture was transferred to MRS broth, which was incubated for 18 h at 30 ºC. The cells were pelleted and washed with sterile saline solution (8.5% NaCl) and used as inoculum.

Preparation and fermentation of bean seeds

Yellow soybeans (*Glycine max* cv. Merit) were obtained from Mang Fong Pacific Trading S.A., and "green soybean" from Biocesta (Spain). Seeds were cleaned and stored in darkness at 4 ºC until use.

Bean seeds were ground in a ball mill (Glen Crestn Ltd., Stanmore, UK), sieved, and the 0.050 to 0.250 mm fraction was collected. Bean flours were suspended in sterile distilled water (in 1:11 proportion). At this point, the suspension was distributed in 4 batches for different treatments. The first batch was immediately frozen (-20 ºC) ("control" or "C" batch). In order to avoid microbial growth, several antimicrobials were added (200 µg/mL natamycin, 100 µg/mL ampicillin, 100 µg/mL chloramphenicol, and 100 µg/mL erythromycin) to batched designed to known the effect of soaking at pH 6.5 ("S6.5" batch) or 4.5 ("S4.5 batch"). To simulate the final pH after lactic acid fermentation of the bean flours, the soaking was also done at pH 4.5 by the addition of HCl to the suspension. The last batch was inoculated with 10% (v/v) of *L. plantarum* CECT 748 inoculum ("Fermented" or "F" batch). Batches S6.5, S4.5, and F were incubated at 30 ºC on an orbital shaker incubator (Unitron, Infors AG, Switzerland) at 150 rpm for 48 h. After the incubation processes, the samples were frozen. At least three independent replicates of each batch were obtained.

Extraction of phenolic compounds

Bean flour (10 g) from the different samples (C, S6.5, S4.5, and F) from Y or G beans, was mixed with 80 ml of a solution of HCl-methanol ($1^0/_{00}$)/water (80:20, v/v), and sonicated for 20 min. The sample was then centrifuged (3,000x*g*, 10 min, 5 ºC). The supernatant was collected and the extraction was repeated two times more. The supernatants from the three extractions were combined and concentrated under vacuum at 30 ºC until the methanol was removed. This aqueous solution was extracted three times with diethyl ether and three times with ethyl acetate. The organic phases were combined and dried for 20 min with anhydrous Na_2SO_4. The extract was then evaporated to dryness at reduced pressure and at 30 ºC; the residue was dissolved in methanol/water (50:50, v/v) and filtered through a 0.45 µm cellulose acetate filter (Millipore), before the analyses by high-performance liquid chromatography (HPLC). The extraction was performed in duplicate.

HPLC-PAD and HPLC-ESI/MS analysis

Analysis was carried out on a HPLC-PAD Waters system (Milford. Mass, USA), comprising an autoinjector, a quaternary pump, a photodiode-array detector 2001 and Millennium 32 chromatography manager software. Separation of phenolic compounds was achieved on a reversed phase C18 column Nova-Pak (300 x 3.9 mm, 4 µm).

The analytical conditions were based on those described by Dueñas (2009). A gradient consisting of solvent A (water/acetic acid, 98:2 v/v) and solvent B (water/acetonitrile/acetic acid, 78:20:2 v/v/v) was applied at a flow rate of 1 ml/min from the beginning to 55 min and 1.2 ml/min from this point to the end. The gradient profile was 0-55 min, 100%-20% A; 55-70 min, 20%-10% A; 70-80 min, 10%-5% A; 80-110

min, 100% B. Detection was performed by scanning from 210 to 400 nm with an acquisition speed of 1s. A volume of 25 µl was injected. The samples were analyzed in duplicate.

Mass spectra were obtained using a Hewlett Packard 1100 (Palo Alto, CA) chromatography system equipped with a photodiode array detector (PAD) and a quadrupole mass spectrometer (Hewlett Packard 1100 MSD) with an electrospray interface. Separation conditions and column were the same that referred above for the HPLC-PAD analysis.

Identification and quantification of phenolic compounds

Chromatographic peaks were identified by comparison of retention times and UV spectra with those of standards, and confirmed by HPLC-ESI/MS. Compounds, for which standard were not available, were tentatively identified according to their order of elution, UV spectra by HPLC-PAD and data of HPLC-ESI/MS analysis (Dueñas et. al., 2009). Quantification at 280 nm was made using the external standard calibration curves, with commercial standards, by injection of different volumes of the stock solution over the range of concentration observed for each compounds, using a linear regression for the relationship of area sum versus concentration under the same conditions as for the samples analysed. The compounds tentatively identify were quantified by the calibration curves of the more similar compounds.

Statistical analysis

To validate the work all the experiments were carried out two times. Results are expressed as averages ± standard deviations (SD).

Results

Phenolic composition of yellow soybeans vs. so-called "green soybeans": Isoflavone absence in the green beans

As the specific phenolic composition of green beans sold in Spain (and labelled as "green soybean") has not been described so far, in this study we analyzed the phenolic compounds present in a "green soybean" from a Spanish brand. The composition of the green bean was compared to the profile found in a yellow soybean.

In the chromatographic conditions used in this work, phenolic compounds, non-flavonoids and flavonoids, co-eluted; therefore, the phenolic compounds present in the samples could be identified and quantified in a single chromatographic run. Table 1 showed the wavelength of maximum UV absorption and the molecular ions of the compounds identified in the bean flours from the HPLC-ESI/MS, grouped according to similarity in the phenolic structure. Analysis of MS spectra recorded for each peak together with comparisons of MS, UV spectra and retention times led to the identification of some of the compounds from the chromatographic conditions.

Tables 2 and 3 showed the phenolic composition of yellow soybean (Y) and green bean (G) flours, respectively. In the yellow soybean, phenolic acids, flavanones and isoflavones were detected by HPLC-PAD and HPLC-MS as described previously (Dueñas et al., 2012). Surprisingly, green bean does not contain isoflavones, whereas these compounds are the main phenolic compounds present in yellow soybeans. It is well known that in

soybeans, isoflavones exist in different types of aglycones forms (daidzein, genistein, and glycitein), glucoside forms (daidzin, genistin, and glycitin), and malonyl glucoside and acetyl glucoside forms (Rostagno et al., 2004; Wu et al., 2004). In this study, the most abundant isoflavone in the control sample (Y-C) was daidzin and their derivatives (347.9 μg/g). In addition, genistein and derivatives were found also in high concentration (239.1μg/g). Contrarily, glycitin and their derivatives were present in the lower concentration (37.3 μg/g). Isoflavones were present mainly in the glucoside form (301 μg/g), being the acetyl- and malonyl-derivatives present in similar concentrations (166.95 and 156.35 μg/g, respectively). It is interesting to note, that no acetyl glycitin was detected in the yellow soybean flour sample. In addition to isoflavones, other flavonoids, such as flavanones (naringenin and eriodictyol derivatives) and flavonols (kaempferol glucosides) and tryptophan were detected.

As observed in Table 3, isoflavones and kaempferol derivatives were not detected in the green bean flour (G-C). The compounds detected were mainly glycoside forms of flavanones (eriodictyol galactoside, eriodictyol glucoside) and flavones (apigenin hexose, apigenin 8-*C*-glucoside, and luteolin 7-*O*-glucoside) (486.64 μg/g). The most abundant compounds extracted from the G-C samples were the glucosides apigenin 8-C-glucoside (vitexin) (331.09 μg/g) and eriodictyol glucoside (74.85 μg/g). In spite that the analyzed green beans do not contain isoflavones, they contained phenolic compounds which could provide health benefits after consumption.

Modification in the phenolic composition during soaking of beans

It has been described that the lactic fermentation of soybean leads to health benefits (Granito et al., 2005; Khahl et al., 2006); however, this fermentation involved the

microbial metabolism during the soaking of the flour. Lactic acid bacteria are characterized by a fast utilization of the sugars present in the sample leading to a fast decrease in the pH. Therefore, in order to known the specific action of the microorganism, the effects of the soaking at a pH 4.5, similar to the final pH after microbial fermentation, was analyzed. As showed in Table 2, the recovery of phenolic compounds from the Y-S4.5 sample was lower than from the Y-C sample (42%) or to the Y-S6.5 sample (63%). The isoflavone aglycones represent an 8% of the phenolic compounds detected, while in the Y-S6.5 sample, they increased to a 13%. The most noteworthy change that could be observed in the Y-S4.5 sample is a surprisingly high proportion of malonyl glucoside isoflavone derivatives, which constitutes a 48% of the total phenolic compounds detected. Soaking at pH 4.5 increases malonyl glucoside isoflavone extraction as these compounds represent a 25% or 32% of the phenolics present in the Y-C or Y-S6.5 samples, respectively. No other phenolic compounds were detected, except low eriodictyol hexose and tryptophan content. We did not observe growth of microorganisms during soaking.

In relation to the green bean (Table 3), the soaking treatment at pH 6.5 (G-S6.5) produced an increase in the phenolic compounds extracted (875.20 versus 503.18 μg/g). The most apparent changes are the decrease in the apigenin 8-C-glucoside (vitexin), the increase of apigenin derivatives, and the appearance of new apigenin derivatives no detected in the control sample (such as apigenin 7-*O*-glucoside, apigenin methylether, apigenin 7-*O*-rutinose, and apigenin 7-*O*-neohesperidose). The eriodictyol glycosides disappeared as a consequence of the soaking treatment at pH 6.5. Most of these modifications were also observed in the soaked sample at pH 4.5 (G-S4.5). Contrarily to the G-S6.5 sample, soaking at pH 4.5 produced an increase in the eriodictyol galactoside which was absent at pH 6.5.

Effects of fermentation in the phenolic composition of beans using *L. plantarum*

In yellow soybean, the content of isoflavone glucoside, malonyl, and acetyl glucoside forms was reduced in favour of the appearance of isoflavone aglycones (74% of the total isoflavone content) (Table 2). Most of the daidzein derivatives were in the aglycone form (92%); however, malonylgenistein was relatively resistant to the bacterial action, representing a 42% of the genistein derivatives present in the *L. plantarum* fermented sample (Y-F).

Similarly to soybean, *L. plantarum* fermentation produced important modifications in the phenolic profile of green bean (Table 3). In relation to eriodictyol derivatives, eriodictyol glucoside abundant in the control sample (G-C) was reduced, and eriodictyol galactoside increased. One of the most important modifications observed as result of the fermentation was the increase in the extracted apigenin derivatives, from 399.23 µg/g in the G-C sample to 1,519.98 µg/g (G-F), accounting only apigenin 8-C-glucoside (vitexin) for 1,228.47 µg/g.

An interesting data is obtained from the tryptophan content of the samples. Tryptophan is an amino acid which eluted in the chromatographic conditions used. In spite that yellow soybean contained higher tryptophan content than green bean (30.42 µg/g versus 14.47 µg/g), the fermentation process applied give a final tryptophan concentration of 54.72 µg/g on the yellow soybean (Y-F) and 155 µg/g on the green bean sample.

Discussion

Beans contain a variety of biologically active compounds. Isoflavones are one group of potential anticarcinogenic compounds that have been extensively studied. Evidence has

shown that soybean consumption has potential health benefits leading to an increase in the soy-derived food products present in the markets. However, a potential fraud to the consumer could be done when non-soybeans are sold as "green soybeans". In Spain, "green soybeans" are widely found in markets leading to consumer confusion. In a previous study, it was demonstrated that a bean labelled as "green soybean" purchased from a local market was a different legume (mungbean (*Vigna radiata* [L] Wilczek) which showed a different protein profile (García-Ruíz et al., 2007).

The results obtained in this work confirmed previous studies which indicated that the so-called "soja verde" (green soybean) found in Spanish markets is a different legume than soybean (García-Ruiz et al., 2007). An additional study need to be performed to provide data about the variety of beans that are sold as "green soybean" in Spain. Obviously, from these results the Spanish way to label these green beans could generate consumer confusion, and therefore, a potential fraud. More interestingly, the healthy benefits of soybean related to the presence of isoflavones, are absent in the "soja verde" analyzed in this study. The absence of these specific phenolic compounds could provide false health expectative for the consumer.

In spite that the so-called "green soybeans" sold in Spanish markets are not true soybeans, being a different legumes devoid of isoflavones, consumer could perform on them different technological processes to modify their phenolic composition.

Processing conditions have a great influence on the profile of phenolic compounds presents in bean seeds. Soaking is one of the steps in the production of bean-derived food products, such as tempeh and tofu. Soaking is used to aid in the dehulling and grinding beans. It has been described that during the soaking process some glycosilated compounds could be hydrolyzed to their aglycones (Nufer et al., 2009). On the other hand, it has been described that the lactic fermentation of soybean leads to health benefits (Granito et al.,

2005; Khahl et al., 2006); however, this fermentation involved the microbial metabolism during the soaking of the flour. Lactic acid bacteria are characterized by a fast utilization of the sugars present in the sample leading to a fast decrease in the pH. Therefore, in order to known the specific action of the microorganism, the effects of the soaking at a pH 4.5, similar to the final pH after microbial fermentation, was analyzed.

In general, fermentation of legumes leads to an improvement in their nutritional value, such as in protein quality, increased palatability, increased levels of B vitamins (Granito et al., 2005). This process also decreased the levels of antinutritional factors present in legume seeds, as phytic acid and flatulence-causing oligosaccharides alpha-galactosides (Doblado et al., 2003). It has been reported that fermentation processes caused a significant increase in the free radical scavenging capacity of legumes, which could be associated with changes in the phenolic composition (Chang et al., 2009; Dueñas et al., 2012).

Fermentation of soybean with *Aspergillus oryzae*, *Rhizopus oryzae*, *Bacillus subtilis*, and *L. plantarum* produced an increase in total phenolic compounds content (Fernandez-Orozco et al., 2007). Tabera et al. (1995) and Bartolomé et al. (1997) reported an increase in phenolic compounds after fermentation of *Lens culinaris*. Dueñas et al. (2005) studied the effect of *L. plantarum* fermentation on the content of phenolic compounds in *Vigna sinensis* flours, and they suggest fermentation as an adequate and effective process for increasing nutritional and biological quality owing to the improvement in the concentration of phenolic compounds. These results could occur because fermentation hydrolyzes complexes of polyphenols to other simpler and biologically more active ones.

Lactobacillus strains are important members of the normal intestinal microbiota in humans and are recognized to be associated with the host´s health. Lactobacilli strains possess enzymes important in the metabolism of phenolic compounds; some of them have been

described from *L. plantarum* (Rodríguez et al., 2009). As a consequence of *L. plantarum* fermentation of yellow soybean *p*-hidroxyphenylacetic acid, *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde were detected in the Y-F sample. Considering that isoflavones were the main phenolic compounds present in the soybean flour, these compounds were severely affected by fermentation (Y-F sample). It has been described that conjugate glycosides are not absorbed intact across the intestine of healthy adults and they need to be hydrolysed releasing isoflavone aglycones, which are more bioactive forms as they could be absorbed by the intestinal microbiota (Setchell et al., 2002). In our study, the content of isoflavone glucoside, malonyl, and acetyl glucoside forms was reduced in favour of the appearance of isoflavone aglycones (74% of the total isoflavone content). Most of the daidzein derivatives were in the aglycone form (92%); however, malonylgenistein was relatively resistant to the bacterial action, representing a 42% of the genistein derivatives present in the *L. plantarum* fermented sample (Y-F).

Fermentation of soybean seeds with *Aspergillus oryzae*, *Rhizopus oryzae* and *Bacillus subtilis* produced also important changes in flavonoids compounds, with a significant formation isoflavone aglycone contents such as daidzein, glycitein and genistein as a consequence of glucosidase activity of microorganism in this process (Dueñas et al., 2012) and Chiou and Cheng (2001) observed an increase in daidzein and genistein content when soybean was fermented with *A. oryzae* to prepare koji. Therefore, this process was shown to be a good way to increase the phenolic content of soybean, which could confer health-promoting effects.

The observed conversion of glycosilated isoflavones into aglycones during fermentation, revealed a β-glucosidase activity on the *L. plantarum* strain used as inoculum. Fermentation of soybean with lactic acid bacteria enhanced the content of aglycones, which are able to bind to estrogen receptor sites and are more physiologically active than

other isoflavone forms (Tsangalis et al., 2002). Therefore, in addition to the probiotic nature of the starter organisms, the increased content of aglycones undoubtly would enhance the health benefits of fermented soybean on consumers. Although isoflavone glycosides could be hydrolyzed to isoflavone aglycones in the gastrointestinal tract by gut microbiota, the rate of hydrolysis varies with an individual and remained unclear. Due to variations on the level of intestinal bacteria through illnesses, diet or age, the intestinal bacteria cannot always be relied upon for glycoside deconjugation in order to release isoflavone aglycones (Otieno and Shah, 2007). Therefore, it is important to provide food with a considerable amount of free isoflavones aglycones, such those produced by fermentation with *L. plantarum*.

Similarly to soybean, *L. plantarum* fermentation produced important modifications in the phenolic profile of green bean (Table 3). In relation to eriodictyol derivatives, eriodictyol glucoside abundant in the control sample (G-C) was reduced, and eriodictyol galactoside increased. One of the most important modifications observed as result of the fermentation was the increase in the extracted apigenin derivatives and it has been proved that vitexin possess many pharmacological activities, such as antispasmodic, anti-inflammatory, antimicrobial, antioxidant/free radical scavenging (Prabhakar et al., 1981; Li et al., 2012), and antithyroid effect (Gaitan et al., 1995).

Several phenolic compounds from green bean were detected for the first time in the G-F sample, as *p*-hydroxyphenylacetic acid, catechin, trans *p*-coumaric acid, apigenin trimethylether, vitexin 2´´ *O*-rhamnoside, luteolin 7-hexoside, and taxifolin. The presence of these compounds is related to the bacterial activity on the green bean flour. In addition to the modification of the phenolic profile, Khalil (2006) described that fermentation with lactobacilli increased nutrient availability of a green bean (mungbean), as increased

protein content, improved digestibility and solubility of the proteins, but also reduced the antinutritional factors.

It has been described that protein may have a protective effect against the degradation of isoflavones upon processing (Nufer et al., 2009). Also, protein content and the level of protein denaturation may affect isoflavone extractability such that the measured amount of isoflavones does not reflect the true amount in the sample. In a previous study, a greater percentage of total isoflavones was extracted from a low protein soy product than form a high protein soy product, after a single extraction, indicating that protein-isoflavone interactions may complicate extractability (Nufer et al., 2009). Our study confirmed this observations, since when proteins are severely hydrolyzed (high free tryptophan content), the sum of total phenolic compounds increased (Table 1 and 2).

Conclusions

Legumes are present in almost every diet throughout the world. Consuming the legumes and related products is becoming popular due to human health concerns. As the legume attracting much attention recently is the soybean, which contains a high concentration of isoflavones, different legumes are erroneously designated as “soybean”. In Spanish markets, the so-called “green soybean” is often found, and could generates consumer confusion since this bean is a different legume and could be devoid of isoflavones. In spite of this potential consumer fraud, the health benefits of both bean seeds could be undoubtly enhanced by lactobacilli fermentation. Fermentation guarantees that every consumer, independently of their intestinal microbiota, could possible derive the health benefits resulting from the formation of different bioactive compounds.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Table 1. Characteristic of UV and mass spectra of phenolic compounds.

Compounds	$\lambda_{max\ (nm)}$	$[M-H]^-$	fragments
Isoflavones			
Daidzein 7-glucoside (daidzin)	249, 313sh	415	253
Malonyl daidzin	250, 301sh	501	457, 253
Acetyl daidzin	252, 301sh	457	253
Daidzein	253	253	
Glycitein 7-glucoside (glycitin)	256, 320	445	283
Malonilglycitin	258, 310	531	283
Glycitein	283	283	
Genistein 7-glucoside (genistin)	262	431	269
Malonylgenistin	259, 320	518	473, 269
Acetyl genistin	260, 315	473	269
Genistein	260	269	
Flavanones			
Naringenin 7-*O*-neohesperidoside	285, 340sh	579	271
Naringenin 7-*O*-rutinoside			
Naringenin 7-*O*-glucoside	284, 330	433	271
Naringenin	288, 326sh	271	
Eriodictyol 7-*O*-galactoside	284,327sh	449	281
Eriodictyol 7-*O*-glucoside	287, 327sh	449	281
Flavones			
Apigenin 7-*O*-neohesperidoside	268, 334	577	431
Apigenin rutinoside	268, 335	577	431
Apigenin hexoside	268, 335	431	271
Apigenin 7-*O*-glucoside	268, 336	431	
Apigenin 8-*C*-glucoside (vitexin)	270, 300sh, 333	431	271
Vitexin 2" *O*-rhamnoside	270, 300sh, 333	577	431
Apigenin methylether	269, 336	283	271
Apigenin trimethylether	268	311	
Luteolin 7-*O*-hexoside	254, 350	447	285
Luteolin 4´-*O*-hexoside	245, 337	447	285
Flavonols			
Kaempferol diglucoside	264, 348	609	285
Kaempferol glucoside	264, 348	447	285
Hydroxybenzoic and hydroxycinnamic compounds			
*p-H*ydroxyphenylacet ic acid	229, 274	157	
p-Hydroxybenzoic acid	255	137	
p-Hydroxybenzaldehyde	286	121	
trans p-Coumaric acid	310	163	
trans p-Coumaric acid derivative	312	329	
Amino acid			
Tryptophan	277	203	

sh – shoulder

Table 2
Content (μg/g) of phenolic compounds in control and processed yellow soybean flours. Results are expressed as averages ± standard deviations (SD).

Compound	Y-C	Y-S6.5	Y-S4.5	Y-F
Isoflavones				
Daidzein 7-*O*-glucoside (daidzin)	181.96±18.56	124.73±5.02	33.92±3.54	15.73±1.78
Malonyl daidzin	11.42±0.65	16.46±0.56	24.94±1.54	nd[a]
Acetyl daidzin	154.53±17.55	320.16±45.01	85.28±11.24	13.55±1.55
Daidzein	nd	109.78±11.85	24.49±0.31	325.51±41.11
Glycitein 7-*O*-glucoside (glycitin)	21.92±1.08	16.95±0.85	6.32±0.72	11.57±1.12
Malonyl glycitin	15.38±2.01	30.54±1.75	14.53±0.88	1.63±0.25
Glycitein	nd	2.96±0.31	nd	25.63±1.74
Genistein 7-*O*-glucoside (genistin)	97.14±11.32	63.39±5.01	19.64±0.58	15.47±2.03
Malonyl genistein	129.55±5.89	274.54±18.02	135.67±4.01	99.97±11.02
Acetyl genistin	12.42±1.52	16.39±1.01	12.77±0.71	7.05±1.02
Genistein	nd	17.78±0.87	4.74±0.83	117.39±8.11
Flavonoids (non isoflavones)				
Eriodictyol 7-*O*-glucoside	14.51±0.95	7.09±1.02	1.61±0.05	nd
Naringenin 7-*O*-neohesperidoside	nd	1.69±0.08	nd	nd
Naringenin 7-*O*- rutinoside	nd	2.26±0.21	nd	nd
Naringenin 7-*O*-glucoside	nd	27.88±1.89	nd	nd
Naringenin	nd	11.29±0.54	nd	7.72±0.99
Kaempferol diglucoside	12.86±0.88	6.78±0.95	nd	20.21±2.01
Kaempferol glucoside	5.71±0.68	3.44±0.28	nd	nd
Hydroxybenzoic and hydroxycinnamic compounds				
p-Hydroxyphenylacetic acid	nd	nd	nd	119.37±9.05
p-Hydroxybenzoic acid	0.45±0.02	nd	nd	10.77±1.87
p-Hydroxybenzaldehyde	nd	nd	nd	11.64±1.56
Amino acids				
Tryptophan	30.42±3.42	87.34±5.02	8.21±0.98	54.72±4.02
Sum of individuals	688.27	1141.45	372.12	857.93

[a]nd, not detected
Values are mean ± SD (n=2)

Table 3
Content (μg/g) of phenolic compounds in control and processed green bean flours. Results are expressed as averages ± standard deviations (SD).

Compound	G-C	G-S6.5	G-S4.5	G-F
Flavonoids				
Catechin	nd	nd	nd	11.47±1.08
Eriodictyol 7-*O*-galactoside	5.49±0.32	nd	14.98±1.04	95.74±3.65
Eriodictyol 7-*O*-glucoside	74.85±2.14	nd	nd	11.83±0.98
Apigenin 7-*O*-neohesperidoside	nd	7.02±0.41	4.52±0.41	nd
Apigenin rutinoside	nd	6.93±0.31	5.72±0.84	nd
Apigenin methylether	nd	39.06±1.05	26.33±0.64	7.52±0.87
Apigenin trimethylether	nd	nd	nd	8.04±1.02
Apigenin hexoside	68.14±3.09	367.04±7.05	466.23±8.65	nd
Apigenin 7-*O*-glucoside	nd	356.04±6.93	130.18±4.01	251.74±6.32
Apigenin 8-*C*-glucoside (vitexin)	331.09±6.75	41.00±1.09	67.16±2.91	1228.47±35.21
Vitexin 2" O-rhamnoside	nd	nd	nd	24.21±1.76
Luteolin 7-*O*-hexoside	7.07±0.35	nd	nd	13.92±0.99
Luteolin 4'-*O*-hexoside	nd	nd	nd	6.15±0.41
Taxifolin	nd	nd	nd	26.14±1.85
Hydroxybenzoic and hydroxycinnamic compounds				
p-Hydroxyphenylacetic acid	nd[a]	nd	nd	10.61±1.34
p-Hydroxybenzoic acid	nd	7.00±0.33	3.61±0.23	5.67±0.53
trans p-Coumaric acid derivative	2.07±0.19	nd	nd	3.63±0.33
trans p-Coumaric acid	nd	nd	nd	3.04±0.31
Amino acids				
Tryptophan	14.47±0.93	51.11±1.85	26.62±1.95	155.00±4.15
Sum of individuals	503.18	875.20	745.35	1863.18

[a]nd, not detected
Values are mean ± SD (n=2)