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# Bioactive Phenolic Compounds of Soybean (*Glycine max* cv. Merit): Modifications by Different Microbiological Fermentations

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In this work, the effect of solid-substrate fermentation with *Aspergillus oryzae*, *Rhizopus oryzae* and *Bacillus subtilis* of soybean seeds on bioactive phenolic compounds was studied. Among the analysed sample extracts several phenolic compounds, hydroxybenzoics, hydroxycinnamics and flavonoids, such as flavonols, flavanones, isoflavones were identified by HPLC-DAD-ESI/MS. The results obtained indicate that fermentation process carried out in seeds inoculated with different microorganisms produced significant changes in flavonoids and phenolic acids contents. A significant increase in the content of phenolic acids was observed in the samples fermented with the different microorganisms with respect to soybean without fermentation and fermented naturally. Fermentation process produced also important changes in flavonoids compounds, with a significant formation in isoflavone aglycone contents such as daidzein, glycitein and genistein as a consequence of glucosidase activity of microorganism in this process, showing significant differences ($p<0.05$) with respect to control. Therefore, this process was shown to be a good way to increase the phenolic content of soybean, which could confer health-promoting effects.

## INTRODUCTION

Soybean (*Glycine max* cv. Merit) is a good source of proteins and bioactive compounds, such as vitamins, carotenoids, saponins and phenolics. The benefits of soybean-based foods for human health are well known, and nowadays the demand for soybean products has increased because of the renewed interest in functional food. Soybeans contain a high concentration of phenolic compounds [Lee *et al*., 2011], phenolic acids and flavonoids, among them the most abundant are isoflavones, the health benefits of which are well recognized in the world [Nagata *et al*., 1998; Setchell & Cassidy, 1999; Hendrich & Murphy, 2001]. Isoflavones are present mainly as glucosides, acetylglucosides and malonylglucosides of genistein, daidzein and glycitein. Isoflavone content varies considerably in soybean depending on different factors, such as cultivars, harvested year, geographical and environmental conditions, maturity, *etc*. [Kim *et al*., 2006a; Lee *et al*., 2008; Malencic *et al*., 2008; Xu & Chang, 2008]. Distribution of isoflavones in the soy parts varied greatly being the germ that had a higher level of isoflavones than cotyledon or soy coat; on the other hand the dominating isoflavones were also different in the three parts of the seed [Yue *et al*., 2010]. Bioactivity of dietary soybean isoflavone differs depending on the chemical forms, and the structure of these compounds is a limiting factor for absorption [Cho *et al*., 2009]. The 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, 2003]. The initial content in the glucosides, daidzin, glycitin and genistin of soybeans are not affected by temperature below 135°C, in the absence of other factors, but the presence of glycosidase enzymes, releases the corresponding aglycones [Park *et al*., 2002; Ribeiro *et al*., 2007].

Several forms of soybean consumption, such as soybean sprouts, pastes, soymilk, soybean oil and tofu are extensively consumed in Asian diets [Kim *et al*., 2006a]. These food products are obtained by different soybean process which could modify the isoflavones content. The knowledge that conjugated forms have less biological effect than aglycones has stimulated the development of technological processes to obtain soybean products rich in isoflavone aglycones by different treatments such as addition of enzymes or by the action

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of different microorganisms [Tsangalis *et al*., 2002; Park *et al*., 2003; Cho *et al*., 2009].

Fermentation is an ancient technology that remains one of the most practical methods for enhancing the nutritional and organoleptic qualities of foods. It has been reported that the fermentation causes a general improvement in the nutritional value of legumes [Zamora & Fields, 1979; Akpapunam & Achinewhu, 1985]. 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 [Frías *et al*., 1996; Granito *et al*., 2005]. This process also decreases the levels of antinutritional factors present in legume seeds, as phytic acid and flatulence-causing oligosaccharides α-galactosides [Alonso *et al*., 2000; 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 [Randhir *et al*., 2004; Dueñas *et al*., 2005]. This process also produced an increase in vitamin E, peroxyl-radical trapping capacity and inhibition of peroxidation, when soybean seed was fermented with different inoculum, as *Aspergillus oryzae*, *Rhizopus oryzae* and *Bacillus subtilis* [Fernández-Orozco *et al*., 2007].

Several microorganisms have been used to carry out the soybeans fermentation, mainly filamentosus fungi, *Bacillus* sp. and lactic acid bacteria. Lin *et al*. [2006] demonstrated that soybeans fermented with filamentous fungi enhanced the phenolic content and radical scavenging activity. Tempeh made from soybean fermented by *Rhizopus oligosporus,* showed great antiradical activity, determined by the DPPH method [Chang *et al*., 2009]. Wardhani *et al*. [2009] described that solid state fermentation of soybeans with *Aspergillus oryzae* for five days provided the best conditions for increasing antioxidant activity in fermented soybean. Choi *et al*. [2007] reported that the fermentation characteristics in Korean soybean paste (*doenjang*) prepared with *Bacillus sp*., increased the bioavailability of isoflavones in this product due to the formation of aglycone forms by β-glucosidase activity. Cho *et al.* [2009] showed that soybean fermentation by *Bacillus pumilus* produced changes in the esterase activity and phenolic content. An increase in the radical scavenging activity was observed, which could be due to increased level of isoflavone aglycones and decrease of its glycosides and phenolic acids.

Soybeans contain also other phenolic compounds different from isoflavones, such as phenolic acids, flavonols, flavanones, *etc*. These compounds could be modified by the fermentation process and therefore the antioxidant activity of the soybean derived [Kim *et al*., 2006a; Lin *et al*., 2006; Malencic *et al*., 2008].

In this work, we have studied the effect of solid-substrate fermentation on the phenolic composition (phenolic acids, flavonols, flavanones and isoflavones) of cracked soybean seeds inoculated with *Aspergillus oryzae*, *Rhizopus oryzae*, *Bacillus subtilis* or fermented by the natural seed microbiota. This study will lead to the selection of an adequate microorganism that could be used as a starter to obtain soybean seeds flour with high increased levels of bioactive phenolic compounds.

## MATERIALS AND METHODS

### Seeds

Soybeans (*Glycine max* cv. Merit) were obtained from Mang Fong Pacific Trading S. A. Seeds were cleaned and stored in darkness at 4ºC until use.

### Preparation of cultures

*Aspergillus oryzae* CECT 2094$^T$ (ATCC 1011), *Rhizopus oryzae* CECT 2340 (ATCC 24563) and *Bacillus subtilis* CECT 39$^T$ (ATCC 6051) were purchased from the Spanish Type Culture Collection (CETC) and used as inocula. Stock cultures were grown and maintained as follows. *A. oryzae* and *R. oryzae* were grown for seven days on potato dextrose agar (Difco Laboratories, Detroit, MI) at 30ºC, and the spores were collected and washed in sterile saline solution and used as inocula. *B. subtilis* was grown aerobically in Brain Hearth Infusion (BHI) broth (Difco Laboratories, Detroit MI) for 18 h at 30ºC. The pelleted cells were washed twice in sterile solution and used as inocula.

### Fermentation of soybean seeds

Sterile soybeans were used to obtain a controlled microbial fermentation. Therefore, several technological processes were applied to raw soybean, such as cracking, soaking, autoclaving and freeze-drying to obtain sterile soybean flours. All these technological processes potentially caused changes in the soybean composition [Shahidi & Naczk, 2004].

Solid-substrate fermentations were carried out using 100 g of cracked soybeans suspended in sterile distilled water (1:2, w/v) for 16 h and autoclaved at 121ºC for 15 min. The sterilized cracked seeds were inoculated with 5% (v/w) of the above cultures containing $10^5$ spores/g of each one microorganism: *A. oryzae* (AF), *R. oryzae* (RF) or *B. subtilis* (BF). The inoculated cracked seeds (30 g) were aseptically distributed over Petri dishes and placed in a climatic incubator (Memmert, Germany) at 30ºC and 90% relative humidity for 48 h. After fermentation, the fermented cracked seeds were autoclaved at 121ºC for 15 min, freeze-dried, milled and passed through a sieve of 0.5 mm mesh diameter. Solid-substrate fermentations on the soybean flours obtained were performed in duplicate. A soybean flour sample was spontaneously fermented by the microbiota present on the seed (NF). A control (C) of cracked seeds of raw soybeans was also prepared and analysed.

### Chemicals and solvents

Solvents used were HPLC grade. The HPLC grade standard compounds, protocatechuic, *p*-hydroxybenzoic, vanillic, syringic, sinapic, *trans p*-coumaric, *p*-hydroxyphenylacetic and *trans*-ferulic acids, *p*-hydroxybenzoic aldehyde, the flavonoids eriodictyol-7-*O*-rutinoside, eriodictyol-7-*O*-glucoside, hesperetin-7-*O*-rutinoside, naringenin, naringenin-7-*O*-neohesperidoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside and *trans*-resveratrol, and the isoflavones genistein, genistein-7-*O*-glucoside, daidzein, daidzein-7-*O*-glucoside, were purchased from Extrasynthèse (Genay, France). Glycitein-7-*O*-glucoside was purchased from PhytoLab (Vestenbergsgreuth, Germany).

**Extraction of phenolic compounds from soybeans**

Soybeans flour (10 g) from the different samples C, NF, AF, RF, and BF was mixed with 80 mL of a solution of HCl--methanol ($1^0/_{00}$)/water (80:20, v/v), and sonicated for 20 min; this procedure was performed three times. The sample was then centrifuged (3,000×g, 10 min, 5°C). The supernatants were combined. An aliquot of this methanol solution (200 mL) was extracted three times with diethyl ether (30 mL) and three times with ethyl acetate (30 mL). The organic phases were combined and dried for 20 min with anhydrous $Na_2SO_4$. The extract was them evaporated to dryness at reduced pressure and at 30°C; the residue was dissolved in methanol/water (50:50, v/v) and filtered through a 0.45 $\mu$m 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 (Waters, Milford, Mass, USA). Separation of phenolic compounds was achieved on a reverse phase C18 column Nova-Pak (300 x 3.9 mm, 4 mm).

The analytical conditions were based on those described by Dueñas *et al*. [2009]. Two mobile phases were employed for elution, solvent A: water/acetic acid (98:2, v/v) and solvent B: water/acetonitrile/acetic acid (78:20:2, v/v/v). 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. The flow rate was 1 mL/min from the beginning to 55 min and 1.2 mL/min from this point to the end. The column was washed with 10 mL of acetonitrile and equilibrated with 25 mL of the initial mobile phase before next injection. Detection was performed by scanning from 210 to 400 nm with an acquisition speed of 1 s. A volume of 25 mL was injected. The samples were analysed 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 were the same that referred above for the HPLC-PAD analysis, except for the flow rate which was fixed at 0.7 mL/min. The ESI source parameters were as follows: drying gas ($N_2$) flow and temperature, 10 L/min and 340°C, respectively; nebulizer pressure, 40 psi; capillary voltage, 4000V. Mass spectra were acquired using in-source collision-induced dissociation mass spectrometry (CID MS), scanning negative ions from *m/z* 100 to *m/z* 2500 using the following fragmentation program: 100 V (m/z < 200), 200 V (m/z 200–1000), 250 V (m/z 1000–2500).

**Identification and quantification of phenolic compounds**

Chromatographic peaks were identified according to retention times, UV spectra, UV spectral parameters and mass spectra compared with commercial standards when available.

Other compounds, for which standards 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*., 2005, 2009].

Quantification 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 identified were quantified by the calibration curves of the more similar compounds.

**Statistical analyses**

Analyses were performed in duplicate, and the data are presented as mean ± standard deviation (SD). Data were analysed by one-way analysis of variance (ANOVA). Significant differences were assessed with an LSD test ($p<0.05$). The statistical analysis was performed using PC software package, SPSS (version 13.0; SPSS Inc., Chicago). Principal Component Analysis was used with the statistical package Statistica version 7.1, Statsoft Inc.

## RESULTS AND DISCUSSION

**Changes in phenolic compounds during microbial fermentation**

Among the analysed sample extracts several phenolic compounds, non-flavonoids hydroxybenzoics, hydroxycinnamics and flavonoids, such as flavonols, flavanones and isoflavones, were identified.

Table 1 presents the wavelength of maximum UV absorption and the molecular ions of identified compounds from HPLC-ESI/MS, grouped according to similarity in phenolic structure. Analysis of MS spectra recorded for each peak together with comparison of $MS^2$, UV spectra and retention times led to identification of some of the compounds from the chromatographic conditions.

Greater qualitative and quantitative differences in the identified phenolic compounds were observed between the control (soybean without fermentation process), soybean fermented by the natural microbiota and seeds inoculated with different microorganisms (Tables 2 and 3). Some of the compounds were formed as a consequence of the technological process of fermentation.

**Hydroxybenzoic and hydroxycinnamic acids**

Protocatechuic, *p*-hydroxibenzoic, vanillic and syringic acids, were identified and quantified in the control sample (Table 1). These compounds were identified by comparison of retention times and UV spectra with those of corresponding standards. The total content of these compounds in this sample, calculated by quantification using HPLC, was 10.72 $\mu$g/g for hydroxybenzoics, where *p*-hydroxybenzoic and vanillic acids, presented the highest concentration (Table 2).

*trans-p*-Coumaric, *cis-p*-coumaric, sinapic and *trans*-ferulic acids were identified as hydroxycinnamic acids, in the sample without fermentation. In this case, the content of hydroxycinnamics was 3.34 $\mu$g/g, being *trans-p*-coumaric acid the most abundant among the identified hydroxycinnamic compounds(Table 2).

TABLE 1. Wavelength of maximum UV absorption and molecular ions of identified phenolic compounds in the soybeans.

| Compounds | λmax (nm) | $[M-H]^-$ | Fragments |
|---|---|---|---|
| ***Hydroxybenzoic and hydroxycinnamic compounds*** | | | |
| protocatechuic acid | 297 | 153 | |
| *p*-Hydroxybenzoic acid | 255 | 137 | |
| *p*-Hydroxybenzoic aldehyde | 286 | 121 | |
| *p*-Hydroxyphenylacetic acid | 229, 274 | 151 | |
| Vanillic acid | 261, 293 | 167 | |
| Syringic acid | 275 | 197 | |
| Sinapic acid | 237, 323 | 223 | |
| *trans p*-Coumaric acid | 310 | 163 | |
| *cis p*-Coumaric acid | 295 | 163 | |
| *trans*-Ferulic acid | 323 | 193 | |
| ***Flavonols and flavanones*** | | | |
| Eriodyctiol-7-rutinoside | 284, 325 | 595 | 287 |
| Hesperetin-7-rutinoside | 284, 330 | 609 | 301 |
| Kaempferol-3-rutinoside | 266, 347 | 593 | 285 |
| Kaempferol 3-glucoside | 266, 349 | 447 | 285 |
| Eriodictyol glycoside | 284, 327sh | | 287 |
| Naringenin-7-neohesperidoside | 284, 330 | 579 | 271 |
| Kaempferol hexose 1 | 266, 348 | 447 | 285 |
| Naringenin | 289, 326 | 271 | |
| ***Stilbene*** | | | |
| *trans*-Resveratrol 3-glucoside | 318 | 369 | |
| ***Isoflavones*** | | | |
| Daidzein derivative 1 | 250, 301sh | | 253 |
| Daidzein 7-glucoside (daidzin) | 256,313 sh | 415 | 253 |
| Glycitein 7-glucoside (glycitin) | 256, 320 | 445 | 283 |
| Daidzein derivative 2 | 250, 301sh | | 253 |
| Genistein derivative 1 | 258, 330 | | 269 |
| Genistein derivative 2 | 258, 330 | | 269 |
| Genistein 7-glucoside (genistin) | 260, 327 | 431 | 269 |
| Genistein derivative 3 | 255,330 | | 269 |
| Daidzein derivative 3 | 250, 301sh | | 253 |
| Daidzein derivative 4 | 250, 301sh | | 253 |
| Daidzein derivative 5 | 250, 301sh | | 253 |
| Daidzein malonylglycoside | 250, 301sh | 501 | 253 |
| Glycitein derivative | 254, 320 | | 283 |
| Genistein derivative 4 | 258, 330 | | 269 |
| Daidzein acetylglycoside | 252, 301sh | 457 | 253 |
| Glycitein malonylglycoside | 258, 310 | 531 | 283 |
| Daidzein | 250, 298 | 253 | |
| Genistein acetylglycoside | 259, 320 | 473 | 269 |
| Genistein malonylglycoside | 258, 320 | 518 | 269 |
| Glycitein | 258, 327 | 283 | |
| Genistein acetylglycoside | 260, 315 | 473 | 269 |
| Genistein derivative 6 | 258, 326 | | 269 |
| Genistein | 260, 326sh | 269 | |

These results agree with these obtained by Kim *et al*. [2006b], who identified some of these compounds in various soybean cultivars, in similar concentrations. These authors found that germination process in dark and light conditions produced changes in these compounds (hydroxycinnamic and hydroxybenzoic acids). Xu & Chang [2008] analysed the phenolic composition of 30 soybean samples, grown in the North Dakota-Minnesota region, and identified also hydroxybenzoic and hydroxycinnamic compounds. The hydroxycinnamics content was higher than hydroxybenzoic compounds. The free phenolic acid contents in the different soybean cultivars ranged from 40.3 to 92.9 μg/g. These results differed from those found by us, due to different cultivar studied and origin.

In general, hydroxybenzoic compounds increased both in soybean flours fermented naturally by only the microorganisms present in the seeds (NF) as in the samples inoculated with *A. oryzae* (AF), *R. oryzae* (RF), *B. subtilis* (BF), mainly *p*-hydroxybenzoic, vanillic and syringic acids. The treatment with *A. oryzae* caused a higher increase in the total hydroxybenzoics; around seven fold with respect to control, followed by the process of fermentation with *R. oryzae* (RF) that produced a five fold increase, although no significant differences were observed with respect to AF. The samples inoculated with *B. subtilis* improved four fold with respect to soybean without fermentation process (control). However, although the content of total hydroxybenzoics in samples fermented with AF was the highest, the content of *p*-hydroxybenzoic, vanillic and syringic acids increased in RF with respect to AF. This was due to the formation of *p*-hydroxyphenylacetic acid in all samples inoculated with microorganisms, excepted in NF, being the soybean fermented with *A. oryzae*, which presented the highest content. Also, it is important to highlight the formation of *p*-hydroxybenzaldehyde in all samples that underwent treatment with microorganisms. Aldehydes formation was also found in other grain-legumes that were submitted to some processing such as fermentation, germination, or addition of different enzymes [Dueñas *et al*., 2005, 2007a, b, 2009; López-Amorós *et al*., 2006].

Hydroxycinnamic compounds also showed differences depending on inoculum used to the fermentation process. A general increase in the total content was observed in the samples fermented, with exception of samples fermented with *R. oryza,* which presented not significant differences with respect to the control sample. Ferulic acid disappeared after fermentation process, both in samples fermented naturally, as in inoculated with the different microorganism (Table 2). *trans-p*-Coumaric acid showed higher content in the samples fermented with the different microorganisms, reaching the maxima concentration in the samples fermented by *A. oryzae* and *B. subtilis.*

**Flavanones and flavonols**

The flavonols, kaempferol 3-*O*-rutinoside, kaempferol 3-*O*-glucoside and the flavanones, eriodictyol-7-*O*-rutinoside, hesperetin-7-*O*-rutinoside (hesperidin), naringenin-7-*O*--neohesperidoside (naringin), naringenin and one eriodictyol hexoside were identified by comparison of their retention times, UV and mass spectral characteristics with library data and commercial standards (Table 1).

TABLE 2. Concentration ($\mu$g/g) of hydroxybenzoics, hydroxycinnamics, flavanones and flavonols in control and fermented with different microorganisms soybean samples.

| Compounds | Control | NF | AF | RF | BF |
|---|---|---|---|---|---|
| | | | ***Hydroxybenzoics*** | | |
| Protocatechuic acid | 1.37±0.03$^{b}$ | nd$^{a}$ | 2.92±0.26$^{d}$ | 2.07±0.14$^{c}$ | nd$^{a}$ |
| *p*-Hydroxybenzoic acid | 3.21±0.15$^{a}$ | 6.27±1.24$^{b}$ | 7.87±0.77$^{bc}$ | 9.45±1.04$^{c}$ | 9.35±0.69$^{c}$ |
| *p*-Hydroxyphenylacetic acid | nd$^{a}$ | nd$^{a}$ | 29.05±1.55$^{c}$ | 13.18±0.83$^{b}$ | 21.75±2.75$^{c}$ |
| *p*-Hydroxybenzaldehyde | nd$^{a}$ | 1.88±0.11$^{c}$ | 1.20±0.15$^{b}$ | 1.11±0.13$^{b}$ | 1.63±0.09$^{c}$ |
| Vanillic acid | 3.92±0.14$^{a}$ | 4.71±1.01$^{a}$ | 14.05±0.67$^{b}$ | 15.41±1.06$^{b}$ | 2.80±0.09$^{a}$ |
| Syringic acid | 2.22±0.01$^{a}$ | 4.76±0.28$^{a}$ | 11.32±1.05$^{b}$ | 17.51±4.23$^{b}$ | 5.18±0.65$^{a}$ |
| **TOTAL** | **10.72 ± 0.24**$^{a}$ | **17.62 ± 0.25**$^{a}$ | **66.41 ± 4.24**$^{c}$ | **58.73 ± 1.02**$^{c}$ | **40.71 ± 2.76**$^{b}$ |
| | | | ***Hydroxycinnamics*** | | |
| *trans p*-Coumaric acid | 1.94±0.21$^{a}$ | 4.19±0.39$^{b}$ | 7.55±0.26$^{c}$ | 2.35±0.93$^{a}$ | 6.37±0.44$^{c}$ |
| *cis p*-Coumaric acid | 0.21±0.05$^{b}$ | 0.70±0.02$^{c}$ | 0.74±0.06$^{c}$ | nd$^{a}$ | nd$^{a}$ |
| Sinapic acid | 0.58±0.01$^{c}$ | 0.27±0.01$^{b}$ | 1.01±0.05$^{d}$ | 1.20±0.07$^{a}$ | nd$^{a}$ |
| *trans*-Ferulic acid | 0.61±0.02$^{b}$ | nd$^{a}$ | nd$^{a}$ | nd$^{a}$ | nd$^{a}$ |
| **TOTAL** | **3.34 ± 0.18**$^{a}$ | **5.16 ± 0.88**$^{b}$ | **9.30 ± 0.25**$^{c}$ | **3.55 ± 0.84**$^{c}$ | **6.37 ± 0.44**$^{b}$ |
| | | | ***Flavanones and flavonols*** | | |
| Eriodictyol 7-rutinoside | 7.78±0.25$^{d}$ | 9.89±0.33$^{e}$ | 3.27±0.22$^{c}$ | nd$^{a}$ | 0.91±0.05$^{b}$ |
| Hesperidin | 5.28±0.27$^{c}$ | nd$^{a}$ | nd$^{a}$ | 2.32±0.01$^{b}$ | 2.32±0.67$^{b}$ |
| Kaempferol 3-rutinoside | 0.79±0.09$^{a}$ | nd$^{a}$ | 4.02±0.81$^{b}$ | 0.98±0.01$^{a}$ | nd$^{a}$ |
| Kaempferol 3-glucoside | nd$^{a}$ | 2.40±0.23$^{b}$ | 2.07±0.82$^{b}$ | 1.08±0.20$^{ab}$ | nd$^{a}$ |
| Eriodictyol glycoside | 1.01±0.06$^{b}$ | nd$^{a}$ | nd$^{a}$ | nd$^{a}$ | nd$^{a}$ |
| Naringin | 0.81±0.02$^{b}$ | nd$^{a}$ | nd$^{a}$ | nd$^{a}$ | nd$^{a}$ |
| Kaempferol glycoside 1 | nd$^{a}$ | 1.39±0.01$^{b}$ | 1.49±0.31$^{b}$ | 2.21±0.74$^{c}$ | nd$^{a}$ |
| Naringenin | 2.22 ±0.32$^{b}$ | 13.10±0.47$^{d}$ | 3.60±0.71$^{bc}$ | 3.27±0.88$^{bc}$ | nd$^{a}$ |
| **TOTAL** | **17.89 ±0.53**$^{c}$ | **26.78 ±1.05**$^{d}$ | **14.45 ±2.66**$^{c}$ | **9.86 ±1.07**$^{b}$ | **3.23 ±1.01**$^{a}$ |
| | | | ***Stilbene*** | | |
| *trans*-Resveratrol glucoside | 0.23±0.01$^{b}$ | nd$^{a}$ | nd$^{a}$ | nd$^{a}$ | nd$^{a}$ |

NF: natural microbiota; AF: *A. oryzae;* RF: *R. oryzae;* BF: *B. subtilis*
nd: no detected; Values are mean ± SD ($n$ = 2)
a,b,c Means values in the same row with different letters are significantly different: LSD ($p$<0.05).

The changes observed in the flavonols identified depended on the microorganism used as inoculum (Table 2). Kaempferol-3-*O*-glucoside was detected neither in control sample nor in soybean fermented with *B. subtilis*. Kaempferol-3-*O*--rutinoside was identified in the control sample in low concentration, and also in the samples fermented with *A. oryzae* and *R. oryzae*.

With regard to flavanones, in sample without fermentation process, the total content of flavanones was 17.10 $\mu$g/g (Table 2). The most abundant flavanone in this sample corresponded to eriodictyol 7-*O*-rutinoside (7.8 $\mu$g/g), which represented 45% of the total identified flavanones in soybean without fermentation process (control).

The presence of flavanones in the whole seed has been described in different soybean cultivars in variable concentrations, naringin in the range of 0–9 $\mu$g/g, hesperidin 0–12 $\mu$g/g and naringenin 0–29 $\mu$g/g [Kim *et al*., 2006a, b]. The difference in the concentrations depends on several factors such as the size of seeds, which suggests that the synthesis and accumulation of these compounds are related to embryo and cotyledon size. In addition, these authors observed that these compounds were mainly located in the seed coat, whose concentration in this part ranged from 1 to 15 $\mu$g/g.

The flavanones content decreased in the samples fermented with the different microorganisms with respect to sample without fermentation process. However, soybean flours fermented naturally by only the microorganisms present in the seeds presented the highest total content in these compounds (22.99 mg/g). Eriodictyol-7-*O*-rutinoside and naringenin remained in the majority of samples, although they underwent quantitative changes that depended on the microorganism used. Thus, soybean fermented with natural microbiota showed an increase of 27% in the total flavanones content, however, the soybean fermented with the different microorganism experimented a significant decrease, being the most pronounced in the sample fermented with *B. subtilis* (Table 2).

**Isoflavones**

Daidzein, daidzein 7-*O*-glucoside (daidzin), glycitein, glycitein 7-*O*-glucoside (glycitin), genistein, genistein 7-*O*--glucoside (genistin), genistein malonylglycoside, genistein

acetylglycoside, and other genistein and daidzein derivatives were identified in soybean samples (Table 1).

The most abundant group of phenolics identified in all studied samples corresponded to isoflavones (Table 3), which represented the highest percentage both in soybean without fermentation process (92%), as in soybean seed fermented naturally (96%) and samples fermented with different microorganisms, *A. oryzae* (89%), *R. oryzae* (86%) and *B. subtilis* (88%).

The identified isoflavones have been fully described and present in the seeds and various soybean products. It has been observed that the content of isoflavones in soybean showed a large range of concentrations depending on several factors, mainly cultivation, the degree of maturation, and the environmental culture conditions, as well as the variety and origin. Various studies have reported the presence of malonyl and acetyl glycosides of daidzein, genistein and glycitein, in the raw soybean seed in diverse concentrations, mainly in raw mature seeds, but mainly the presence of the glycosides was highlighted [Rostagno *et al*., 2004; Wu *et al*., 2004].

Some of the identified compounds such as genistein and daidzein derivatives could correspond to malonylglycoside isomers reported by the authors cited above. Wu *et al.* [2004] have also reported the presence of different isomers of malonylglycosides of genistein, daidzein and glycitein, that were the most common in soybeans seeds. Therefore, the presence of genistein malonylglycoside and genistein acetylglycoside in the control soybean was in agreement with that reported by the mentioned authors.

Various authors have studied that the phenolic composition, mainly the isoflavone contents in soybeans, suffered important changes during the fermentation process [Lin *et al*., 2006; Otieno & Shah, 2007a, b; Xu & Chang, 2008; Cho *et al*., 2009]. The observed modifications affected the content of phenolic compounds, mainly isoflavones, and depended on conditions of the fermentation process.

TABLE 3. Concentration ($\mu$g/g) of isoflavones in control and fermented with different microorganism soybeans samples.

| Compounds | Control | NF | AF | RF | BF |
|---|---|---|---|---|---|
| Daidzein derivative 1 | 2.86±0.22$^{b}$ | nd$^{a}$ | nd$^{a}$ | nd$^{a}$ | nd$^{a}$ |
| Daidzein 7-glucoside (daidzin) | 186.9±9.63$^{d}$ | 210.4±16.02$^{d}$ | 143.7±22.3$^{c}$ | 55.74±4.32$^{b}$ | 15.01±0.79$^{a}$ |
| Daidzein derivative 2 | nd$^{a}$ | nd$^{a}$ | nd$^{a}$ | 52.54±1.33$^{b}$ | 1.18±0.31$^{a}$ |
| Daidzein derivative 3 | nd$^{a}$ | 0.77±0.11$^{a}$ | 14.82±1.08$^{c}$ | nd$^{a}$ | 4.43±0.15$^{b}$ |
| Daidzein derivative 4 | 0.94±0.05$^{a}$ | 95.53±10.61$^{b}$ | 4.36±0.92$^{a}$ | nd$^{a}$ | 4.84±0.31$^{a}$ |
| Daidzein derivative 5 | 1.38±0.25$^{b}$ | nd$^{a}$ | 14.97±0.31$^{d}$ | nd$^{a}$ | 7.5±0.91$^{c}$ |
| Malonyl-daidzin | nd$^{a}$ | 7.09±0.35$^{c}$ | 9.34±0.63$^{c}$ | 6.85±1.62$^{c}$ | 3.64±0.67$^{b}$ |
| Acetyl-daidzin | nd$^{a}$ | 20.02±1.71$^{c}$ | 35.77±6.38$^{d}$ | 9.37±2.07$^{b}$ | nd$^{a}$ |
| Daidzein | 12.12±0.41$^{a}$ | 213.12±79.57$^{c}$ | 181.11±19.18$^{b}$ | 85.65±13.81$^{b}$ | 173.25±2.61$^{b}$ |
| **TOTAL daidzein compounds** | **204.09±11.90$^{a}$** | **546.91±98.16$^{c}$** | **403.94±51.65$^{b}$** | **210.15±20.97$^{a}$** | **209.85±1.71$^{a}$** |
| | | | | | |
| Glycitein 7-glucoside (glycitin) | 13.72±1.03$^{b}$ | 17.85±1.37$^{c}$ | 18.07±0.94$^{c}$ | 11.27±0.09$^{a}$ | 13.29±0.56$^{ab}$ |
| Glycitein derivative | nd$^{a}$ | 2.17±0.11$^{c}$ | 1.09±0.11$^{b}$ | nd$^{a}$ | nd$^{a}$ |
| Malonyl-glycitin | nd$^{a}$ | 3.45±0.08$^{c}$ | 1.88±0.25$^{b}$ | 0.62±0.05$^{a}$ | 0.67±0.04$^{a}$ |
| Glycitein | 0.50±0.02$^{a}$ | 36.32±4.68$^{c}$ | 20.37±4.01$^{b}$ | 23.02±0.29$^{b}$ | 18.79±0.63$^{b}$ |
| **TOTAL glycitein compounds** | **14.22±1.26$^{a}$** | **59.79±6.26$^{c}$** | **41.41±4.84$^{b}$** | **34.91±0.18$^{b}$** | **32.75±0.45$^{b}$** |
| | | | | | |
| Genistein derivative 1 | nd$^{a}$ | nd$^{a}$ | 18.34±1.01$^{b}$ | nd$^{a}$ | nd$^{a}$ |
| Genistein derivative 2 | nd$^{a}$ | nd$^{a}$ | 0.65±0.06$^{c}$ | 0.09±0.01$^{b}$ | nd$^{a}$ |
| Genistein 7-glucoside (genistin) | 107.04±5.81$^{b}$ | 183.69±22.54$^{b}$ | 66.25±0.88$^{a}$ | 51.96±1.33$^{a}$ | 62.24±2.19$^{a}$ |
| Genistein derivative 3 | nd$^{a}$ | 5.18±0.68$^{c}$ | 1.21±0.25$^{b}$ | nd$^{a}$ | nd$^{a}$ |
| Genistein derivative 4 | nd$^{a}$ | nd$^{a}$ | 2.5±0.72$^{b}$ | nd$^{a}$ | nd$^{a}$ |
| Genistein acetylhexoside | 8.38±0.51$^{b}$ | 63.61±4.11$^{d}$ | 20.95±5.27$^{c}$ | 7.77±1.08$^{ab}$ | 0.19±0.02$^{a}$ |
| Malonyl-genistin | 2.56±0.09$^{a}$ | 67.06±3.95$^{b}$ | 1.97±0.06$^{a}$ | 0.63±0.03$^{a}$ | 0.78±0.03$^{a}$ |
| Acetyl-genistin | 1.81±0.23$^{a}$ | 10.92±0.37$^{b}$ | 23.62±1.66$^{c}$ | 9.39±2.15$^{b}$ | 1.98±0.06$^{a}$ |
| Genistein derivative 5 | 23.53±1.68$^{c}$ | 27.34±2.09$^{c}$ | 12.13±0.13$^{b}$ | nd$^{a}$ | nd$^{a}$ |
| Genistein | 17.72±0.91$^{a}$ | 321.53±58.01$^{d}$ | 154.73±22.22$^{c}$ | 108.48±14.09$^{bc}$ | 60.78±5.02$^{ab}$ |
| **TOTAL genistein compounds** | **161.02±9.51$^{a}$** | **679.88±82.34$^{c}$** | **302.35±37.83$^{b}$** | **178.33±16.61$^{a}$** | **125.97±8.49$^{a}$** |

NF: natural microbiota; AF: *A. oryzae;* RF: *R. oryzae;* BF: *B. subtilis* ; nd: no detected;
Values are mean ± SD ($n$ = 2)
a,b,c Means values in the same row with different letters are significantly different: LSD ($p$<0.05).

Greater differences in the identified isoflavones were observed between the control and fermented soybean seeds. Table 3 shows the concentration of isoflavones in soybean sample without fermentation process and samples fermented with different microorganisms. Due to the high number of identified isoflavones in the samples, Table 4 shows the total concentration of aglycones and derivatives isoflavones in order to achieve a better clarification of the results. An increase in the total concentration of isoflavone content was observed in the fermented samples. It is important to highlight the increase in isoflavone aglycones content such as daidzein, genistein and glycitein, in all soybean samples after fermentation process (naturally and with different inocula). Therefore, this increase n the aglycones content after fermentation process could be associated to the decrease in the glucosides of these isoflavones. This could be due to the glycosidase activity inherent in the microorganisms used to carry out the fermentation process.

However, a higher number of isoflavones was detected in the different fermented soybeans, than soybean sample without fermentation process, such as malonyl glycosides of daidzein and glycitein and acetyl glycoside of daidzein, along with other derivatives, fundamentally derived from genistein. These derivatives could correspond to glycosilation of the corresponding aglycone with different sugars such as galactose, and to isomers of the acetyl and malonylglycosides of genistein, daidzein and glycitein, which were identified in the control sample. The behaviour of these derivates was different according to microorganisms used as inoculum. Thus, malonyldaidzin, acetyldaidzin and malonyl-glycitin were not detected in the sample without fermentation process and were formed in variable quantities. No significant differences were observed in the NF, AF and RF in malonyl-daidzin content with respect to control sample. Acetylgenistin increased after fermentation process in the treatments NF, AF and RF, however no significant differences were found in malonylgenistin content between soybean seed without fermentation process and the samples fermented with *A. oryzae*, *R. oryzae* and *B. subtilis*, increasing in soybean seed fermented naturally.

As it can be observed in Table 4, the levels of daizein, glycitein and genistein increased markedly after fermentation, with 7.1 to 17.6 fold for daidzein, 37.6 to 72.6 for glycitein and 3.4 to 18.2 for genistein, with respect to the control. The highest increase of aglycones corresponded to the natural fermentation (NF), in which daizein, glycitein and genistein increased 17.6, 72.6 and 18.2 fold respectively, when compared to the control.

Previous studies carried out by our research group in legumes after different processes, such as fermentation, addition of enzymes and germination, reported similar behaviour to that found in this work, with increases and decreases being observed through the study period [Dueñas *et al.*, 2005, 2007a, b, 2009]. This great variation of these compounds could be explained by the complex biochemical metabolism of seeds during treatment. As well as, the activation of the endogenous enzymes in the soybean seeds, together with the enzymes produced by each microorganism used in the fermentation process could bring out synergic and/or antagonist effects depending on the type of compounds.

TABLE 4. Concentrations ($\mu$g/g) in the control (C) and fermented samples of grouped identified.

| Grouped compounds | Control (C) | NF | AF | RF | BF |
|---|---|---|---|---|---|
| Daidzein | 12.12 | 213.12 | 181.11 | 85.65 | 173.25 |
| Daidzein derivatives | 191.97 | 333.79 | 222.83 | 124.5 | 36.6 |
| Glycitein | 0.50 | 36.32 | 20.37 | 23.02 | 18.79 |
| Glycitein derivatives | 13.72 | 23.47 | 21.04 | 11.89 | 13.96 |
| Genistein | 17.72 | 321.53 | 154.73 | 108.48 | 60.78 |
| Genistein derivatives | 143.32 | 358.35 | 147.62 | 69.85 | 65.19 |
| Hydroxybenzoics | 10.72 | 17.62 | 66.41 | 58.73 | 40.71 |
| Hydroxycinnamics | 3.34 | 5.16 | 9.3 | 3.55 | 6.37 |
| Flavonols + flavanones | 17.89 | 26.78 | 8.85 | 4.35 | 3.23 |

F: natural microbiota; AF: *A. oryzae;* RF: *R. oryzae;* BF: *B. subtilis*; nd: not detected phenolic compounds.

Studies carried out using a variety of microorganisms reflected that the liberation of isoflavone aglycones depended on the glucosidase activity inherent in each of them, although factors such as temperature, pressure, action time, *etc*., might influence this reaction [Pham & Shah 2009]. Thus, *Bifidobacterium animalis, Lactobacillus casei* and *Lactobacillus acidophilus* actions in soymilk resulted in an increase in the aglycone concentrations by 5.87, 6.07 and 5.94 fold respectively [Otieno & Shah, 2007b].

Cho *et al*. [2009] found that the fermentation of *cheonggukjang* by *Bacillus pumilius* considerably increased the content in isoflavone aglycones and diminished the isoflavone glycosides, as a result of the β-glucosidase and esterase activities. They also observed a noticeable increase in the radical DPPH scavenging activity after the fermentation.

**Stilbenes**

One stilbene *trans*-resveratrol-3-*O*-glucoside was identified in low concentration in the sample without the fermentation process. This compound disappeared after the fermentation process in seeds inoculated with *A. oryzae*, *R. oryzae* and *B. subtilis* as well as in soybean seeds fermented naturally by only the microorganisms present in the seeds.

In order to obtain a better interpretation of the results, the analysis of principal components was carried out, where the concentration of the identified phenolic compounds in the soybean samples, control (C) and fermented NF, AF, RF and BF were considered. Principal components analysis was done on the concentrations of the identified compounds grouped by their chemical structure (Table 4) in order to achieve a better clarification of the results.

Four eigenvalues of covariance matrix were extracted. The first two accounting for 97.41% of total variance (Table 5), first of them explains 91.74% and the second explains 5.67% of total variance. Projection of the two first variables (Figure 1) showed three groups of compounds which presented high relationship in each one. First group corresponded to genistein and its derivatives, the second to daidzein and its derivatives, and the third to the rest of compounds

TABLE 5. Extracted eigenvalues of covariance matrix.

| | Eigenvalue | % Total variance | Cumulative Eigenvalue | Cumulative % |
|---|---|---|---|---|
| 1 | 42666.52 | 91.74 | 42666.52 | 91.74 |
| 2 | 2637.86 | 5.67 | 45304.38 | 97.41 |
| 3 | 1031.66 | 2.22 | 46336.04 | 99.63 |
| 4 | 171.21 | 0.37 | 46507.25 | 100.00 |

(glycitein+glycitein derivatives, hydroxybenzoics, hydroxycinnamics and flavonols+flavanones). In the third group, the hydroxybenzoics showed the smallest relationship with the rest of compounds of this group. In projection, samples C and BF presented a high correlation and they seem to be rather similar, they were inversely correlated with samples AF and RF; sample NF showed a high inverse correlation with RF.

The sample fermented with *Aspergillus oryzae* (AF) presented the highest relationship with all compounds, but mostly with the third group of compounds. Sample RF was the most related to hydroxybenzoics, and that fermented with the natural microbiota (NF) was most related to the aglycones genistein and daidzein and their derivatives, but it was different of sample AF. The samples C and BF were the most related to the third group of compounds (Figure 1). As it can be observed, the natural microbiota of soybeans (NF) showed the highest relation with the isoflavone aglycones, genistein, daidzein and the derivatives of them, which corresponds to the highest concentration of these compounds in the sample.

From our results, it can be deduced that the fermentation process carried out with natural microbiota or different microorganisms caused significant changes in the phenolic composition of soybean. Non-flavonoids (hydroxybenzoic and hydroxycinnamic compounds) presented a maximum

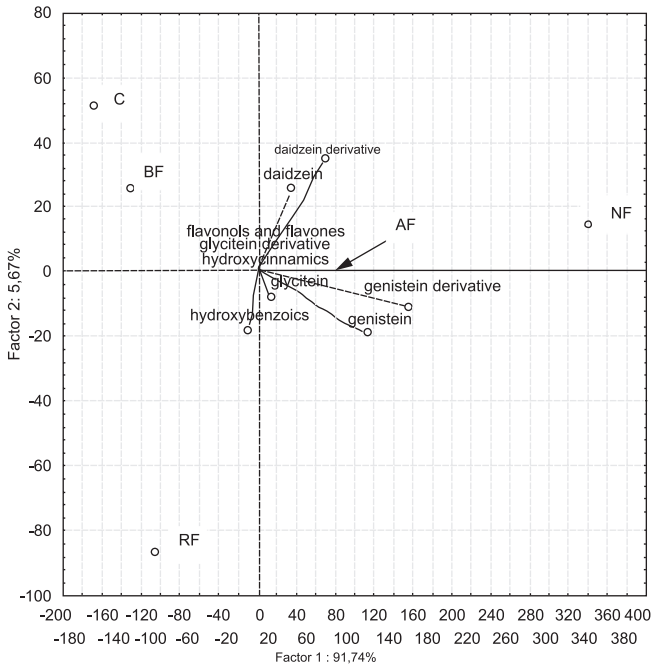


FIGURE 1. Biplot chart of the first and second principal components of the polyphenolic compounds in the control (C) and fermented (NF, AF, RF, BF) soybeans.

of concentration when fermentation was carried out with *A. oryzae* inoculum. Contents of flavonoids, mainly isoflavones, increased in the soybean samples fermented naturally by only the microorganisms present in the seeds and in samples fermented with *A. oryzae* and *R. oryzae*. It is important to highlight the increase in the isoflavone aglycones content, as well as a decrease in glycosides, observed in all fermented samples.

This great variation of these compounds could be explained by the complex biochemical metabolism of seeds during the fermentation process, due to the enzymes production by each microorganism used as inoculum could be different. As well as, the activation of the endogenous enzymes in the soybean seeds, together with the enzymes produced by each microorganism used in the fermentation process could bring out synergic and/or antagonist effects depending on the type of compounds.

## CONCLUSIONS

Fermentation process caused significant changes in the phenolic composition of soybean seeds, and therefore, it could also affect the beneficial biological effects (antioxidant properties) associated with these components. These changes could be due to enzymes' production and activation by the microbiota used in order to perform the fermentation process, bringing out complex biochemical metabolism of soybean during this process. In general, the fermentation process produced a significant increase in the levels of the phenolic acids and flavonoids, mainly aglycones isoflavones. Therefore, the significant occurrence of these bioactive phenolic compounds in the studied soybean flours makes them useful for the food industry, as beyond their nutrition functions they were shown to confer health-promoting effects.

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## REFERENCES

1. Akpapunam M.A., Achinewhu S.C., Effects of cooking, germination and fermentation on the chemical composition of Nigerian cowpea (*Vigna unguiculata*). Qual. Plant. Plant Foods Hum. Nutr., 1985, 35, 353–358.
2. Alonso R., Aguirre A., Marzo F., Effects of extrusion and traditional processing methods on antinutrients and *in vitro* digestibility of protein and starch in faba and kidney beans. Food Chem., 2000, 68, 159–165.
3. Chang C.T., Hsu C.K., Chou S.T., Chen Y.C., Huang F.S., Chung Y.C., Effect of fermentation time on the antioxidant activities of tempeh prepared from fermented soybean using *Rhizopus oligosporus*. Int. J. Food Sci. Tech., 2009, 44, 799–806.
4. Cho K.M., Hong S.Y., Math R.K., Lee J.H., Kambiranda D.M., Kim J.M., Islam S.A., Yun M.G., Cho J.J., Lim W.L., Yun H.D.,

Biotransformation of phenolics (isoflavones, flavanols and phenolic acids) during the fermentation of *cheonggukjang* by *Bacillus pumilus* HY1. Food Chem., 2009, 114, 413–419.

5. Choi Y.M., Kim Y.S., Ra K.S., Suh H.J., Characteristics of fermentation and bioavailability of isoflavones in Korean soybean paste (*doenjang*) with application of *Bacillus* sp. KH-15. Int. J. Food Sci. Tech., 2007, 42, 1497–1503.
6. Doblado R., Frías J., Muñoz R., Vidal-Valverde C., Fermentation of *Vigna sinensis* var. carilla flours by natural microflora and *Lactobacillus* species. J. Food Protect., 2003, 66, 2313–2320.
7. Dueñas M., Fernández D., Hernández T., Estrella I., Muñoz R., Bioactive phenolic compounds of cowpeas (*Vigna sinensis* L.). Modifications by fermentation with natural microflora and with *Lactobacillus plantarum* ATCC 14917. J. Sci. Food Agric., 2005, 85, 297–304.
8. Dueñas M., Hernández T., Estrella I., Changes in the content of bioactive polyphenolic compounds of lentils by the action of exogenous enzymes. Effect on their antioxidant activity. Food Chem., 2007a, 101, 90–97.
9. Dueñas M., Hernández T., Estrella I., Influence of the action of exogenous enzymes on the polyphenolic composition of pea. Effect on the antioxidant activity. Eur. Food Res. Technol., 2007b, 225, 493–500.
10. Dueñas M., Hernández T., Estrella I., Fernández D., Germination as a process to increase the polyphenols content and antioxidant activity of lupin seeds (*Lupinus angustifolius* L.). Food Chem., 2009, 117, 599–607.
11. Fernández-Orozco R., Frías J., Muñoz R., Zielinski H., Piskula M.K., Kozlowska H., Vidal-Valverde C., Fermentation as a bioprocess to obtain functional soybean flours. J. Agric. Food Chem., 2007, 55, 8972–8979.
12. Frías J., Vidal-Valverde C., Kozlowska H., Tabera J., Honke J., Hedley C.L., Natural fermentation of lentils. Influence of time, flour concentration and temperature on the kinetics of monosaccharides, disaccharides and α-galatosides. J. Agric. Food Chem., 1996, 44, 579–584.
13. Granito M., Torres A., Frías J., Guerra M., Vidal-Valverde C., Influence of fermentation on the nutritional value of two varieties of *Vigna sinensis*. Eur. Food Res. Technol., 2005, 220, 176–181.
14. Hendrich S., Murphy P.A., Isoflavones: source and metabolism. 2001, *in:* Handbook of Nutraceuticals and Functional Foods (ed. R.E.C. Wildman). Boca Raton FL, CRC Press, USA, pp. 55–75.
15. Kim E.H., Kim S.H., Chung J.I., Chi H.Y., Kim J.A., Chung I.M., Analysis of phenolic compounds and isoflavones in soybean seeds [*Glycine max* (L) Merill] and sprouts grown under different conditions. Eur. Food Res. Technol., 2006a, 222, 201–208.
16. Kim E.H., Kim S.H., Chung J.I., Chi H.Y., Kim J.A., Chung I.M., A correlation between the level of phenolic compounds and the antioxidant capacity in cooked-with-rice and vegetables soybean (*Glycine max* L.) varieties. Eur. Food Res. Technol., 2006b, 224, 259–270.
17. Lee S.J., Kim J.J., Moon H.I., Ahn J.K., Chun S.Ch., Jung W.S., Lee O.K., Chung I.M., Analysis of isoflavones and phenolic compounds in Korean soybean [*Glycine max* (L.) Merrill] seeds of different seed weights. J. Agric. Food Chem., 2008, 56, 2751–2758.
18. Lee J.H., Jeon J.Y., Kim S.G., Kim S.H., Chun T., Imm J.Y., Comparative analyses of total phenols, flavonoids, saponins and antioxidant activity in yellow soy beans and mung beans. Int. J. Food Sci. Tech., 2011, 46, 2513–2519.
19. Lin C.-H., Wei Y.-T., Chou C.-C., Enhanced antioxidant activity of soyben koji prepared with various filamentous fungi. Food Microbiol., 2006, 23, 628–633.
20. López-Amorós M.L., Hernández T., Estrella I., Effect of germination on legume phenolic compounds and their antioxidant activity. J. Food Comp. Anal., 2006, 19, 277–283.
21. Malencic D., Maksimovic Z., Popovic M., Niladinovic J., Polyphenol content and antioxidant activity of soybean seed extracts. Biores. Technol., 2008, 99, 6688–6691.
22. Nagata C., Takatsuka N., Inaba S., Kawakawi N., Shimizu H., Effect of soymilk consumption on serum estrogen concentrations in premenopausal Japanese women. J. Natl. Cancer Inst., 1998, 90, 1830–1835.
23. Otieno D.O., Shah N.P., Endogenous β-glucosidase and β-galactosidase activities from selected probiotic microorganisms and their role in isoflavone biotransformation in soymilk. J. Appl. Microbiol., 2007a, 103, 910–917.
24. Otieno D.O., Shah N.P., A comparison of changes in the transformation of isoflavones in soymilk using varying concentrations of exogenous and probiotic-derived endogenous β-glucosidases. J. Appl. Microbiol., 2007b, 103, 601–612.
25. Pham T.T., Shah N.P., Hydrolysis of isoflavone glycosides in soy milk by β-galactosidase and β-glucosidase. J. Food Biochem., 2009, 33, 38–60.
26. Park Y.H., Alenscar S.M., Aguiar C.L., Mascrenhas H.A.A., Scamparini A.R.P., Conversion of malonyl β-glucoside isoflavones found in some cultivars of Brazilian soybeans. Ciênc. Tecnol. Aliment., 2002, 22, 130–135.
27. Park Y.K., Lui M.C.Y., Aguiar C.L., Production of enriched isoflavone aglycones during processing of soy protein isolates and soy protein concentrates. 2003, *in:* IFT Annual Meeting Book of Abstracts. Chicago II. Institute of Food Technologists, pp. 215–221.
28. Randhir R., Vattem D. Shetty K., Solid-state bioconversion of fava bean by *Rhizopus oligosporus* for enrichment of phenolic antioxidants and L-DOPA. Innov. Food Sci. Emerg. Technol., 2004, 5, 235–244.
29. Ribeiro M.L.L., Mandarino J.M.G., Carrao-Panizzi M.C., De Oliveira M.C.N., Campo C.B.H., Nepomuceno A.L., Ida E.I., Isoflavone content and β-glucosidase activity in soybean cultivars of different maturity groups. J. Food Comp. Anal., 2007, 20, 19–24.
30. Rostagno M.A., Palma M., Barroso C.G., Pressurized liquid of isoflavones from soybeans. Anal. Chim. Acta, 2004. 522, 169–177.
31. Setchell K.D.R., Cassidy A., Dietary isoflavones: biological effects and relevance to human health. J Nutr., 1999, 129 (suppl) 758S-767S.
32. Setchell K.D.R., Brown N.M., Zimmer-Nechemias L., Brashear W.T., Wolfe B.E., Kirschner A.S., Heubi J.E., Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. Am. J. Clin. Nutr., 2002, 76, 447–453.
33. Setchell K.D.R., Brown N.M., Desai P.B., Ziummer-Nechimias L., Wolfe B., Jakate A.S., Creutizinger V., Heubi J.E., Bioavailability, disposition, and dose-response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes. J Nutr., 2003, 133, 1027–1035.

34. Shahidi F., Naczk M., Phenolic compounds of major oilseeds and plant oils. 2004, *in*: Phenolics in Food and Nutraceuticals. CRC Press, Boca Raton, Florida, USA, pp. 83–103.
35. Tsangalis D., Ashton J.F., Mcgill A.E.J., Shah N.P., Enzymatic transformation of isoflavone phytoestrogens in soymilk by β-glucosidase producing bifidobacteria. J. Food Sci., 2002, 67, 3104–3113.
36. Wardhani D.H., Vázquez J.A., Pandiella S.S., Mathematical modelling of the development of antioxidant activity in soybeans fermented with *Aspergillus oryzae* and *Aspergillus awamori* in the solid state. J. Agric. Food Chem., 2009, 57, 540–544.
37. Wu Q., Wang M., Sciarappa W.J., Simon J.E., LC/UV/ESI-MS analysis of isoflavones in Edamame and Tofu soybeans. J. Agric. Food Chem., 2004, 52, 2763–2769.
38. Xu B., Chang S.K.C., Characterization of phenolic substances and antioxidant properties of food soybeans grown in the North Dakota-Minnesota region. J. Agric. Food Chem., 2008, 56, 9102–9113.
39. Yue X., Abdallah A.M., Xu Z., Distribution of isoflavones and antioxidant activities of soybean cotyledon, coat and germ. J. Food Process. Pres., 2010, 34, 795–806.
40. Zamora A.F., Fields M.L., Nutritive quality of fermented cowpeas (*Vigna sinensis*) and chickpeas (*Cicer arietinum*). J. Food Sci., 1979, 44, 234–236.

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