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Spectroscopic determination of metabolic and mineral changes of soya-chunk mediated by *Aspergillus sojae*

K.M. Maria John [a], Gansukh Enkhtaivan [a], Jiho Lee [a], Muthu Thiruvengadam [b], Young-Soo Keum [a], Doo Hwan Kim [a,*]

[a] Department of Bioresources and Food Science, Konkuk University, Seoul 143-701, Republic of Korea
[b] Department of Applied Bioscience, Konkuk University, Seoul 143-701, Republic of Korea

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ABSTRACT

Time dependent changes of primary (GC–MS), isoflavones (LC–MS) and minerals (ICP-OES) content of fermented soya-chunk were compared with un-fermented (0H) soya-chunk and presented. Results revealed that the amino acid content increased gradually based on the fermentation time; whereas the maltose, sucrose and fructose contents were reduced due to the fungal growth. The glucosides changed extensively during the initial fermentation time resulting in augmentation of aglycones and phytoalexins. This affects the antioxidant potential whereas the DPPH and ABTS of 0H showed lowest activity (18.15% and 54.92%) and increased quite high with fungal fermentation (45.81% and 93.47%). The calcium (0.55%), magnesium (0.47 mg/kg), nickel (5.17 mg/kg l^{-1}), and copper (8.33 mg/kg l^{-1}) content were increased during the fermentation and in a decrease of iron and aluminium contents. Findings suggest that the soya-chunk prepared by fungal fermentation will improve the antioxidant and mineral content and hence their nutritional property will be enhanced for humans.

1. Introduction

Soybean is found to be one of the major food sources for many Asian countries. Due to its high protein and metabolic content it serves as oral antioxidant source to humans. These seeds contain high levels of isoflavones which are known for their weak oestrogenic potential, and the cause for menopausal complaints and osteoporosis in humans (Messina, 1999; Wang & Murphy, 1994). Particularly the isoflavones were converted into phytoalexins during various stress and fermentation conditions and showed high antioxidant, antiestrogenic and antiproliferative potential (Ng et al., 2011). That, microbial fermentation alters the bioavailability of such isoflavones were well studied before and illustrated the improvements on antioxidant, melanogenesis and inhibition of angiogenesis (Jeon, Seo, Shin, & Lee, 2012; Lee, Lee, Jung, & Lee, 2013; Shin & Lee, 2013). Because of these reasons many naturally fermented soy foods such as meju, doenjang and chungkukjang is used in South Korea and many other Asian countries.

India is one of the major countries where vegetarianism is practiced (almost 31%). They eat only the vegetable based products, among them soya-chunk a processed soy based food was very famous because of their taste and health effects. In southern-India many dishes based on soymeal (soya-chunk) such as soya curry, cutlet, pulao and kurma are widely used by the vegetarians (http://naveenanangai.blogspot.kr/2013/02/soya-chunks-or-soya-wadi-curry.html). This soy product is also commonly called as "vegetable chicken" among the vegetarians. Even in biryani a famous Indian rice based food soya chunk was added instead of meat because of its taste and resemblance to meat. These soy products are rich in various biochemical molecules such as proteins, calcium, isoflavones hence these products are accepted as good for health. Even though the soya-chunk was in use for a long time the scientific evidences on their bioavailability of metabolites and their effects are unknown. In our screening study it was clear that the soya-chunks antioxidant activity was very less than other fermented soy products (screening data was not included in this paper). Based on our previous studies *Aspergillus sojae* (AS) improves the metabolic and antioxidant potential of soybean (Maria John, Jung, Lee, Kim, & Lee, 2013). To improve the metabolic and antioxidant content of soya-chunk, AS mediated fermentation was tried.

Microbial fermentation, which alters the secondary metabolites profile, was evidently studied in the crops like soy bean, green tea and black tea (Kim et al., 2013; Maria John, Thiruvengadam, Enkhtaivan, & Kim, 2014; Maria John et al., 2013). The isoflavone

* Corresponding author. Tel.: +82 2 450 3740; fax: +82 2 2201 3738.
E-mail address: kimdh02@hanmail.net (D.H. Kim).

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classes such as glucosides have poor absorbability in human intestine when compared to their corresponding aglycones (Izumi et al., 2000). In the fermented soy foods such as cheonggukjang (Baek et al., 2010; Kim et al., 2011; Park et al., 2010), douche (Fan, Zhang, Chang, Saito, & Li, 2009), meju (Kang et al., 2011; Lee et al., 2012) and tofu (Wu, Wang, Sciarappa, & Simon, 2004), the level of glucosides changed to aglycones and hence their antioxidant along with absorbability of human intestine improved. In this case the level of glucosides in soya-chunk and their changes during microbial interaction will be helpful to improve the product quality in terms of increase in aglycones metabolites and antioxidant potential. In addition the mineral content and their changes during fungal fermentation also help for the high nutritive soy based food.

2. Materials and methods

2.1. Samples and preparations

Soy-chunks (1 kg) were purchased from commercial market from India and were soaked for 10 h with distilled water. After soaking, samples were collected separately and the water was removed by squishing, and then they were crumbled using mixer. 10 g of the sample was separated and served as control (0H). The remaining samples were autoclaved for sterilising the samples. After autoclaving 10 g (AU) of samples were separated to examine the metabolic changes. The food grade fungus *A. sojae* (AS) was inoculated onto the autoclaved samples (10 g each) in separate petri plates. The samples were placed under incubation at 37 °C for a period of 5 days. Each day, 6 petri plates were removed from the incubator and placed in a deep freezer (−80 °C) and served as 1D, 2D, 3D, 4D and 5D samples. All the samples were lyophilised and powderised before the analysis.

2.2. Primary metabolites sample preparation and analysis by GC–MS

Lyophilised samples (0H, AU and 1D–5D) were weighed 100 mg and extracted by adding 1 mL of methanol: water: chloroform (2.5:1:1) containing norvaline as an internal standard. After shaking under a Retsch ball mill at 30/s (5 min) followed by sonication (10 min) the samples were centrifuged at 5000 rpm at 20 °C for 8 min. From the supernatant 600 μL was separated and were made up to 1 mL by adding 400 μL of water. From this extract 400 μL of the samples was vacuum dried and used for the derivatisation. Methoxyamine hydrochloride in pyridine (20 mg/mL) of 200 μL was added to the extract followed by incubation (90 min) at 30 °C. To this mixer 100 μL of N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was added and were incubated at 37 °C, for 30 min (Maria John et al., 2013).

Shimadzu GC–MS system (QP-2010 SE) with an auto injector (AOC 20i) equipped with a Rxi-5silMS capillary column (30 m length × 0.25 mm i.d × 0.25 μM film thickness) was used for the analysis. GC conditions were as follows: injector temperature was 250 °C, injection volume of 1 μL. Oven temperature program starts from 80 °C for 2 min followed by 300 °C from 2 to 15 min with 10 °C/min hold and was finally held for 3 min with MS, ionisation at −70 volts. Mass range was set at 50–600 *m*/*z* with 10 spectra per second acquisition rate.

2.3. Secondary metabolites sample preparation and analysis by LC–ESI-MS

The secondary metabolites were analysed by using HP1100 LC system connected to Varian 500-MS ion-trap mass spectrometer from Varian Technologies (Palo Alto, CA, USA). Water and acetonitrile acidified with 0.1% formic acid served as mobile A and B. The gradient flow as follows: 5–60% of B in 30 min followed by 100% in 35 min. 100% B for 45 min followed by 5% in 50 min and maintained 5% up to 60 min. The flow rate was 0.2 mL/min and the injection volume was 10 μL. Mass spectra were obtained using electrospray ionisation in positive and negative modes within a range of *m*/*z* 100–1000. The operating parameters were as follows: needle voltage is 3500 volts; capillary voltage, 80 volts; drying gas temperature, 350 °C; drying gas pressure, 30 psi (nitrogen); and nebuliser gas pressure, 40 psi (air).

2.4. Data processing and multivariate analysis

The GC–MS (*.qgd) and LC–MS data (*.xms) files were converted to netCDF (*.cdf) format using inbuilt data bridge software in case of GC–MS and Vx Capture (version 2.1; Adron systems, Adron, USA) in case of LC–MS. The converted files were aligned for the retention correction, normalised peak intensities and masses by using metAlign software package (http://www.metalign.nl). The resulting aligned file was processed for multivariate statistical analysis using SIMCA-P+ 12.0 (Umetrics, Umea, Sweden) software. The data sets were log-transformed prior to partial least squares discriminant analysis (PLS-DA). The metabolites were selected based on variable importance in the projection (VIP) values (value >8.0) and *p*-value (<0.05) statistics.

For the correlation studies, Pearson's correlation coefficient test using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA) was used. Pare-wise metabolite-antioxidant effects were plotted and compared by heat map using MEV software version 4.8 (multiple array viewer, http://www.fm4.org/).

2.5. Inductively coupled plasma-optical emission spectrometry (ICP-OES) analysis

The minerals such as phosphoric acid (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), nickel (NI), iron (Fe), copper (Co), manganese (Mn), aluminium (Al) and zinc (Zn) contents were analysed by using ICP-OES (Inductively Coupled Plasma, IRis Intrepid, Thermo Elemental Co., UK). The sample analysis was performed by following the procedure of Sahan, Basoglu, and Gucer (2007). Samples (0.5 g) were digested using the Milestone MLS 1200 (Italy) microwave digestion system with 6 mL of HNO_3 by the following digestion program: 250 W (2 min), 0 W (2 min), 250 W (6 min), 400 W (5 min) and 600 W (5 min). After cooling at room temperature, sample solutions were transferred into 50 mL polyethylene flasks. 1 mL of internal standard solution (1 mg l^{-1}) was added to the samples followed by diluted to 25 mL. The analyses were performed at the following flow rates: (a) plasma gas of 15 L min^{-1}, (b) auxiliary gas of 1 L min^{-1}, and (c) sample of 0.8 mL min^{-1}. The mineral eluates were monitored at different wavelengths: 393.366 (85) nm-Ca, 228.616 (147) nm-Co, nm-P, A324.754 (103) Cu, 259.940 (129) nm-Fe, 766.491(44) nm-K, 285.213 (117) nm-Mg, 257.610 (130) nm-Mn, 588.995 (57) nm-Na, 213.856 (157) nm-Zn. All chemical analyses were carried out in triplicate on each sample (AOAC, 1990).

2.6. DPPH and ABTS free radical scavenging assay

1,1-Diphenyl-2-picrylhydrazyl (DPPH) and azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) scavenging potential of the samples were analysed by following the procedure of Maria John et al. (2014). In brief the sample extracts (20 μL) were added to 180 μL of DPPH (200 μM in ethanol) followed by a 20-min incubation at room temperature. The absorbance was measured at 515 nm using a micro plate reader.