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Effect of acetic acid treatment on isoflavones and carbohydrates in pickled soybean

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ABSTRACT

Acetic acid treatment improves the physical, chemical, and physiological properties of soybeans. This study investigated the changes in isoflavone and carbohydrate profiles in four soybean cultivars subjected to different acetic acid treatment durations. The sum of total isoflavone content of the pickled soybeans and the pickling solution was increased by 30–93% after acetic acid treatment for 30 days. In the comparison to four isoflavone groups of the untreated soybean, malonyl glucosides were decreased by 17–41%, whereas glucosides were increased by 54–160% in the pickled soybeans. In particular, aglucones were found in the pickled soybeans, but not in the untreated soybeans. Following acetic acid treatment, the total carbohydrate content in the pickled soybeans was 50–65% less than that of untreated soybeans. In particular, oligosaccharides and sucrose in the pickled soybeans decreased significantly (>50%) within the first 24 h. This study provides important information on the changes in isoflavone and certain carbohydrates profiles during soybean pickling, and may be useful to produce functional soybean-based foods containing more bioactive compounds.

1. Introduction

Soybean (*Glycine max* (L.) Merrill) is a popular health food source due to its various nutritive and nonnutritive components, including essential amino acids, fatty acids, protease inhibitors, phytosterols, saponins, phenolic acids, and phytic acid (Anderson & Wolf, 1995; Messina, 1999). The consumption of soybeans or soyfoods provides health benefits to the consumer, and help in the prevention and relief of several conditions, including cardiovascular disease, different types of cancer, osteoporosis, and menopausal symptoms. For example, the intake of soy phytochemicals or peptides is reported to reduce the occurrence of certain cancers, including prostate, breast, and endometrial cancers (De Mejia & De Lumen, 2006; Lee et al., 2003; Yamamoto et al., 2003). In particular, isoflavones, which are abundant in soybeans, have a positive effect on both hormone-dependent and independent diseases, and are thought to contribute to the low incidence of breast cancer in Asian women (Coward, Barnes, Setchell, & Barnes, 1993; Kim, Peterson, & Barnes, 1998; Setchell, 1998).

The isoflavone content of soybeans is affected by various genetic and environmental conditions, including variety, crop year, and geographical location. Furthermore, food processing, such as fermentation, heating, soaking, deforming, pressurizing, acidity (pH), and enzymatic hydrolysis also influence the isoflavone composition and content (Chung, Yu, Park, & Kim, 2014; Coward, Smith, Kirk, & Barnes, 1998; Wang & Murphy, 1994). For example, due to de-glycosylation by microorganisms, fermented soyfoods like Chunggukjang (a Korean soya paste) and Sufu (a Chinese fermented tofu) typically contain larger amounts of aglucones compared to raw soybean or non-fermented soyfoods. *Bacillus subtilis*, which is used to make Natto (a Japanese fermented soybean dish), produces isoflavone derivatives called succinyl-β-glucosides that have beneficial physiological functions in humans (Chung et al., 2012; Park et al., 2010; Yin, Li, Li, Eizo, & Masayoshi, 2004). In addition, acetic acid treatment of soybeans is reported to increase their total isoflavone content, especially that of aglucone-type isoflavones (Eom, Kim, Choi, Cha, & Kim, 2006; Han, Hong, & Kim, 2007).

Soybeans contain various carbohydrates with bioactive and nutritional functions. Raffinose and stachyose, which are classified as $(galactose)_n$–glucose–fructose polysaccharides of the raffinose family, are the main oligosaccharides found in soybeans (Prashanth & Mulimani, 2005). Soybean oligosaccharides have also been reported to have several health benefits, including improvement of carbohydrate and lipid metabolism in humans. For example, oligosaccharides are reduced to glucose, triglyceride, cholesterol, and/or phospholipids in the blood (Chen, Liu, Zhu, Xu, & Li, 2010; Mussatto & Mancilha, 2007). In addition, oligosaccharides may have positive effects on the human intestinal environment by acting as prebiotics (Qiang, YongLie, & QianBing, 2009). However, it may be desirable to remove oligosaccharides from soy foods owing to concerns about symptoms such as flatulence after soyfood intake (Prashanth & Mulimani, 2005).

Changes in the isoflavone profiles of soybeans and soyfoods have been studied extensively (Chung et al., 2012; Chung et al., 2014;

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Coward et al., 1998; Han et al., 2007; Yin et al., 2004). However, to the best of our knowledge, there is a lack of information concerning the changes in isoflavone composition and content of soybeans and soyfoods following acid treatment. Soybeans pickled in vinegar are called vinegar bean, and while the dish has long been consumed as a healthy food in Korea, it has only recently attracted research attention due to its potential health benefits, such as control of lipid metabolism, antioxidant activity, and anti-cancer potential (Eom et al., 2006). Despite this interest, there has been no comprehensive analysis of the change in soybean isoflavone content and composition during pickling (Kim & Park, 2009; Park, Kim, Kim, Suk, & Kim, 2007).

Therefore, the aim of the present study was to describe the effects of pickling on the bioactive ingredients in soybean. Specifically, four soybean cultivars were treated with common brewed vinegar for one month. Thereafter, the time-dependent changes of 12 isoflavones and 5 carbohydrates in soybean were measured. The results of our study extend our knowledge of the effects of the pickling process on isoflavone and carbohydrate profiles during pickled soybean production. These results may be useful for the production and improvement of soybean-based functional foods.

2. Materials and methods

2.1. Soybean and vinegar materials

Four soybean cultivars (Wonheuk, Dawon, Seoritae with blue cotyledons, and Seoritae with yellow cotyledons) were obtained from the Rural Development Administration of Korea in 2014 and were used to make the pickled soybeans used in this study. Wonheuk and Dawon cultivars are classified as small-grain bean sprouts, and both have a black seed coat and yellow cotyledon. Two native cultivars of Seoritae that also belong to the small grain variety have long been used as medicinal soybeans in Korea. Both Seoritae cultivars have a black seed coat, whereas one has a blue cotyledon and the other has a yellow cotyledon. The brewed vinegar (fermented malt vinegar with a total acidity of 6–7%, Ottogi, Gyeonggi-Do, Korea) was purchased from a local market near Konkuk University, Seoul Korea.

2.2. Chemicals and reagents

All solvents were of HPLC grade and were obtained from Fisher Scientific Korea Ltd. (Seoul, Korea). Glacial acetic acid, 0.1 N hydrochloric acid (HCl), and 0.1 N oxalic acid (extra pure grade) were purchased from either J. T. Baker (Phillipsburg, NJ, USA) or Daejung Chemicals and Metals (Gyeonggi-Do, Korea). Twelve isoflavone standards were obtained to identify isoflavone types in the samples of interest. Aglucone and β-glucoside were purchased from LC Laboratories (Woburn, MA, USA; Assay, ≥99.0%), while malonyl- and acetyl-β-glucosides were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan; Assay, ≥90.0%). All isoflavone standards were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA) and stored below −20 °C as stock solutions. The isoflavone standards were subsequently diluted to the required concentrations, ranging from 0.1 μg/mL to 200 μg/mL, depending on the isoflavone concentration of the sample of interest. Fructose, raffinose, and stachyose were purchased from Sigma-Aldrich (Assay ≥98.0%). Glucose and sucrose were purchased from Fluka (Buchs, Switzerland, Assay ≥99.5%). All carbohydrate standards were diluted in distilled water for each range depending on the required carbohydrate concentrations of the samples of interest.

2.3. Pickled soybean preparation

To make pickled soybean on a laboratory scale, 10 g of soybeans (approximately 80 to 100 soybean grains) was combined with 30 mL of brewed vinegar. Five experiments (1, 5, 10, 20, and 30 days, $n = 3$ per experiment) were set up in order to evaluate the effects of acetic acid treatment duration on isoflavone and carbohydrate profiles. All experiments were maintained at room temperature. Untreated raw soybeans, which were in dry conditions, were used as the control group. After each acetic acid treatment period, the pickled soybeans were separated from the pickling solution, and subsequently lyophilized and pulverized. Prior to pulverization, the pickled soybean mass was measured for the correction of isoflavone and carbohydrate quantification (*see* Section 2.7: *Quantification of isoflavones and carbohydrates*). Each pickling solution was replenished to a total volume of 30 mL using brewed vinegar. All samples were stored in a deep freezer (−70 °C) until isoflavone and carbohydrate analysis.

2.4. Extraction for isoflavones analyses

For extraction of isoflavones, 1 g of pulverized soybeans was extracted with an acidified acetonitrile (ACN) solution comprising 10 mL ACN and 2 mL 0.1 N HCl. After stirring at 200 rpm for 2 h at room temperature with a shaker (Green SSeriker VS-203D, Vision Scientific Co., Ltd., Daejeon, Korea), each extract was filtered with Whatman No. 42 filter paper (GE Healthcare Companies, Seoul, Korea). The filtrate was then evaporated by a rotary vacuum evaporator to below 35 °C (EYELA N1200AV-W w/CCA-1111, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The resulting residue was reconstituted with 5 mL of 80% methanol and filtered again through a 0.2-μm syringe membrane filter (13 mm, Hydrophilic PTFE, E.CHROM Science Inc., Daegu, Korea).

For isoflavone analysis of the pickling solution, it was diluted 5-fold with 80% methanol. To eliminate solid matrix particles present in the pickling solution it was centrifuged at 4 °C and 3200 rpm for 10 min (VS-6000CFi, Vision Scientific Co., Ltd.), and then filtered through a syringe membrane (Chung et al., 2012).

2.5. Extraction for carbohydrate analyses

For carbohydrate extraction, the pulverized soybean sample (0.1 g) was extracted with a mixture of 5 mL ethanol and 2 mL water. After stirring at 200 rpm for 30 min at room temperature with a shaker, 1 mL of 0.1 N oxalic acid was added, followed by 5 min further stirring. The extract was then brought to a volume of 20 mL with water, after which 10 mL chloroform was added. After centrifugation at 4 °C and 3000 rpm for 10 min, the supernatant was filtered through a Nylon syringe membrane. For carbohydrate analysis of the pickling solution, the solution was diluted 60-fold with 25% ethanol. The resulting solution was centrifuged at 4 °C and 3000 rpm for 10 min and filtered through a Nylon syringe membrane (Oh et al., 2012).

2.6. Instrumental analysis of isoflavones and carbohydrates

An Agilent 1260 infinity HPLC system was used for isoflavone analysis. A reverse phase column (YMC-Pack ODS AM-303, C18 (4.6 mm × 250 mm, 5 μm), YMC Korea Co., Ltd.) was used to separate the 12 isoflavones and was maintained at 25 °C throughout the analysis. The mobile phase consisted of 0.1% glacial acetic acid in water (solvent A) and 0.1% glacial acetic acid in ACN (solvent B). A previously reported elution gradient with slight modification was in order to achieve better resolution of the 12 isoflavones (Chung et al., 2012). Specifically, the initial ratio of solvent A to solvent B in the mobile phase was 85:15 (*v*/*v*), with a flow rate of 1 $mL \cdot min^{-1}$. Solvent B was increased from 15 to 20% over 25 min and then increased from 20 to 30% over an additional 30 min. Then, solvent B was maintained at 30% for 5 min. Thereafter, solvent B was returned to the initial ratio over 5 min. The total HPLC analysis time was 65 min, with 10 min being used for re-equilibration. The sample injection volume was 20 μL and the UV wavelength was set at 254 nm. Some representative chromatograms are shown in the supplementary materials (Figs. S1 and S2).

An Alltech 2000 ES evaporative light-scattering detector (ELSD, Alltech Associates, Deerfield, IL, USA), equipped with a SDV 30 plus