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Effects of oven-drying, roasting, and explosive puffing process on isoflavone distributions in soybeans

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ABSTRACT

Distributions of isoflavones in soybeans treated with oven-drying, roasting, or explosive puffing were analysed using high-performance liquid chromatography (HPLC). As oven-drying time increased from 0 to 120 min at 100 °C, concentration (μmol/g) of malonyl derivatives of isoflavones decreased and β-glucosides increased significantly with over 0.99 coefficient of determination (R^2) ($P < 0.05$). Roasting at 200 °C for 7, 14, and 21 min and explosive puffing at 490, 588, and 686 kPa decreased malonyl derivatives significantly and increased acetyl-β-glucosides and β-glucosides significantly ($P < 0.05$). Total isoflavones (TI) in 21 min roasted and 686 kPa puffed soybeans decreased by 25.46% and 10.42%, respectively, while TI in 120 min oven-dried soybeans was not significantly different ($P > 0.05$) compared to untreated samples. Regression analysis showed that malonyl-β-genistin had higher slopes of decreases (μmol/g/min) than malonyl-β-daidzin in oven-dried soybeans. This is the first report on the effects of explosive puffing and the changes of isoflavone profiles in soybeans.

1. Introduction

Isoflavones, phytoestrogenic compounds found in soybeans and soy foods, have received considerable attention due to their health beneficial function (Hendrich et al., 1999; Klein, Perry, & Adair, 1995; Teede et al., 2003; Zheng & Zhu, 1999). A total of 12 isoflavones were found in raw soybeans and distribution of these isoflavones in soy and soy foods is influenced by many factors including crop year, crop location, storage period, processing conditions, processing type, and the presence of microorganisms with β-glucosidase activity (Coward, Smith, Kirk, & Barnes, 1998; Hendrich et al., 1998; Riedl et al., 2007).

Soybeans are consumed in various types of foods through diverse processing methods such as conventional cooking with high moisture content, fermentation, frying, baking, and roasting. Effects of processing on the isoflavone profiles are reviewed by Uzzan and Labuza (2004) and Shimoni (2004). Changes of isoflavone distributions in soy foods are dependent on the processing conditions. Raw soybeans are composed of about 70–80% of malonyl-β-glucosides, 5% of acetyl-β-glucosides, 25% β-glucosides, and less than 2% aglycones (Lee et al., 2004). Conventional thermal treatment decreases malonyl derivatives into β-glucosides via intra-conversion while aglycones have higher heat resistance (Shimoni, 2004). Dry heat treatment such as frying, toasting, or baking process increases the formation of acetyl derivatives of isoflavones through decarboxylation from malonyl derivatives (Toda, Sakamoto, Takayanagi, & Yokotsuka, 2000; Uzzan & Labuza, 2004). Fermentation with microorganisms or natural products containing high β-glucosidase activity converts β-glucosides into corresponding aglycones (Murphy et al., 1999; Yang, Chang, & Lee, 2006).

Roasting and explosive puffing are widely used processing methods for cereal products, fruits, and vegetables (Hwang, Kim, Park, & Yang, 2007; Payne, Taraba, & Saputra, 1989). In Korea, roasting is commonly used to produce sesame oil and to prepare roasted seeds including soybeans, barley, ginkgo, or chestnuts (Hwang et al., 2007; Seog, 2002). Roasting has been used to deactivate antinutritional components in soybeans and to give characteristic flavour and brown colour to final products (Im, Choi, & Choi, 1995). Explosive puffing process, which uses a rotating cylinder with high temperature flame, has sudden release of water vapour pressure leading explosive puffing of cereals such as corn, rice, and soybeans. Puffed grains endure dehydration, starch gelatinisation, increase of the product volume, and textural changes (Hoke et al., 2007). However, the effects of explosive puffing process on the changes of isoflavones are not reported in the literature to our best knowledge.

The objectives of this study were to monitor the changes of isoflavone profiles in soybeans treated with oven-drying, roasting, or explosive puffing process, respectively.

2. Materials and methods

2.1. Materials

Soybeans were purchased from a local grocery market (Seoul, Korea). Twelve isoflavone standard compounds were purchased

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from Wako Chem. Co. (Osaka, Japan) and formononetin was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). HPLC-grade methanol, acetonitrile, HCl, and acetic acid were purchased from Fisher Scientific (Fairlawn, NJ, USA).

2.2. Oven-drying process

Oven-drying is a typical process to reduce moisture contents in seeds. One hundred grams of raw soybeans were mixed with 200 mL tap water at room temperature for 12 h. The soaked raw soybeans were oven-dried at 100 °C for 0, 30, 60, and 120 min using a forced-air dry oven (Win Science, Seoul, Korea).

2.3. Roasting process

Raw soybeans were put in a drum of a coffee roaster (Genesis Co. Ltd., Gyeonggi, Korea), roasted at the temperature of 200 °C with continuous rotating of the drum and sampled at 0, 7, 14, and 21 min.

2.4. Explosive puffing process

Raw soybeans were puffed using a cylindrical puffing machine. One hundred grams of raw soybeans were put in a cylindrical drum in the puffing machine and heated with high-temperature flame. When the inner pressure of cylinder was reached at 490, 588, and 686 kPa, the inner pressure was explosively released and samples were recovered. Conditions of explosive puffing were chosen according to the suggestion of a puffing manager. Generally, explosive puffing with inner pressure of 588 kPa was used for the preparation of edible puffed soybeans.

2.5. Isoflavone extraction and analysis

Isoflavone analysis was according to Lee et al. (2004). Briefly, one gram of ground samples was mixed with a mixture of 2 mL of 0.1 N HCl, 7 mL acetonitrile, and 3 mL deionised water. Samples were shaken for 2 h using a shaker (Jeio Tech, Seoul, Korea) and centrifuged at 2,208 g for 10 min (Hanil, Incheon, Korea). One millilitre of supernatant was dried under a flow of nitrogen gas and stored at −40 °C until use. Formononetin was added as an internal standard to confirm the recovery of isoflavone during the extraction procedure.

Isoflavones in soybean extracts were analysed using a high performance liquid chromatograph equipped with an ultraviolet detector (Hitachi, Tokyo, Japan). A 4 μm Waters Novapak C_{18} reversed-phase HPLC column (150 mm × 3.9 mm I.D.) with a Novapak C_{18} stationary phase guard column and a 0.5 μm pre-column filter from Vydac (Hesperia, CA, USA) was used as stationary phase. Mobile phase was a mixture of 1% (v/v) acetic acid in water (solvent A) and 100% acetonitrile (solvent B) at a flow rate of 0.6 mL/min. The gradient of mobile phase was 85% solvent A from 0 to 5 min, decrease of solvent A up to 65% from 5 to 44 min, increase of solvent A up to 85% from 44 to 45 min, and then re-equilibration of solvent A at 85% for 5 min. Injection volume was 10 μL and isoflavones in eluent was detected at 260 nm. Isoflavones were identified based on the retention times of all 12 standard compounds (Yang et al., 2006). Quantification of isoflavones was calculated using calibration curves prepared from HPLC peak areas of each isoflavone.

2.6. Statistical analysis

The data were analysed statistically by ANOVA and Duncan's multiple range test using SPSS software program (SPSS Inc., Chicago, IL, USA). A P value <0.05 was considered significant.

3. Results and discussion

3.1. Quantification of 12 standard isoflavone compounds

Calibration curves of 12 isoflavone standards were prepared and linearity (R^2), slopes, and y-intercept from calibration curves of each isoflavone are summarised in Table 1. All 12 isoflavones were successfully isolated and the concentration of each isoflavone was determined. The slopes of daidzein, genistein, and glycitein were 1.184×10^{11}, 1.444×10^{11}, and 1.000×10^{11} (peak areas/mol), respectively, and those of daidzin, genistin, and glycitin were 0.949×10^{11}, 1.245×10^{11}, and 0.995×10^{11} (peak areas/mol), respectively. Generally, aglycones had higher slopes than corresponding malonyl derivatives and β-glucosides in current analysis conditions.

César et al. (2006) reported that the linearity of genistein, daidzein, and glycitein in methanol solutions between the peak areas and the injected mass were 6706.7, 4918.4, and 4878.5, respectively, and genistein had the highest slope. Our study showed that slopes of calibration curves were in the decreasing order of genistein, daidzein, and glycitein, which agrees with the report of César et al. (2006). The slopes of each isoflavone may vary depending on many analysis factors including mobile phase, stationary phase, detecting systems, and units of quantification such as peak areas/mol and peak areas/g.

3.2. Isoflavone profiles during processing

Isoflavone distribution of oven-dried, roasted, or explosively puffed soybeans are shown in Table 2. Total isoflavones (TI) in oven-dried soybeans at 0, 30, 60, and 120 min were 6.92, 7.20, 7.22, and 6.77 μmol/g soy, respectively (Table 2). Significant decreases in TI were not observed during oven-drying for 120 min at 100 °C ($P > 0.05$). As roasting time increased from 0 to 7, 14, and 21 min, TI in soybeans were 7.03, 5.07, 5.42, and 5.24 μmol/g, respectively (Table 2). TI amongst 7, 14, and 21 min roasted soybeans were not significantly different ($P > 0.05$) while significant changes were observed in TI between roasted and unroasted soybeans ($P < 0.05$). TI in explosively puffed soybeans at 0, 490, 588, and 6.86 kPa were 5.76, 5.28, 4.95, and 5.16 μmol/g, respectively (Table 2) and TI amongst 490, 588, and 686 kPa samples were not significantly different ($P > 0.05$). Like the roasting process, TI between explosively puffed and untreated samples was significantly different ($P < 0.05$). Loss of TI from 21 min roasting and explosive puffing at 686 kPa were 25.46% and 10.42%, respectively. Roasting caused more decreases in TI than explosive puffing process, which may be due to the higher treatment temperature. Oven-drying did not decrease TI significantly whereas roasting caused significant decreases of TI in soybeans. Coward et al.

Table 1
Linearity (R^2), slopes, and y-intercept from calibration curves of 12 isoflavones

Isoflavone standards	Linearity (R^2)	Slope ($\times 10^{11}$) (peak areas/mol)	y-Intercept ($\times 10^5$) (peak areas)
Daidzein	0.9995	1.184	−0.531
Daidzin	0.9997	0.949	−0.382
Acetyl-β-daidzin	0.9999	1.020	−0.629
Malonyl-β-daidzin	0.9846	0.790	−1.316
Genistein	0.9999	1.444	−1.679
Genistin	0.9991	1.245	−2.148
Acetyl-β-genistin	1.0000	1.467	−0.539
Malonyl-β-genistin	0.9998	0.953	−1.294
Glycitein	0.9995	1.000	−0.678
Glycitin	0.9999	0.995	−0.618
Acetyl-β-glycitin	0.9996	1.143	−0.949
Malonyl-β-glycitin	0.9977	0.865	−1.588