Food Chemistry 112 (2009) 316–320

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

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

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ARTICLE INFO

Article history:
Received 21 January 2008
Received in revised form 4 May 2008
Accepted 20 May 2008

Keywords:
Isoflavones
Processing effects
Oven-drying
Roasting
Explosive puffing

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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0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.05.065

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

Table 2
Distribution of isoflavones in oven-dried, roasted, or explosively puffed soybeans

Samples[a]	DE	DI	ADI	MDI	GE	GI	AGI	MGI	GY	GYI	AGTI	MGYI	TI
μmol Isoflavones/g soy													
0 min O[a]	0.04[b]	0.27	0.26	1.31	0.10	0.46	0.01	3.59	0.01	0.48	0.06	0.32	6.92cd[d]
30 min O	0.09	0.63	0.29	1.10	0.25	0.86	nd [c]	3.32	0.02	0.27	0.07	0.28	7.20d
60 min O	0.11	0.86	0.27	0.89	0.28	1.31	nd	2.81	0.04	0.30	0.12	0.23	7.22d
120 min O	0.07	1.30	0.21	0.53	0.22	2.10	0.01	1.69	0.09	0.34	0.12	0.11	6.77c
0 min R[a]	0.10	0.53	0.29	1.31	0.11	0.44	0.03	3.96	0.01	0.10	0.09	0.05	7.03cd
7 min R	0.09	1.05	0.96	0.13	0.11	0.78	1.32	0.21	0.02	0.22	0.12	0.05	5.07a
14 min R	0.14	1.21	1.00	0.08	0.20	0.87	1.42	0.03	0.05	0.22	0.14	0.05	5.42ab
21 min R	0.20	1.22	0.95	0.08	0.32	0.75	1.17	0.03	0.11	0.21	0.14	0.05	5.24a
0 kPa P[a]	0.06	0.48	0.24	0.55	0.21	1.60	0.01	2.54	0.01	0.04	0.03	0.01	5.76b
490 kPa P	0.01	0.77	0.67	0.06	0.09	2.10	1.36	nd	0.02	0.09	0.06	0.05	5.28a
588 kPa P	0.01	0.72	0.70	0.06	0.09	1.83	1.29	nd	0.02	0.11	0.08	0.05	4.95a
686 kPa P	0.01	0.81	0.74	0.06	0.10	1.81	1.27	nd	0.02	0.19	0.10	0.05	5.16a

[a] *Abbreviations:* O, oven-drying; R, roasting; P, puffing; DE, daidzein; DI, daidzin; ADI, acetyl-β-daidzin; MDI, malonyl-β-daidzin; GE, genistein; GI, genistin; AGI, acetyl-β-genistin; MGI, malonyl-β-genistin; GY, glycitein; GYI, glycitin; AGYI, acetyl-β-glycitin; MGYI, malonyl-β-glycitin; TI, total isoflavones.
[b] Average of triplicates ($n = 3$).
[c] nd, not detected.
[d] Different letters are significant amongst treatment at 0.05.

(1998) reported that TI in the food under normal cooking conditions was not reduced whereas food under excessive heating resulted in increases in aglycones and decreases in TI. Xu, Wu, and Godber (2002) studied the changes of daidzin, genistin, and glycitin in model systems at 95–215 °C and found no significant changes up to 110 °C, but isoflavone glucosides degraded above 135 °C greatly. They reported that daidzin, genistin, and glycitin decreased about 65%, 74%, and 98% of original concentration, respectively, after 3 min of heating at 215 °C. In this study, temperature of oven-drying and roasting were 100 and 200 °C, respectively. Therefore, TI in oven-dried soybeans was not changed while roasting decreased TI significantly.

Relative percentage of isoflavone profiles during oven-drying, roasting, and explosive puffing are shown in Table 3. Oven-drying process decreased malonyl derivatives and increased β-glucosides and aglycones significantly ($P < 0.05$). Malonyl-β-glucosides decreased from 75.57% to 34.36% while β-glucosides and aglycones increased from 17.59 to 55.14 and from 2.18% to 5.59% for 120 min, respectively (Table 3).

In roasting process, malonyl-β-glucosides decreased from 75.67% to 3.11% while acetyl-β-glucosides and β-glucosides increased from 5.77 to 43.30 and from 15.30% to 41.56%, respectively, for 21 min at 200 °C. Aglycones changed from 3.26% to 12.04% for 21 min roasting. Coward et al. (1998) showed that dry heat processing such as toasting or extrusion, led to the formation of acetyl-β-glucosides from malonyl-β-glucosides through decarboxylation. Toda et al. (2000) roasted soybeans for 0, 5, 10, and 20 min at 200 °C and reported the significant increases of acetyl-β-glucosides and β-glucosides up to10 min roasting and a slight decrease of acetyl-β-glucosides at 20 min.

Similar to the roasting process, explosive puffing caused a significant decrease in malonyl derivatives and significant increase in acetyl derivatives and β-glucosides ($P < 0.05$). Malonyl-β-glucosides decreased from 53.62% to 2.03% while acetyl-β-glucosides and β-glucosides increased from 4.75 to 41.02 and from 36.75% to 54.54%, respectively, through 686 kPa explosive puffing treatment. Interestingly, aglycones did not increase during the explosive puffing process, which implies that temperature of explosive puffing may not be high enough to cleave glycosidic linkage between β-glucopyranose and aglycones under current experiment conditions. Although effects of diverse processing types and methods on the isoflavone distribution have been reported in the literature, this is the first report on the explosive puffing and isoflavone profiles in soybeans.

3.3. Regression analyses of isoflavones in soybeans during processing

Regression analyses of isoflavones in soybeans treated with oven-drying are shown in Fig. 1 and Table 4. Regression analyses were conducted based on the isoflavone concentration (μmol/g) and relative percentage (%) (Table 4). Changes of malonyl derivatives and β-glucosides were highly correlated with the processing time. Malonyl derivatives decreased and β-glucosides increased with the regressions of $y = -0.024x + 5.332$ ($R^2 = 0.993$) and

Table 3
Relative isoflavone concentration (%) of oven-dried, roasted, or explosively puffed soybeans

Samples	Aglycones	β-Glucosides	Acetyl-β-glucosides	Malonyl-β-glucosides
0 min O[a]	2.18a[b]	17.59a	4.66a	75.57f
30 min O	5.01e	24.51 b	5.15ab	65.33e
60 min O	5.90g	34.06c	5.38bc	54.67d
120 min O	5.59f	55.14e	4.92ab	34.36c
0 min R[a]	3.26c	15.30a	5.77c	75.67f
7 min R	4.44d	40.37d	47.47g	7.72b
14 min R	7.23h	42.46d	47.27g	3.03a
21 min R	12.04i	41.56d	43.30f	3.11a
0 kpa P[a]	4.88e	36.75c	4.75a	53.62d
490 kPa P	2.30ab	56.15e	39.67d	1.89a
588 kPa P	2.32ab	53.78e	41.83e	2.07a
686 kPa P	2.42b	54.54e	41.02e	2.03a

[a] *Abbreviations:* O, oven-drying; R, roasting; P, explosive puffing.
[b] Different letters are significant amongst treatment at 0.05.

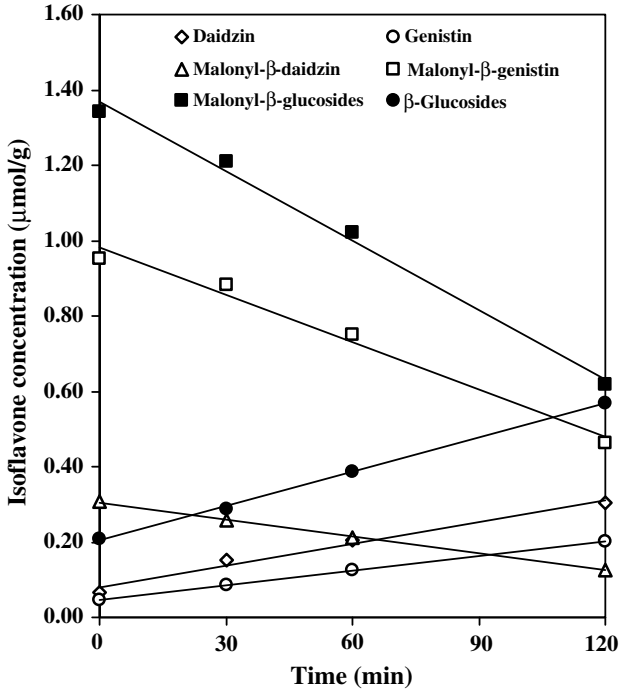

Fig. 1. Changes of isoflavones in oven-dried soybeans. Regression equations of isoflavones are summarised in Table 4.

Table 4
Linearity (R^2), slopes, and y-intercept of selected isoflavones in soybeans treated with 0, 30, 60, and 120 min oven-drying at 100 °C

Isoflavones	Slope		y-Intercept		Linearity (R^2)
	(%)	μmol/g/min[a]	(%)	μmol/g[a]	
Malonyl derivatives	−0.469	−0.024	102.145	5.332	0.993
β-Glucosides	1.761	0.021	97.190	1.176	0.998
Malonyl-β-genistin	−0.452	−0.016	103.231	3.706	0.983
Malonyl-β-daidzin	−0.495	−0.006	99.084	1.298	0.998
β-Genistin	2.983	0.013	100.434	0.462	0.999
β-Daidzin	3.097	0.008	120.740	0.326	0.985

[a] Unit of slopes and intercepts.

$y = 0.021x + 1.176$ ($R^2 = 0.999$), respectively, when x is processing time and y is isoflavone concentration (μmol/g). Based on the slopes of changes, about 87.5% of degraded malonyl derivatives turned into β-glucosides. Results of this study imply that the concentration of malonyl derivatives and β-glucosides in oven-dried soybeans can be predicted.

Concentrations (μmol/g) of malonyl-β-genistin and malonyl-β-daidzin in soybeans decreased during oven-drying with the regressions of $y = -0.016x + 3.706$ ($R^2 = 0.984$) and $y = -0.006x + 1.298$ ($R^2 = 0.998$), respectively, while those of genistin and daidzin increased with the regressions of $y = 0.013x + 0.462$ ($R^2 = 0.999$) and $y = 0.008\ x + 0.326$ ($R^2 = 0.986$), respectively (Table 4). Malonyl-β-genistin was degraded faster than malonyl-β-daidzin and genistin formed faster than daidzin during oven-drying based on the regression slopes of concentration (μmol/g). However, this result may not indicate that genistin derivatives are more sensitive to heat treatment than daidzin derivatives in oven-dried soybeans. To determine the heat stability of daidzin and genistin derivatives in soybeans during oven-drying at 100 °C, regression analysis with relative percentage (%) was conducted. Regression slopes (%) for the decreases of malonyl-β-genistin and malonyl-β-daidzin were −0.452 and −0.495, respectively, and those for the increase of genistin and daidzin were 2.983 and 3.097, respectively, which implies that malonyl-β-daidzin was degraded faster than malonyl-β-genistin and daidzin was formed as fast as genistin in oven-dried soybeans. The higher regression slopes (μmol/g/min) of genistin formation may be due to the higher concentration of genistin derivatives than daidzin derivatives in soybeans (Table 2).

Generally, the concentration of genistin derivatives in raw soybeans was higher than those of daidzin derivative (Kim et al., 2007; Lee et al., 2004; Sakthivelu et al., 2008) and it is expected that malonyl-β-genistin have higher regression slopes of decreases (μmol/g/min) than malonyl-β-daidzin if thermal degradation of isoflavones follow first-order kinetics. Chien, Hsieh, Kao, and Chen (2005) reported that malonyl-β-genistin in model system decreased with a first-order reaction to acetyl-β-genistin during dry heating at 100 °C.

Regression analysis on the isoflavone distribution from roasting and explosive puffing was not shown because changes of isoflavone profiles were not observed clearly during treatments and the coefficient of determination was relatively low.

The stability of isoflavones has been reported in model systems and in many different foods including soymilk, tofu, and fermented foods (Chien et al., 2005; Eisen, Ungar, & Shimoni, 2003; Kevin, Baraem, Carlos, & Kirby, 2006; Kim et al., 2007; Shimoni, 2004; Uzzan & Labuza, 2004; Yang et al., 2006). Generally, the degree of thermal energy applied and the amount of moisture in samples play important roles in the changes of total isoflavones and distribution of isoflavones in unfermented soy foods.

The stability of daidzin and genistin derivatives was different depending on the experimental conditions. Eisen et al. (2003) reported that genistin in soymilk decreases with typical first-order kinetics and the rate constant ranging from 0.437–3.871 to 61–109 days^{-1} at 15–37 and 70–90 °C, respectively, while daidzin was relatively stable during storage at 15–37 °C. Xu et al. (2002) showed that the thermal stability of daidzin was higher than that of genistin in model system. However, according to Kevin et al. (2006), loss of daidzin derivatives was higher than that of genistin derivatives in model systems with diverse pH and temperature. They reported that malonyl-β-, and acetyl-β-daidzin changed more at 100 °C than malonyl-β-, and acetyl-β-genistin in model system with pH 2, 7, and 10 (Kevin et al., 2006). In this study, malonyl-β-genistin decreased with higher regression slope (μmol/g/min) than malonyl-β-daidzin while malonyl-β-daidzin had higher regression slope (%) than malonyl-β-daidzin in oven-dried soybeans at 100 °C.

4. Conclusions

All 12 standard isoflavone compounds were isolated, calibration curves were prepared, and the concentration of each isoflavone was determined. Distribution of isoflavone profiles during oven-drying, roasting, or explosive puffing was analysed and the stability of each isoflavone was compared during oven-drying. Malonyl-β-genistin in oven-dried soybeans showed higher regression slopes of decreases (μmol/g/min) than malonyl-β-daidzin. However, malonyl-β-daidzin had higher slopes of decreases (%) than malonyl-β-genistin in percentage units, which implies that malonyl-β-daidzin may be more heat sensitive than malonyl-β-genistin in oven-dried soybeans at 100 °C. Roasting and explosive puffing decreased malonyl derivatives significantly and increased acetyl derivatives and β-glucosides significantly ($P < 0.05$). Roasting caused more loss in total isoflavones than oven-drying and explosive puffing, which may be due to the higher temperature of treatment.

Acknowledgements

This study was financially supported by Seoul R&BD Program (10625) and authors greatly appreciate for the support.

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