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Stability of Soybean Isoflavone Isomers According to Extraction Conditions

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Abstract: Stability of soybean isoflavone isomers according to extraction conditions such as temperature, pH, and extracting solvents was investigated. Heating induced three chemical reactions to occur for malonyl derivatives of isoflavones, namely decarboxylation of malonyl groups into acetyl derivatives, deesterification of malonyl residues, and hydrolysis of β-glycosidic bonds. Among the twelve isoflavone isomers, change in concentrations of acetylglycosides were most pronounced: Acetyl derivatives were present only in trace amounts in unheated hypocotyls, but the content increased dramatically during heating. As for the glycosides, concentrations of daidzin and glycitin increased due to heat treatment, though that of genistin remained almost unchanged. Heat decomposition rates and the patterns differed among the three malonyl derivatives. After 120 min at 80°C, the relative concentrations of daidzin, glycitin and genistin were increased from 9.2%, 12.4% and 3.3% to 19.3%, 21.9% and 6.2%, respectively. When crude isoflavones were solubilized in glycine buffer (pH 10.0) and incubated at 80°C, deesterification occurred faster than at pH 7.0. When the pH of isoflavone solution was increased, the malonylglycosides were hydrolyzed to their respective glycosides at increased rate. Both acetyl and aglycone forms were unchanged and only de-esterification reactions occurred. At the acidic pH, malonylglycosides were much stable both at 60 and 80°C. However at pH 10, 80°C and 1 hr, 75-80% of malonylglycosides were transformed to their deesterified glycosides. When isoflavones were extracted with 60% aqueous ethanol at 60°C, isoflavone isomers were stable and the deesterification reactions did not occur in these conditions. However, at 80°C deesterification of malonylglycosides occurred significantly with 15-20% of malonylglycosides being hydrolyzed into their respective glycosides. This experiment showed that malonylglycosides undergo decomposition when heated or exposed to alkaline conditions. Also, aqueous ethanol was preferred to aqueous methanol as solubilizing media for obtaining extract with minimum degradation of malonylglycosides.

Keywords : hypocotyls, isoflavones, isomers, stability

Introduction

Content and composition of isoflavone isomers in soybean foods are affected by soybean variety, methods of processing, and addition of other components.[1] Fermented soy foods contain predominantly isoflavone aglycones, whereas in nonfermented soy foods, isoflavones are present mainly as β-glycosides.[2,3] Dwyer *et al.*[4] determined the content of isoflavone in tofu, soy drink and soy-based formulas. Tofu contained the highest amounts of isoflavones while soy drink contained lesser amounts, and soy-based formulas were devoid of isoflavones. Heat processing, enzymatic hydrolysis, and fermentation can significantly alter the isomeric distribution of these isoflavones, since malonylglycosides of isoflavones were heat-labile and unstable.[5] During heat treatment, some malonyl isoflavones were transformed into acetylglycosides, which were isolated from toasted defatted soy flakes.[6] Heat treatment during extrusion processing in the manufacture of soy granule and texturized vegetable protein produced an appreciable amount of acetylgenistin and acetyldaidzin.[1] Ingolf *et al.*[7] investigated the effects of temperature and time on the changes in the profile of isoflavones during thermal processing of tofu. Wang *et al.*[8] reported that the best conditions for the hydrolysis of isoflavone glycosides in soybean and its processed products into their aglycones are found to be in 1 N HCl for 2 hr at 98-100°C. At a higher HCl concentration, the content of genistein tended to decrease due probably to the degradation by HCl.

Although many researchers have investigated the composition of isoflavone isomer in soybean and its

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processed products, the factors that affect the composition of isoflavone were not fully elucidated. In this paper, we investigated effects of various extracting conditions on stability of isoflavone isomers.

Materials and Methods

Materials

Soybean hypocotyl used for extracting isoflavone was obtained from Dr. Chung's Food Co., Ltd (Cheongju, Korea), a local soymilk manufacturer. HPLC column used for isoflavone quantification was ODS A303 (4.6 × 250 mm, YMC, U.S.A). Twelve isoflavone standards were obtained from Fujicco (Kobe, Japan).

Extraction of Isoflavones

Isoflavone in soybean hypocotyl was quantified by extracting with aqueous methanol.[9,10] First soybean hypocotyl was ground with blender and filtered through a 100-mesh sieve, and 2.0 g was weighed and mixed with 20 m*l* of 60% methanol at 40°C for 1 hr with stirring. The mixture was centrifuged for 15 min at 3000xg. The residue was resuspended in 10 m*l* of 60% methanol and subsequently recentrifuged. The supernatants obtained were pooled and vacuum dried at room temperature for further studies.

Quantification of Ioflavone

Crude isoflavones obtained were solubilized in 60% methanol and filtered through a 0.45 μm membrane filter before HPLC analysis. HPLC analysis was carried out using Agilent system (Agilent 1100 series, Agilent Technologies Inc., Palo Alto, CA) with ODS A303 (4.6 × 250 mm, YMC, U.S.A) column. Detector used was UV detector at 254 nm, and the flow rate of the solvent was 1.0 m*l*/min. A linear HPLC gradient was composed of (A) 0.1% glacial acetic acid in H_2O and (B) 0.1% glacial acetic acid in acetonitrile. Following injection of 20 μ*l* of sample, solvent B was increased from 15% to 35% over 50 min, and then held at 35% for 10 min.

Effect of Dry Heating on Composition of Isoflavone in the Hypocotyls

Time dependent effects of dry heat on stability of isoflavone isomers were investigated by heating the hypocotyls in oven at 105°C for various time. Heat-induced chemical reaction was quantified by extracting isoflavone isomers from the powdered hypocotyl using 60% aqueous methanol which was subsequently analyzed using HPLC.

Deesterification of Malonyl Residues in the Aqueous System

Crude isoflavones were solubilized in distilled water with different pH at the concentration of 1%. pH of isoflavone solutions were fixed with either 0.1 M HCl or NaOH. Effects of solution pH and incubation time on stability of malonyl residues were analyzed using HPLC. For time dependent change in concentrations of isoflavone isomers at pH 7.0 and 10.0, 0.2 M phosphate buffer and glycine buffers were used, respectively.

Deesterification of Malonyl Residues in Aqueous Alcohols

Changes in composition of isoflavone isomers in the presence of aqueous alcohols were studied Briefly, 2.0 g powdered hypocotyl was suspended in 20 m*l* aqueous alcohols and changes in compositions isoflavone isomers at different alcohol concentrations were investigated during incubation time of 1 hr using HPLC.

Results and Discussions

Effect of Dry Heat on the Composition of Isoflavone in the Hypocotyls

As shown in Fig. 1, malonyl glycosides were the main components of raw soybean hypocotyls and dry heating the hypocotyls induced three chemical reactions; decarboxylation of malonyl group into acetyl derivatives, deesterification of malonyl residue, and hydrolysis of β-glycosidic bonds. According to Fig. 1, changes in concentrations of acetylglycosides were most pronounced; acetyl derivatives were present only in trace amounts in unheated hypocotyls, but the content dramatically increased by heating. Among the three glycosides, concentrations of daidzin and glycitin increased due to heat treatment, though that of genistin remained almost unchanged. Concentrations of such aglycones as genistein and daidzein were not

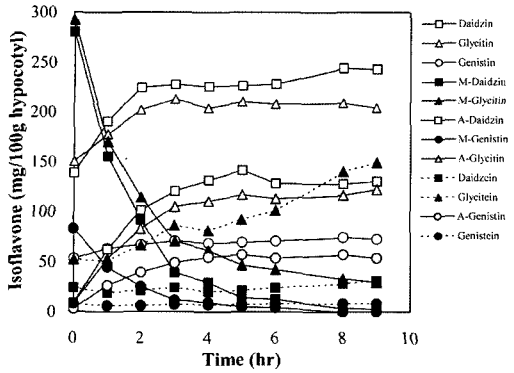

Fig. 1. Changes in composition of isoflavone isomers during dry heating. Soybean hypocotyls were heated in the dry oven at 105°C.

altered during heating though that of glycitein was increased. Heat decomposition rates and the patterns differed between the three malonyl derivatives. For example, 90% of malonylglycitin initially present was transformed into acetylglycitin, glycitin, and glycitein, with the ratio of the decomposed derivatives being 4:2:3 (wt:wt:wt) during heating.

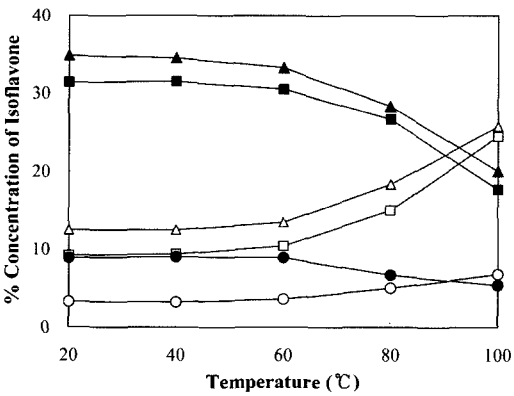

Fig. 2. Effect of reaction temperature on the hydrolysis of malonylglycosides of isoflavone. Isoflavone solution (pH 7.0) was incubated for 1 hr. Daidzin (□), glycitin (△), genistin (○), malonyl-daidzin (■), malonyl-glycitin (▲), malonyl-genistin (●).

Malonyldaidzin was mostly transformed to acetyldaidzin (45%), and daidzin (40%) while daidzein was formed in lesser amount (3%). As for malonylgenistin, the main decompositon products were acetylgenistin (40%) and genistin (20%).

Deesterification of Malonyl Residue of Isoflavones in the Aqueous System

Fig. 2 shows the effects of heating isoflavone extracts on stability of isoflavone isomers. Powdered crude extracts of isoflavones (which were extracted from soy hypocotyls using 60% aqueous methanol) were solubilized in 0.2 M phosphate buffer (pH 7) at the concentration of 1% at different temperatures. Concentrations of isoflavone isomers were expressed as percentage of the total isoflavones (relative percentage). When isoflavones were boiled for 1 hr, some 40% of malonylglycosides were converted into their respective glycosides at 100°C. When the aqueous solution of isoflavone was autoclaved at 121°C for 15 min at pH 7.0, approximately 70% of malonylglycosides were converted into their respective glycosides, though total amount of isoflavones were virtually unchanged. Table 1 showed

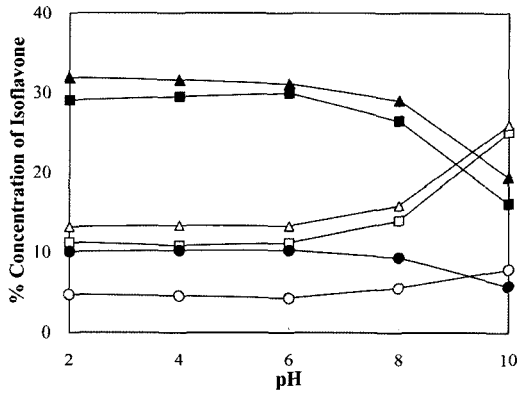

Fig. 3. Effect of pH on the hydrolysis of malonylglycosides. Isoflavone solution was reacted at 60°C for 1 hr. Daidzin (□), glycitin (△), genistin (○), malonyl-daidzin (■), malonyl-glycitin (▲), malonyl-genistin (●).

Table 1. Effect of autoclave at 121°C for 15min on the hydrolysis of malonylglycosides

	Isoflavone content (mg/100 m*l*)						
	Daidzin	Glycitin	Genistin	M-daidzin	M-glycitin	M-genistin	Total
Heated Sample	19.1	18.8	7.1	5.5	6.5	2.0	59.0
Control	9.7	11.0	3.8	18.4	20.0	6.4	69.3

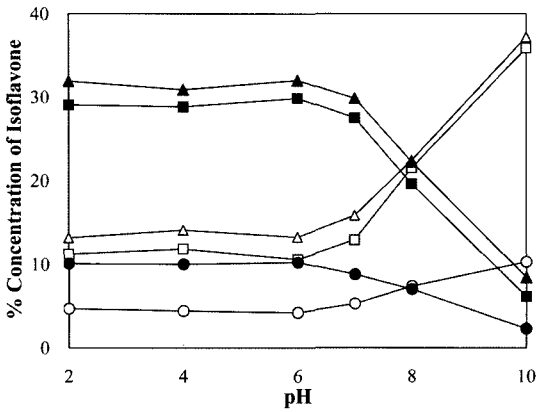

Fig. 4. Effect of pH on the hydrolysis of malonylglycosides when isoflavone solution was reacted at 80°C for 1 hr. Daidzin (□), glycitin (△), genistin (○), malonyl-daidzin (■), malonyl-glycitin (▲), malonyl-genistin (●).

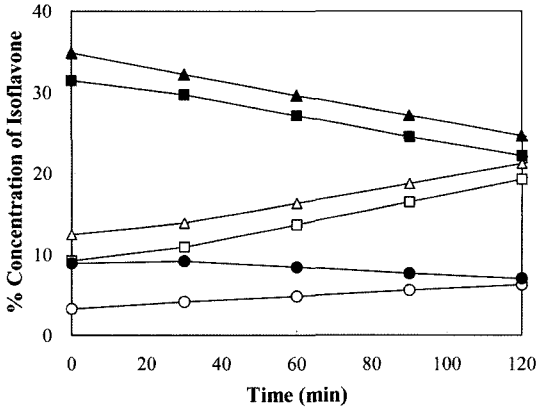

Fig. 5. Hydrolysis of malonylglycosides with time when one percentage of crude isoflavone solution (pH 7.0) was reacted at 80°C. Daidzin (□), glycitin (△), genistin (○), malonyl-daidzin (■), malonyl-glycitin (▲), malonyl-genistin (●).

that high heat treatment caused malonylglycosides to be unstable at pH 7.0 .

Fig. 3 and 4 show the conversion of malonylglycosides into their respective glycosides with increase of pH at 60°C and 80°C for 1 hr, respectively. When the pH of isoflavone solutions were above pH 7.0, the malonylglycosides were hydrolyzed to their respective glycosides. Both acetyl and aglycone forms were unchanged and only de-esterification reactions occurred. At the acidic pH, malonylglycosides were very stable

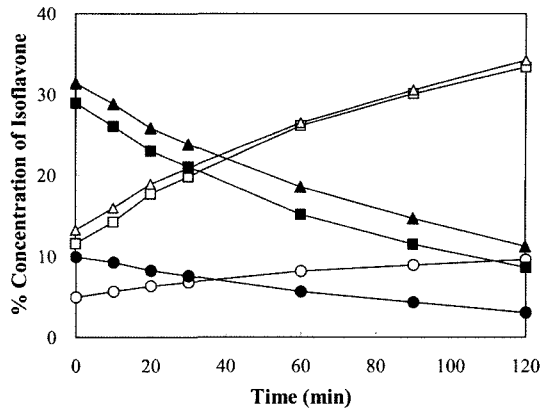

Fig. 6. Hydrolysis of malonylglycosides with incubation time at pH 10.0, 80°C. Daidzin (□), glycitin (△), genistin (○), malonyl-daidzin (■), malonyl-glycitin (▲), malonyl-genistin (●).

both at 60 and 80°C. However at pH 10, 80°C and 1 hr, 75-80% of malonylglycosides were transformed to their deesterified glycosides. These results show that malonylglycosides showed an increased susceptibility to degradation at the exposure to alkaline environment.

Stability of crude isoflavones was investigated at pH 7.0 (Fig. 5) and pH 10.0 (Fig. 6) at temperature of 80°C. The initial relative concentrations of malonyldaidzin, malonylglycitin and malonylgenistin were 31.4, 34.8 and 8.9%, respectively. After 120 min at 80°C (Fig. 5), the relative concentrations of daidzin, glycitin and genistin increased from 9.2%, 12.4% and 3.3% to 19.3%, 21.9% and 6.2%, respectively. About 20-30% of malonylglycosides was deesterified into their respective glycoside forms, i.e. daidzin, glycitin and genistin under the conditions. However, the concentration of acetyl and aglycone were not changed. It is speculated that in aqueous environment, only deesterification reaction occurred during heat treatment. When crude isoflavone was solubilized in 0.2 M glycine buffer (pH 10.0) at 80°C, deesterification occurred faster than at pH 7.0 (Fig. 6). The relative concentrations of malonyldaidzin, malonylglycitin and malonylgenistin were changed from 28.9%, 31.4% and 9.9% to 8.6%, 11.2% and 3.0%, respectively, after incubation for 120 min at 80°C. The relative concentrations of daidzin, glycitin and genistin

increased from 11.6%, 13.2% and 5.0% to 33.4%, 34.2% and 9.6%, respectively. About 65-70% of malonylglycosides was deesterified into their respective glycoside. The concentrations of acetyl and aglycone forms remained virtually unchanged during heating.

Change in Composition of Isoflavone Isomers during Extraction with Different Aqueous Alcohols

Changes in composition of isoflavone isomers in the presence of aqueous alcohol were investigated at different temperature and time. Stability of isoflavones in the presence aqueous alcohols were investigated because ethanol and methanol were normally used as the extraction solvents. When isoflavones were extracted with 60% of aqueous ethanol at 60°C, change in isoflavone composition was not significant with time (Fig. 7). Deesterification reaction did not occur in these conditions. However, at 80°C deesterification of malonylglycosides occurred slightly with 15-20% of malonylglycosides being hydrolyzed into their respective glycosides (Fig. 8). Fig. 9 shows that the concentration of malonylglycosides decreased with increase in the concentration of ethanol during extraction from hypocotyl powder at 80°C, 1 hr. This is probably due to the reduced extraction of malonylglycosides since malonylglycosides are most hydrophilic among the isoflavone isomers. Total amount of isoflavones extracted was also reduced when ethanol concentration was greater than 80%. During extraction of isoflavone glycosides

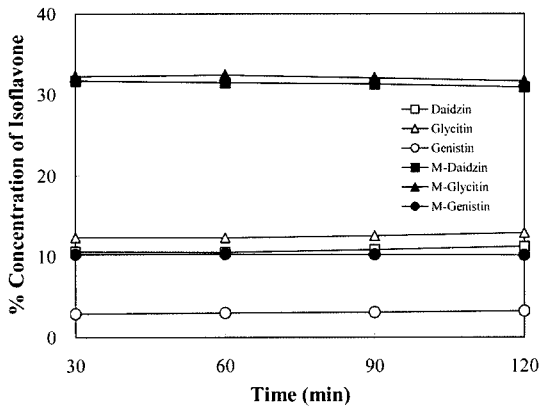

Fig. 7. Change in composition of isoflavones isomers during extraction with 60% aqueous ethanol at 60°C.

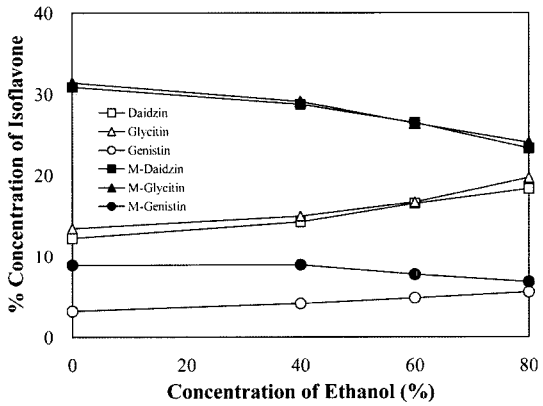

Fig. 9. Effect of ethanol concentration on the change of composition of isoflavones isomers during extraction with aqueous ethanol at 80°C.

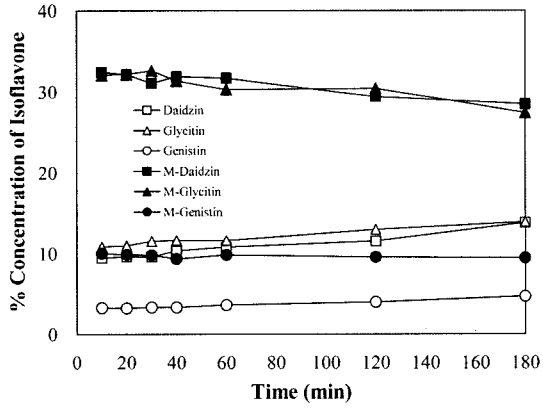

Fig. 8. Change in composition of isoflavones isomers during extraction with 60% aqueous ethanol at 80°C.

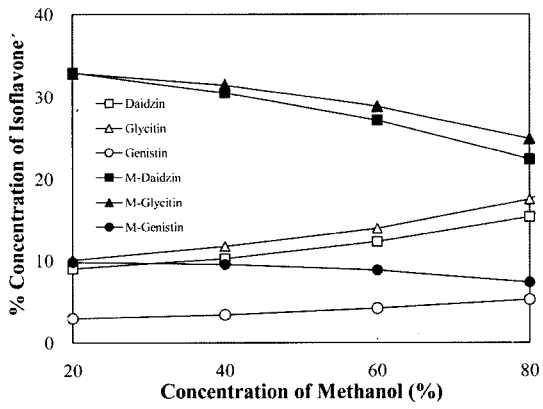

Fig. 10. Change of composition of isoflavones isomers during extraction with aqueous methanol at 80°C.

with aqueous methanol rather than ethanol, more of malonylglycosides were converted to their glycosides (Fig. 10). Even though the trend was similar between methanol and ethanol, conversion percentage of malonylglycosides to their respective glycosides was higher with aqueous methanol.

This result confirmed that malonylglycosides undergo decomposition when heated or exposed to alkaline conditions. Also, aqueous ethanol was preferred to aqueous methanol as solubilizing media for obtaining extract with minimum degradation of malonylglycosides.

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