Changes in the Profile of Genistein, Daidzein, and Their Conjugates during Thermal Processing of Tofu†

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Profiles of genistein, daidzein, genistin, daidzin, and their acetyl- and malonyl-β-glycosides were determined in tofu as affected by temperature and time. Tofu was heated in water at 80, 90, and 100 °C for 0 (control), 10, 20, 30, and 40 min, and the contents of the isoflavones of interest were quantified using reversed-phase HPLC. Total isoflavone content decreased most likely due to leaching of isoflavones into the water. Because the content of the isoflavones of the genistein series was little affected by the treatments, the decrease in the total isoflavone content was almost exclusively due to a decrease of the daidzein series. Changes in the profile of the daidzein series suggest little decarboxylation of the malonylglycoside to the acetylglycoside, but considerable de-esterification of the malonyl- and acetylglycoside to the β-glucoside. Strongly temperature dependent decreases of the aglycon suggest possible thermal degradation of daidzein in addition to losses due to leaching.

Keywords: *Tofu; thermal processing; isoflavones; genistein; genistin; daidzein; daidzin; HPLC*

INTRODUCTION

Soybeans are a rich source of isoflavones. The main isoflavones found in soybeans are genistein, daidzein, and glycitein, each of which exists in four chemical forms, as an aglycon (genistein, daidzein, and glycitein), a β-glucoside (genistin, daidzin, and glycitin), an acetylglucoside (6″-*O*-acetylgenistin, 6″-*O*-acetyldaidzin, and 6″-*O*-acetylglycitin), and a malonylglucoside (6″-*O*-malonylgenistin, 6″-*O*-malonyldaidzin, and 6″-*O*-malonylglycitin) (*1*). Because there is indication that the aglycons might be more bioactive (*2*), knowledge of the effect of processing on the exact composition of isoflavones and their four forms is important (*3*). Isoflavones are the most common estrogenic compounds found in plants and have been shown to possess antimicrobial and insecticidal properties (*4*) and to prevent and reduce the risks of various cancers (*5*−*7*). Isoflavones, and genistein in particular, inhibit the activity of protein tyrosine kinases (*8*), which, together with a modulation of the transforming growth factor (TGF) β-1 signaling pathways, might be the major mechanisms of action by which isoflavones reduce the risk of cancer (*9*). In addition, isoflavones may have a role in decreasing the risk of cardiovascular diseases (*10*) by reducing the level of total cholesterol as well as low-density lipoprotein (LDL) cholesterol (*11*). Other health benefit claims as reviewed by Kurzer and Xu (*1*) include reduction in postmenopausal symptoms and risks of osteoporosis in women.

In raw soybeans the three families of genistein, daidzein, and glycitein are found in a ratio of approximately 6:3:1, respectively (*12*). Unprocessed soybeans contain 1.2−4.2 mg of total isoflavones/g of soybean, with large variation due to variety, crop year, and growth location (*13*). Because soybeans are consumed only after being processed into either fermented products, such as soy sauce, or unfermented products such as tofu, Wang and Murphy (*14*) determined losses of isoflavones during processing of soybeans to manufacture tempeh, tofu, and soy protein isolate. They observed that 61, 44, and 53% of total isoflavones were lost in manufacturing these products, respectively. Fukutake et al. (*15*) noted that the amount of genistein was higher in fermented soy foods than in nonfermented foods, which was attributed to the cleavage of the β-glycosyl bond in genistin by microorganisms during fermentation to form genistein. However, the total isoflavone content usually is higher in nonfermented soy products than in fermented ones (*12*).

Tofu is one of the more accepted soy products in the United States. The tofu analyzed in Wang and Murphy's (*12*) study contained 0.532 mg of total isoflavones/g of tofu. In another study, total mean isoflavone content in raw tofu was 0.297 mg/g, whereas cooked tofu contained 0.258 mg/g (*16*). Coward et al. (*17*) reported 0.031 and 0.015 mg/g of genistein and 0.249 and 0.269 mg/g of genistin in tofu of two different brands, respectively. The daidzein and daidzin contents of the first tofu brand were 0.016 and 0.249 mg/g respectively, whereas those of the second tofu brand were 0.015 and 0.269 mg/g, respectively.

Heating causes a change in the conjugation profile of the isoflavones in soy products. Coward et al. (*3*) reported that baking and frying of isolated soy protein and textured vegetable proteins did not alter the total isoflavone contents but changed the profiles of individual isoflavones due to conversion of the malonyl conjugates. Moist heat increased the content of the β-glucoside conjugates, whereas dry heat caused an

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increase in the acetyl conjugate. Conversion to the aglucone and a decrease in total isoflavone content were observed only when the food was heated excessively. Similarly, Mahungu et al. (*18*) observed that dry heat during extrusion processing of soy products increased acetyl conjugates and decreased malonyl conjugates due to decarboxylation of the malonyl conjugate. However, they also noted a decrease of the total isoflavone content due to extrusion processing. Davies et al. (*19*) studied the effect of storage on soy protein isolate and genistein. They observed that genistein reacted with lysine as well as auto-degraded to form Maillard browning products when stored for extended periods, and a higher storage temperature was found to increase the degradation rates of genistein. Although isoflavones are generally considered to be heat-stable, Franke et al. (*16*) observed a 13% decrease in raw versus cooked tofu, which was attributed to leaching of isoflavones into the water. They concluded that the isoflavone content in soyfoods was a function of the variety of soybean used, the storage conditions of raw materials and products, and the processing parameters employed during manufacturing.

These studies underscore the importance of quantifying the total content and the individual forms of isoflavones in all types of processed soy foods before any claims about the health benefits of their consumption due to phytoestrogen content can be made. Tofu is a very popular food among the Asian population and is gaining popularity among Americans as well, due to the associated health claims. Although the initial amount of isoflavones in various soy foods may be high, prolonged thermal processing and holding in liquid environments, such as in a restaurant setting, may affect the concentration and the profile of isoflavones. In the present study, changes in the concentrations of the major isoflavones in tofu during prolonged heating in water were determined. Glycitein and its conjugates were not determined as they account for only ~5% of the total isoflavones (*20*) and because quantities of the various glycitein conjugates were at or below the limit of detection, which made proper quantitation impossible.

MATERIALS AND METHODS

Tofu Samples. Tofu was procured from a local Chinese grocery store. It was of homemade style and not a branded product. In the store, the block of tofu was stored in water at refrigerated temperatures (4 °C). The coagulant used in the manufacture was calcium sulfate, and the firmness grade was "firm". The tofu for all three replicates came from the same tofu batch to avoid introducing variation due to differences in isoflavone content from different tofus.

Chemicals. HPLC grade methanol, acetic acid, acetonitrile, and water were used as solvents for the study and were procured from Fisher Scientific (Pittsburgh, PA). Genistein, genistin, daidzein, and the sodium salt of fluorescein (internal standard) were obtained from Sigma Chemical Co. (St. Louis, MO). Daidzin was bought from LC Laboratories (Woburn, MA).

Cooking Conditions and Sample Preparation. Freshly bought tofu was cut into 25 mm cubes, each weighing 21.94 ± 0.61 g, and cooked at three different temperatures of 80, 90, and 100 °C. At each temperature the holding times were 0 (control), 10, 20, 30, or 40 min. Around 600 mL of water was filled into a 1000 mL beaker and heated to the designated temperature and maintained at that temperature. Four cubes were placed into the beaker, and a cube was randomly withdrawn with a slotted spoon at each 10 min time interval. The individual cubes were dipped into 1 °C water for 3 min to rapidly cool them and then transferred to plastic cups, capped with a lid, labeled, and frozen at −20 °C prior to isoflavone extraction.

Isoflavone Extraction. Isoflavones in tofu were extracted by modifying the extraction procedure of Wang et al. (*21*). Frozen tofu samples were ground to a fine crumbly state using a pestle and mortar, and ~5 g was accurately weighed and mixed with 15 mL of 80% methanol while still frozen. The tofu−methanol mixture was stirred for 30 min with the aid of a magnetic stir plate at room temperature. Then the slurry was centrifuged for 15 min at 5000*g*. The supernatant was filtered through Whatman No. 1 filter paper into a 50 mL volumetric flask. The residue in the centrifuge tube was resuspended in ~10 mL of 80% methanol by vortexing for 1 min. It was again centrifuged at 5000*g* for 5 min, and the resulting supernatant was added to the previous supernatant after filtering through a Whatman No. 1 filter paper as before. One milliliter of disodium fluorescein solution (1 mg/mL) was added to the filtrates as internal standard, and the filtrates were made to volume with 80% methanol. Finally, the sample solution was filtered again through a 0.45 μm nylon membrane filter (Alltech, Deerfield, IL) before HPLC analysis.

For recovery studies and determination of analytical precision, known quantities of genistein (200 μg) and genistin (200 μg) were added to 2 g of tofu and extracted using the same protocol as described above.

Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC). A Perkin-Elmer (Norwalk, CT) HPLC system equipped with a series 410 LC pump, a Phenomenex (Torrance, CA) W-Porex 10 C18 reversed phase column, 5 μm, 25 cm × 4.6 mm i.d., and a UV−vis detector (LC-95, Perkin-Elmer) set at 254 nm was used for the separation and subsequent detection of genistein, genistin, daidzein, and daidzin in tofu. A linear gradient system of 0.1% glacial acetic acid in water (A) and 0.1% glacial acetic acid in acetonitrile (B) was programmed from 90% A/10% B to 72% A/28% B over a 35 min run time at a flow rate of 1.7 mL/min. Column temperature was maintained at 35 °C using a Bio-Rad column heater (Bio-Rad, Hercules, CA).

Standard Curves. Standard curves were prepared for genistein, genistin, daidzein, and daidzin. Different standards of genistein, genistin, daidzein, and daidzin were prepared at concentration levels of 2.67, 5.34, 8.00, 10.67, and 13.34 μg/mL for genistein and genistin and at 3.34, 6.67, 10.00, 13.34, and 16.67 μg/mL for daidzein and daidzin, with 66.67 μg/mL of fluorescein added as the internal standard. The final volume was made to 7.5 mL with 80% methanol. Standards were filtered through a 0.45 μm membrane filter (Alltech) before HPLC analysis. Linear regression resulted in correlation coefficients of 0.995 for genistein, 0.999 for genistin, 0.998 for daidzein, and 0.992 for daidzin. Although standards for the acetyl- and malonyl-β-glycosides of genistin and daidzin were not available, Kudou et al. (*22*) showed that the molar extinction coefficient of the malonyl conjugate approximates that of the β-glycoside; thus, the standard curves of genistin and daidzin were used for quantifying the acetyl and malonyl conjugates.

Statistical Analyses. A randomized complete block design with three replicates was used. An analysis of variance and Fisher's LSD were utilized to discriminate between the different temperature levels and holding times. All analyses were done using SAS, release 6.12 (*23*).

RESULTS AND DISCUSSION

Precision and Recovery Studies. The coefficients of variation were determined to be 2.58 and 6.15% for genistein and genistin, respectively. The percent recoveries for genistein and genistin were 71.46 and 89.29%, respectively. Fukutake et al. (*15*) reported 90 and 91% recoveries for genistein and genistin, respectively, in soybean. However, because the main goal of the study was to determine treatment effects instead of actual amounts, data have not been adjusted for recovery.

Table 1. Influence of Temperature and Time of Processing on Total Isoflavones (Micrograms per Gram) in Tofu ($n = 3$)[a]

	holding time				
temp (°C)	0 min	10 min	20 min	30 min	40 min
80	$^{x}204.4_{a}$	$^{x}204.2_{a}$	$^{x}185.8_{ab}$	$^{y}162.0_{c}$	$^{xy}169.6_{bc}$
90	$^{x}204.4_{a}$	$^{xy}193.2_{ab}$	$^{x}184.6_{ab}$	$^{x}186.4_{ab}$	$^{x}174.5_{b}$
100	$^{x}204.4_{a}$	$^{y}175.1_{b}$	$^{x}164.1_{bc}$	$^{z}131.2_{d}$	$^{y}147.3_{cd}$

[a] Means in the same row within a temperature treatment with no common subscripts are different ($P \leq 0.05$). Means in the same column within a holding time treatment with no common superscripts are different ($P \leq 0.05$).

Influence of Processing on Total Isoflavones. The total isoflavone content was determined as the sum of all conjugates of the two main isoflavone families, genistein and daidzein, that were determined in this study. Analysis of variance showed significant results for both variables, time and temperature. A significant decrease in total isoflavones was seen over time with a greater decrease at higher temperatures (Table 1). Reports in the literature usually show that isoflavones are rather stable compounds affected by heat only in regard to their specific conjugated form (*3*). However, processes involving liquids, such as washing and soaking (*14*) and boiling (*16*), have been shown to reduce isoflavone content due to leaching. Thus, it is likely that the decrease in overall isoflavone content observed in this study is due to leaching of isoflavones into the water. However, previous studies considered only the effect of processing, independent of time, whereas this study shows that losses increase over time and that this effect is temperature dependent.

Influence of Processing on the Genistein Series. More than 90% of the genistein was found to be in conjugated forms (Table 2), which is similar to a report by Coward et al. (*17*). However, of the conjugates, the β-glucoside was considerably higher than the malonyl- or acetylglycoside, which is similar to a report by Coward et al. (*3*) but higher than those reported previously by others (*12*, *16*). No specific pattern of conversion of malonyl- or acetylglycosides to the β-glucoside or the aglycon was observed. In fact, the genistein series was little affected by processing time and temperature, because in general no significant decrease in the total genistein content or the individual glycosides was observed, with the exception of very small decreases after 40 min at 80 °C for the acetylglycoside, genistin, and total genistein contents. Because no corresponding increases in the aglycon were observed, these small decreases can most likely be attributed to losses due to leaching instead of molecular conversions.

Influence of Processing on the Daidzein Series. It is obvious from Table 3 that the decrease in total isoflavone content is exclusively due to a decrease of daidzein and its conjugates. The concentration of the aglycon, daidzein, at 40% of the total daizein series is slightly higher than that of the glucoside, daidzin, and the malonylglycoside, whereas the concentration of the acetylglycoside contributes only between 5 and 7% of the total daizein. The decrease in daidzein can be observed for all forms with the exception of the β-glucoside and is exacerbated by increasing temperatures. Whereas at 80 °C almost 80% of the total daidzein is still present after 40 min of heating, only 47% is left after 40 min at 100 °C. The decrease in the aglycon was slightly less than, but similar, in regard to temperature to that of the malonylglycoside, and both were very much temperature dependent, with a greater decrease with increasing temperature. However, the decrease in acetylglycoside did not seem to be affected by temperature, and the decrease in the β-glucoside, daidzin, was marginal.

The decrease in aglycon is most likely due to leaching into the water. However, this study was not designed as a full mass balance study, so the water was not analyzed for isoflavone content. In most studies, isoflavones have been found to be fairly heat stable at temperatures well above 100 °C in dry and moist heat (*3*, *14*). However, an overall loss of isoflavones observed in an extrusion study (*18*) could not be explained by leaching alone. In this study, leaching is the most likely explanation for the loss in aglycon, but even though no supporting information beyond being hypothesized by Mahungu et al. (*18*) could be found, decomposition, specifically of daidzein, cannot completely be ruled out. This hypothesis is supported by the fact that temperature played a significant role in the reduction of daizein. In addition, tofu pieces did not appear to be disinte-

Table 2. Influence of Temperature and Time of Processing on Profile of Genistein, Genistin, Acetylgenistin, Malonylgenistin, and the Sum of All Forms of Genistein (Micrograms per Gram) in Tofu ($n = 3$)[a]

		holding time				
compound	temp (°C)	0 min	10 min	20 min	30 min	40 min
genistein	80	$^{x}6.725_{a}$	$^{xy}6.655_{a}$	$^{y}6.442_{a}$	$^{x}5.888_{a}$	$^{x}5.998_{a}$
	90	$^{x}6.725_{bc}$	$^{x}7.690_{ab}$	$^{x}8.888_{a}$	$^{x}6.418_{c}$	$^{x}6.346_{c}$
	100	$^{x}6.725_{ab}$	$^{y}5.966_{b}$	$^{y}7.431_{a}$	$^{x}6.523_{ab}$	$^{x}5.544_{b}$
genistin	80	$^{x}67.738_{a}$	$^{y}69.821_{a}$	$^{y}66.116_{ab}$	$^{y}60.775_{bc}$	$^{z}59.085_{c}$
	90	$^{x}67.738_{b}$	$^{x}76.720_{a}$	$^{x}77.226_{a}$	$^{x}78.920_{a}$	$^{y}68.671_{b}$
	100	$^{x}67.738_{bc}$	$^{x}76.674_{a}$	$^{x}73.659_{ab}$	$^{y}64.323_{c}$	$^{x}77.399_{a}$
6″-*O*-acetylgenistin	80	$^{x}5.063_{a}$	$^{x}3.046_{ab}$	$^{x}3.516_{ab}$	$^{x}3.509_{ab}$	$^{x}2.523_{b}$
	90	$^{x}5.063_{a}$	$^{x}5.011_{a}$	$^{x}5.126_{a}$	$^{x}3.752_{a}$	$^{x}4.055_{a}$
	100	$^{x}5.063_{a}$	$^{x}4.172_{a}$	$^{x}5.051_{a}$	$^{x}5.758_{a}$	$^{x}4.213_{a}$
6″-*O*-malonylgenistin	80	$^{x}2.875_{b}$	$^{x}4.024_{ab}$	$^{x}7.338_{a}$	$^{x}5.284_{ab}$	$^{x}6.132_{ab}$
	90	$^{x}2.875_{a}$	$^{x}4.778_{a}$	$^{xy}5.697_{a}$	$^{xy}4.782_{a}$	$^{x}5.109_{a}$
	100	$^{x}2.875_{a}$	$^{x}3.245_{a}$	$^{y}3.008_{a}$	$^{y}1.578_{a}$	$^{x}2.852_{a}$
total genistein	80	$^{x}82.40_{a}$	$^{y}83.55_{a}$	$^{y}83.41_{a}$	$^{y}75.46_{a}$	$^{y}73.74_{b}$
	90	$^{x}82.40_{b}$	$^{x}94.20_{a}$	$^{x}96.94_{a}$	$^{x}93.87_{a}$	$^{x}84.18_{b}$
	100	$^{x}82.40_{ab}$	$^{xy}90.06_{a}$	$^{xy}89.15_{a}$	$^{y}78.18_{b}$	$^{xy}90.01_{a}$

[a] Means in the same row within a temperature treatment with no common subscripts are different ($P \leq 0.05$). Means in the same column within a holding time treatment with no common superscripts are different ($P \leq 0.05$).

Table 3. Influence of Temperature and Time of Processing on Profile of Daidzein, Daidzin, Acetyldaidzin, Malonyldaidzin, and the Sum of All Forms of Daidzein (Micrograms per Gram) in Tofu ($n = 3$)[a]

		holding time				
compound	temp (°C)	0 min	10 min	20 min	30 min	40 min
daidzein	80	$^{x}48.975_{a}$	$^{x}46.337_{ab}$	$^{x}41.678_{b}$	$^{x}34.505_{b}$	$^{x}41.095_{b}$
	90	$^{x}48.975_{a}$	$^{y}35.937_{b}$	$^{y}33.481_{b}$	$^{x}34.854_{b}$	$^{y}32.424_{b}$
	100	$^{x}48.975_{a}$	$^{z}28.365_{b}$	$^{z}25.322_{b}$	$^{y}16.898_{c}$	$^{z}14.988_{c}$
daidzin	80	$^{x}32.228_{a}$	$^{xy}34.063_{a}$	$^{x}30.511_{ab}$	$^{y}26.836_{b}$	$^{x}26.902_{b}$
	90	$^{x}32.228_{a}$	$^{y}31.239_{a}$	$^{x}32.144_{a}$	$^{x}31.345_{a}$	$^{x}30.892_{a}$
	100	$^{x}32.228_{ab}$	$^{x}35.690_{a}$	$^{x}30.844_{b}$	$^{y}23.229_{c}$	$^{x}30.413_{b}$
6″-*O*-acetyldaidzin	80	$^{x}7.866_{ab}$	$^{x}9.151_{a}$	$^{x}5.845_{bc}$	$^{x}5.540_{bc}$	$^{x}4.678_{c}$
	90	$^{x}7.866_{a}$	$^{x}7.469_{a}$	$^{x}5.223_{a}$	$^{x}5.038_{a}$	$^{x}4.996_{a}$
	100	$^{x}7.866_{a}$	$^{y}3.476_{b}$	$^{x}4.766_{b}$	$^{x}3.213_{b}$	$^{x}3.738_{b}$
6″-*O*-malonyldaidzin	80	$^{x}32.947_{a}$	$^{x}31.126_{ab}$	$^{x}24.346_{abc}$	$^{x}19.647_{c}$	$^{x}23.204_{bc}$
	90	$^{x}32.947_{a}$	$^{xy}24.356_{ab}$	$^{xy}16.801_{b}$	$^{x}21.241_{b}$	$^{x}22.002_{b}$
	100	$^{x}32.947_{a}$	$^{y}17.557_{b}$	$^{y}13.972_{b}$	$^{y}9.708_{b}$	$^{y}8.156_{b}$
total daizein	80	$^{x}122.0_{a}$	$^{x}120.7_{ab}$	$^{x}102.4_{bc}$	$^{x}86.53_{c}$	$^{x}95.88_{c}$
	90	$^{x}122.0_{a}$	$^{y}99.00_{b}$	$^{xy}87.65_{b}$	$^{x}92.48_{b}$	$^{x}90.31_{b}$
	100	$^{x}122.0_{a}$	$^{y}85.09_{b}$	$^{y}74.90_{b}$	$^{y}53.05_{c}$	$^{y}57.30_{c}$

[a] Means in the same row within a temperature treatment with no common subscripts are different ($P \leq 0.05$). Means in the same column within a holding time treatment with no common superscripts are different ($P \leq 0.05$).

grated any more at 100 °C than at 80 °C; thus, leaching of daidzein alone does not seem to be a sufficiently logical explanation for the observation that significantly more daidzein was lost when tofu was heated at 100 °C as compared to 80 °C. However, because there is little evidence for thermal degradation in the literature, matrix effects need to be considered potential contributors as well.

Chemically, formation of the acetylglycoside from the malonylglycoside is possible by heat-induced decarboxylation, which has been considered to be the cause of an observed increase in acetylglycosides in a study investigating extrusion effects on isoflavones (*18*) and was shown in other studies as well (*12*, *21*). However, although the malonylglycoside showed the greatest decrease from almost 33 to 8 μg/g after heating at 100 °C for 40 min, no corresponding increase in acetylglycoside could be observed, indicating that decarboxylation of the malonylglycoside to the acetylglycoside occurred most likely only to a limited extent, if at all. On the other hand, only little or no decrease of the glucoside, daidzin, was observed, which was most likely because any losses that may have been due to leaching were offset by the formation of the β-glucoside by the deesterification of the malonyl- and acetylglycosides. This conclusion is in agreement with previous reports by Coward et al. (*3*, *17*).

CONCLUSIONS

Thermal processing of tofu can influence the profile of the major isoflavones in a significant manner. Time and temperature of heating have important effects on the content of isoflavones. Whereas it has been shown that malonyl daidzin is more labile than malonyl genistin (*21*), the large difference in behavior of the genistein series versus the daidzein series could not be explained from this study and warrants further investigation. In addition, although this study was not able to conclusively show the specific reasons for the decrease in the various forms of the daidzein series, there is indication that daidzein and its various forms might be heat labile in addition to being lost by leaching during cooking. Thus, isotope labeling might be useful in determining the decomposition patterns of the various forms of isoflavones over extended processing times. In conclusion, processing parameters have a strong influence on the profile of isoflavones, and future research extending this work to other soy products and the effects of other parameters such as pH and salt as well as determining the physiological availability of the various chemical forms of the isoflavones is needed.

LITERATURE CITED

(1) Kurzer, M. S.; Xu, X. Dietary phytoestrogens. *Annu. Rev. Nutr.* **1997**, *17*, 353−381.

(2) Onozawa, M.; Fukuda K.; Ohtani, M.; Akaza, H.; Sugimura, T.; Wakabayashi, K. Effects of soybean isoflavones on cell growth and apoptosis of the human prostatic cancer cell line LNCaP. *Jpn. J. Clin. Oncol.* **1998**, *28* (6), 360−363.

(3) Coward, L.; Smith, M.; Kirk, M.; Barnes, S. Chemical modification of isoflavones in soyfoods during cooking and processing. *Am. J. Clin. Nutr.* **1998**, *68* (Suppl.), 1486s−1491s.

(4) Boland, G. M.; Donnelly, M. X. Isoflavonoids and related compounds. *Nat. Prod. Rep.* **1998**, *15*, 241−260.

(5) Peterson, G.; Barnes, S. Genistein inhibition of the growth of human breast cancer cells: independence from estrogen receptors and the multi-drug resistance gene. *Biochem. Biophys. Res. Commun.* **1991**, *179*, 661−667.

(6) Peterson, G.; Barnes, S. Genistein and biochanin A inhibit the growth of human prostate cancer cells but not epidermal growth factor receptor tyrosine autophosphorylation. *Prostate* **1993**, *22*, 335−345.

(7) Yanagihara, K.; Ito, A.; Toge, T.; Numoto, M. Antiproliferative effects of isoflavones on human cancer cell lines established from the gastrointestinal tract. *Cancer Res.* **1993**, *53*, 5815−5821.

(8) Booth, C.; Hargreaves, D. F.; Hadfield, J. A.; McGown, A. T.; Potten, C. S. Isoflavones inhibit intestinal epithelial cell proliferation and induce apoptosis in vitro. *Br. J. Cancer* **1999**, *80*, 1550−1557.

(9) Kim, H.; Peterson, T. G.; Barnes, S. Mechanisms of action of the soy isoflavone genistein: emerging role for its effects via transforming growth factor β signaling pathways. *Am. J. Clin. Nutr.* **1998**, *68* (Suppl.), 1418S−1425S.

(10) Burke, G. L.; Anthony, M.; Vitolins, M. Dietary soy proteins and lipids: a strategy for primary prevention of cardiovascular disease? *Curr. Opin. Endocrin. Diabet.* **1996**, *3*, 508−513.

(11) Carroll, K. K.; Kurowska, E. M. Soy consumption and cholesterol reduction: a review of animal and human studies. *J. Nutr.* **1995**, *125*, 594−597S.
(12) Wang, H. J.; Murphy, P. A. Isoflavone content in commercial soybean foods. *J. Agric. Food Chem*. **1994**, *42*, 1666−1673.
(13) Wang, H. J.; Murphy, P. A. Isoflavone composition in American and Japanese soybeans in Iowa: effects of variety, crop year, and location. *J. Agric. Food Chem*. **1994**, *42*, 1674−1677.
(14) Wang, H. J.; Murphy, P. A. Mass balance study of isoflavones during soybean processing. *J. Agric. Food Chem*. **1996**, *44*, 2377−2383.
(15) Fukutake, M.; Takahashi, M.; Ishida, K.; Kawamura, H.; Sugimura, T.; Wakabayashi, K. Quantification of genistein and genistin in soybeans and soybean products. *Food Chem. Toxicol.* **1996**, *34*, 457−461.
(16) Franke, A. A.; Hankin, J. H.; Yu, M. C.; Maskarinec, G.; Low, S. H.; Custer, L. J. Isoflavone levels in soy foods consumed by multiethnic populations in Singapore and Hawaii. *J. Agric. Food Chem*. **1999**, *47*, 977−986.
(17) Coward, L.; Barnes, N. C.; Setchell, K. D. R.; Barnes, S. Genistein, daidzein, and their β-glycoside conjugates: Antitumor isoflavones in soybean foods from American and Asian diets. *J. Agric. Food Chem*. **1993**, *41*, 1961−1967.
(18) Mahungu, S. M.; Diaz-Mercado, S.; Li, J.; Schwenk, M.; Singletary, K.; Faller, J. Stability of isoflavones during extrusion processing of corn/soy mixture. *J. Agric. Food Chem*. **1999**, *47*, 279−284.
(19) Davies, C. G. A.; Netto, F. M.; Glassenap, N.; Gallagher, C. M.; Labuza, T. P.; Gallaher, D. D. Indication of Maillard reaction during storage of protein isolates. *J. Agric. Food Chem*. **1998**, *46*, 2485−2489.
(20) Barnes, S.; Coward, L.; Kirk, M.; Sfakianos, J. HPLC-mass spectrometry analysis of isoflavones. *Proc. Soc. Exp. Biol. Med*. **1998**, *217* (3), 254−262.
(21) Wang, C.; Ma, Q.; Pagadala, S.; Sherrard, M. S.; Krishnan, P. G. Changes in isoflavones during processing of soy protein isolates. *J. Am. Oil Chem. Soc.* **1998**, *75*, 337−341.
(22) Kudou, S.; Fleury, Y.; Welti, D.; Magnolato, D.; Uchida, T.; Kitamura, K.; Okubo, K. Malonyl isoflavone glycosides in soybean seeds (*Glycine max*. Merrill). *Agric. Biol. Chem*. **1991**, *55*, 2227−2233.
(23) SAS Institute Inc. *SAS/STAT*, release 6.12; Cary, NC, 1996.

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