Chemical modification of isoflavones in soyfoods during cooking and processing[1–3]

Lori Coward, Michelle Smith, Marion Kirk, and Stephen Barnes

ABSTRACT   The principal chemical forms of isoflavones in soybean are their 6″-*O*-malonyl-β-glucoside (6*O*MalGlc) conjugates. Experiments were carried out to determine the best conditions for extraction of isoflavones from soyfoods and the effects of commercial processing procedures and of cooking on isoflavone concentrations and composition. Hot alcohol extraction of ground soybeans deesterified 6*O*MalGlc conjugates. Although room temperature extraction slowed the conversion, extraction at 4 °C for 2–4 h led to the highest yield of 6*O*MalGlc conjugates and the lowest proportion of β-glucoside conjugates. Analysis of soyfood products by reversed-phase HPLC–mass spectrometry revealed that defatted soy flour that had not been heat treated consisted mostly of 6*O*MalGlc conjugates; in contrast, toasted soy flour contained large amounts of 6″-*O*-acetyl-β-glucoside conjugates, formed by heat-induced decarboxylation of the malonate group to acetate. Soymilk and tofu consisted almost entirely of β-glucoside conjugates; low-fat versions of these products were markedly depleted in isoflavones. Alcohol-washed soy-protein concentrates contained few isoflavones. Isolated soy protein and textured vegetable protein consisted of a mixture of all 3 types of isoflavone conjugates. Baking or frying of textured vegetable protein at 190 °C and baking of soy flour in cookies did not alter total isoflavone content, but there was a steady increase in β-glucoside conjugates at the expense of 6*O*MalGlc conjugates. The chemical form of isoflavones in foods should be taken into consideration when evaluating their availability for absorption from the diet.   *Am J Clin Nutr* 1998;68(suppl):1486S–91S.

KEY WORDS   Genistein, daidzein, glycitein, glucosides, solvent extraction, isoflavones, food processing, malonylglucoside conjugate, acetylglucoside conjugate, soyfoods

INTRODUCTION

There is an apparent association between increased soy consumption and reduced cancer risk (1). In addition, a meta-analysis confirmed that soy protein lowers blood cholesterol concentrations (2), a finding observed almost 20 y before in rabbits (3) and in patients with hypercholesterolemia (4). The number of investigators carrying out both animal studies (5–10) and human clinical trials (11–13) with soy products has increased and, as a result, there is considerable interest in the soybean isoflavones and their potential chemopreventive effects.
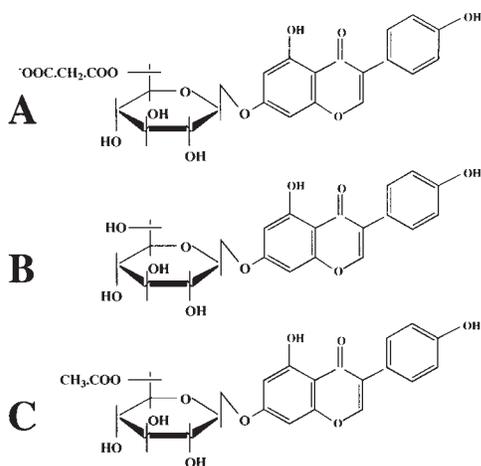
Before 1990, the composition of isoflavones in soyfoods was thought to be largely determined by whether the foods were fermented (14, 15). Fermented soyfoods such as miso and tempeh contain the unconjugated isoflavone aglucones, whereas nonfermented soyfoods [soymilk, tofu, soy flour, soy-protein concentrate, and isolated soy protein (ISP)] contain their β-glucoside conjugates (**Figure 1**). Isoflavones at that time were recovered for analysis by extraction into hot, aqueous organic solvents such as acetonitrile, ethanol, or methanol. However, in 1991 it was found that extraction of soybeans with these solvents without the use of heat led to a different set of isoflavone glucosides, identified as the 6″-*O*-malonyl-β-glucoside (6*O*MalGlc) conjugates (Figure 1) (16). Careful investigation of soyfoods with reversed-phase HPLC–mass spectrometry (HPLC-MS) and revised extraction protocols showed that most soyfoods contained mixtures of the β-glucoside, 6*O*MalGlc, and 6″-*O*-acetyl-β-glucoside (6*O*AcGlc) conjugates (Figure 1) (17, 18). The 6*O*AcGlc conjugates had been previously identified in toasted soy flour (19).

Because the chemistry of the isoflavone conjugates is altered during the commercial processing of soy into food products, we set out to determine *1*) whether the revised extraction procedures in use permit quantitatively reliable measurement of the different isoflavone conjugate types, *2*) to what extent the consumer's cooking the soyfood further alters the isoflavone glucoside conjugate composition, and *3*) whether the isoflavone moiety is stable under these conditions. To properly interpret data, it is crucial that we know exactly what forms of isoflavones are consumed by the subjects in a clinical trial. The proportion of each chemical type of isoflavone absorbed into the blood remains poorly understood, although it was proposed that the aglucones are absorbed readily from the upper small

FIGURE 1. Chemical structures of the glucoside conjugates of genistein found in soyfoods. A, 6''-*O*-malonyl-β-glucoside; B, β-glucoside; C, 6''-*O*-acetyl-β-glucoside.

intestine, that the β-glucoside conjugates are absorbed from the distal small intestine after hydrolysis to the aglucone, and that the 6*O*MalGlc and 6*O*AcGlc conjugates are absorbed from the large intestine after hydrolysis (20).

MATERIALS AND METHODS

Materials

HPLC-grade methanol and acetonitrile and ammonium acetate were purchased from Fisher Scientific (Norcross, GA). Trifluoroacetic acid was purchased from Sigma Chemical Company (St Louis). The disodium salt of fluorescein was purchased from JT Baker (Phillipburg, NJ). Genistin was isolated from a soy-protein extract prepared by Protein Technologies International (St Louis); genistein was made by acid hydrolysis of genistin (21). Daidzin was isolated from soy molasses (21) and daidzein was purchased from LC Labs (Woburn, MA). Soymilks were provided by West-Soy Foods (Los Angeles). ISP was provided by Protein Technologies International. Ground soybeans were provided by Bill Wiebold, University of Missouri at Columbia. Toasted soy flour and soy molasses were provided by Archer Daniels Midland Co (Decatur, IL). Tofu, soy flour, and textured vegetable protein (TVP) were purchased at a local health food store.

Extraction conditions

Soyfoods (0.5 g) were mixed with 5 mL 80% aqueous methanol with 1 mg fluorescein as an internal standard (17). Milk samples (1 mL) were mixed with 4 mL 80% methanol containing 1 mg fluorescein. Extraction was carried out by tumbling the mixture for 2–24 h at room temperature (22–23°C) or 4°C or shaking in a water bath for 2–4 h at 80°C. Samples of each were centrifuged at 16000 × *g* for 5 min at 4°C before HPLC analysis.

Cooking conditions

Soy flour was either mixed with water to make a dough or mixed with wheat flour, sugar, butter, baking soda, and eggs to make a cookie. Both preparations were baked in a conventional oven at 190°C for 0–30 min. TVP was hydrated in boiling water and either

fried over medium heat for 0–20 min or baked at 170°C for <60 min. Samples were stored frozen at −20°C until analyzed.

HPLC analysis

Reversed-phase HPLC analysis of isoflavones and their glucoside conjugates was carried out on a 22 cm × 4.6 mm internal diameter Brownlee Aquapore C$_8$ column with a 1.5 cm × 3.2 mm internal diameter RP-C$_8$ NewGuard precolumn (Rainin, Woburn, MA). Samples were eluted at a flow rate of 1.5 mL/min by using a gradient of 0–30% eluant B (95% acetonitrile with 0.1% trifluoroacetic acid) over 30 min. The column was washed with 100% eluant B for 5 min and equilibrated with eluant A (5% acetonitrile with 0.1% trifluoroacetic acid) for 5 min between runs. The eluted isoflavones were detected at 262 nm (**Figure 2**). Quantitative data for daidzein, daidzin, genistein, and genistin were obtained from comparison with known standards. Kudou et al (16) showed that the molar extinction coefficients of the 6*O*MalGlc conjugates approximate those of the β-glucoside conjugates, so the 6*O*AcGlc and 6*O*MalGlc conjugates were calculated from the β-glucoside standards. Glycitein (7,4′-dihydroxy-6-methoxyisoflavone) and its conjugates were calculated by using daidzein and its conjugates because standards were not available. Data are expressed as means ± SDs.

Mass spectrometry analysis

The identity of each isoflavone conjugate was confirmed by HPLC-MS. Analyses were performed on an API III triple-quadrupole mass spectrometer (PE-Sciex, Concorde, Canada) equipped with 2 Macintosh Quadra 950 computers (Apple Computer Inc, Cupertino, CA) for data analysis. The isoflavones were separated by reversed-phase HPLC on a 10-cm × 4.6-mm internal diameter Aquapore C$_8$ column at a flow rate of 1.0 mL/min by using a linear 0–50% gradient of acetonitrile in the presence of 10 mmol ammonium acetate/L. Positive ions were introduced into the mass spectrometer after their generation by atmospheric pressure chemical ionization caused by a corona discharge needle in the heated nebulizer interface of the instrument (17). Each conjugate produced a [M$^+$H]$^+$ ion and the aglucone fragment.
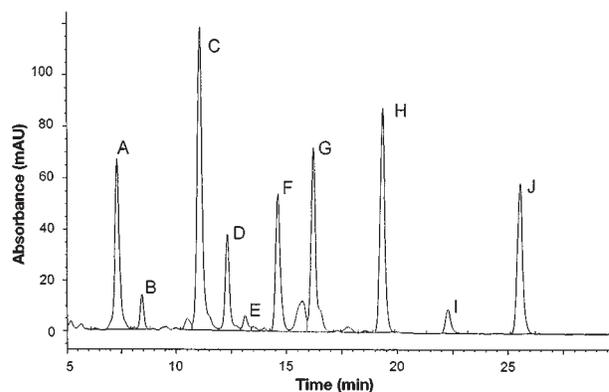
FIGURE 2. Reversed-phase HPLC results of the isoflavone conjugates in textured vegetable protein. A, daidzin; B, glycitin; C, genistin; D, 6''-*O*-malonyldaidzin; E, 6''-*O*-malonylglycitin; F, 6''-*O*-acetyldaidzin; G, 6''-*O*-malonylgenistin; H, 6''-*O*-acetylgenistin; I, genistein; J, fluorescein (internal standard); AU, absorbance unit.

TABLE I
Effect of extraction temperature on isoflavone concentrations in 80% aqueous methanol extracts of around soybeans[1]

Isoflavone and temperature	6OMalGlc	βGlc	6OAcGlc	Aglucone	Total
			$\mu g/g$		
Daidzein					
4°C	352.1 ± 5.0	55.6 ± 0.8	73.4 ± 0.6	ND	481.1
Room temperature	302.5 ± 1.3	71.0 ± 0.1	71.8 ± 0.7	ND	445.3
80°C	69.6 ± 7.3	393.7 ± 9.3	ND	ND	463.3
Genistein					
4°C	545.9 ± 8.8	57.2 ± 0.5	5.4 ± 0.1	ND	608.5
Room temperature	467.5 ± 3.7	87.9 ± 0.2	4.3 ± 0.1	3.8 ± 0.1	563.5
80°C	69.6 ± 7.3	393.7 ± 9.3	ND	ND	463.3
Glycitein					
4°C	139.5 ± 3.3	42.1 ± 0.4	ND	ND	181.6
Room temperature	125.7 ± 1.7	48.7 ± 1.4	ND	ND	174.4
80°C	ND	150.8 ± 2.3	ND	ND	150.8

[1] $\overline{x}$ ± SD. Concentrations are in aglucone units. 6OMalGlc, 6''-O-malonyl-β-glucoside; βGlc, β-glucoside; 6OAcGlc, 6''-0-acetyl-β-glucoside; ND, none detected.

RESULTS

Extraction conditions

Isoflavone β-glucoside conjugates were extracted from soybeans at 80°C, at room temperature, and at 4°C for 2–72 h. Quantitative and reproducible recovery of the isoflavone glucosides was achieved after 2 h (data not shown). Extraction at 4°C gave the highest concentration of 6OMalGlc conjugates and the lowest concentration of β-glucoside conjugates (**Table 1**). At room temperature, a slight conversion of the 6OMalGlc conjugates to the β-glucoside conjugates was observed. Extraction at 80°C caused extensive conversion of the 6OMalGlc conjugates to the β-glucoside conjugates but not to the 6OAcGlc conjugates or aglucones. Although the composition of the individual β-glucoside conjugates was drastically altered by temperature, the total amount of isoflavones extracted was constant. The 6OMalGlc conjugates were stable at 4°C for 24 h but prolonged storage also caused conversion to the β-glucoside conjugates (**Figure 3**).

Soyfoods

Soy flour, toasted soy flour, ISP, tofu, low-fat tofu, soymilk, low-fat soymilk, nonfat soymilk, and TVP were extracted at 4°C. Soy flour, which is prepared by grinding soybeans into a powder, contained predominantly the 6OMalGlc conjugates (**Table 2**). Toasting the flour increased the content of both the β-glucoside and 6OAcGlc conjugates. ISP is prepared by first solubilizing the proteins at an alkaline pH to separate them from the insoluble carbohydrates and then precipitating the proteins at their isoelectric point (pH 5). After the mixture is neutralized, the protein is spray-dried to produce the commercial product. Isoflavones were found in ISP as a mixture of the 6OMalGlc and β-glucoside conjugates, with a small amount of the 6OAcGlc conjugates and aglucones. TVP, prepared by extrusion technologies on soy flour, soy-protein concentrates, or ISP, contained a combination of all 3 conjugates, 30% occurring as the 6OAclGlc conjugates. Tofu and soymilk, both of which are processed by hot aqueous extractions, contained predominantly the β-glucoside conjugates (**Table 3**). Low-fat and nonfat soymilk also contained predominantly β-glucoside conjugates but were relatively depleted of isoflavones, by 57% and 88%, respectively. Low-fat tofu was depleted of β-glucoside conjugates by 40%, but a slight increase was seen in the 6OAcGlc conjugates and aglucones.

Cooked foods

When TVP, which contains each of the glucoside conjugates, was baked, the amounts of 6OAcGlc conjugates and aglucones present were unchanged, but the 6OMalGlc conjugates slowly degraded to the β-glucoside conjugates. When TVP was fried, however, increases in the 6OAcGlc conjugates and aglucones, as well as the β-glucoside conjugates, were observed (**Figure 4**). Under normal cooking times (60 min for baking and 7.5 min for frying), the total isoflavones recovered did not decrease once an increase due to the loss of water during cooking was taken into account. When soy flour was baked as a dough, the water in the dough regulated the internal temperature of the dough and 6OMalGlc conjugates decreased only slightly over the 30 min of cooking. When ingredients such as sugar and butter were added, the 6OMalGlc conjugates were degraded much more rapidly (**Figure 5**). When the cookie was ideally cooked (after 7.5 min), the amounts
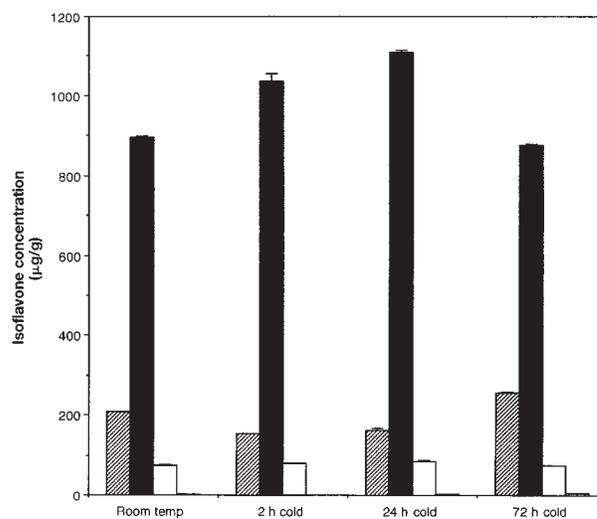
FIGURE 3. Mean (±SD) total concentrations of isoflavone glucoside conjugates from ground soybeans extracted with 80% aqueous methanol at room temperature (temp) for 2 h or at 4°C (cold) for 2 and 24 h, or after storage at 4°C for 24 or 72 h. For each set of bars, from left to right, the bars represent β-glucosides, 6''-O-malonyl-β-glucosides, 6''-O-acetyl-β-glucosides, and aglucones. n = 2.

TABLE 2
Isoflavone concentrations in soy products after cold 80% aqueous methanol extraction[1]

Food and isoflavone	6OMalGlc	βGlc	6OAcGlc	Aglucone	Total
			$\mu g/g$		
TSF					
Daidzein	402.0 ± 5.7	609.6 ± 6.4	331.8 ± 4.8	ND	1343.4
Genistein	525.9 ± 6.7	665.0 ± 6.8	296.4 ± 1.1	22.2 ± 0.2	1509.5
Glycitein	110.9 ± 4.0	131.6 ± 1.4	ND	ND	242.5
Soy flour					
Daidzein	570.9 ± 6.9	110.7 ± 1.0	148.1 ± 0.8	ND	829.7
Genistein	657.8 ± 6.6	106.6 ± 3.8	59.3 ± 0.5	10.8 ± 0.3	834.4
Glycitein	111.6 ± 1.9	31.3 ± 0.3	ND	ND	142.9
ISP					
Daidzein	366.2 ± 0.1	232.5 ± 10.5	190.6 ± 1.7	ND	789.3
Genistein	721.2 ± 3.0	403.9 ± 2.2	95.2 ± 1.8	37.7 ± 0.2	1258.0
Glycitein	82.4 ± 1.8	31.8 ± 0.3	ND	ND	114.2
TVP					
Daidzein	195.4 ± 8.3	375.3 ± 7.5	306.9 ± 4.5	42.1 ± 2.0	919.7
Genistein	247.9 ± 8.5	467.8 ± 11.5	327.8 ± 6.3	48.6 ± 0.7	1092.1
Glycitein	33.5 ± 2.1	64.9 ± 0.8	ND	ND	98.4

[1] $\bar{x}$ ± SD. 6OMalGlc, 6''-0-malonyl-β-glucoside; βGlc, β-glucoside; 6OAcGlc, 6''-0-acetyl-β-glucoside; TSF, toasted soy flour; ND, none detected; ISP, isolated soy protein; TVP, textured vegetable protein.

of β-glucoside conjugates were more than doubled. If the cookie was dry and slightly burnt (after 15 min), the 6OAcGlc conjugates and aglucones were increased. As seen for the TVP, under normal cooking conditions total isoflavone concentrations were constant. If the cookie was charred, the isoflavone concentrations were decreased (L Coward et al, unpublished observations, 1996).

DISCUSSION

This study showed that the isoflavone glucoside conjugates were easily altered during extraction, processing, and cooking. The 6OMalGlc conjugates were unstable when exposed to heat. Aqueous heating, as seen during extraction with aqueous methanol, caused conversion to the β-glucoside conjugates. Room temperature extraction also led to a loss of the 6OMalGlc
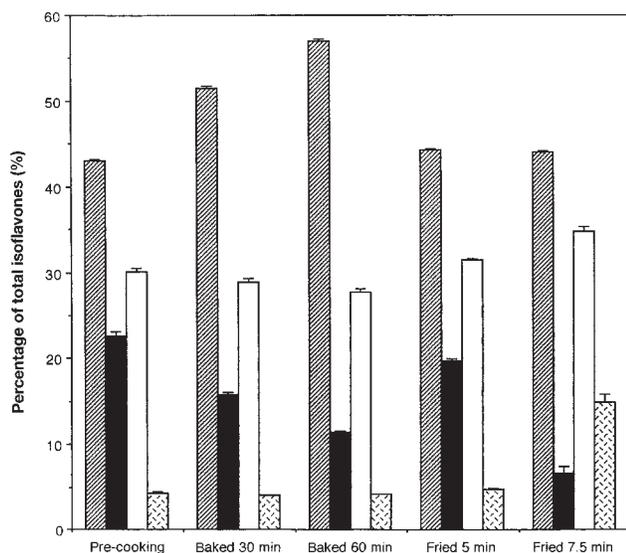
FIGURE 4. Effect of cooking styles and cooking times on the mean (±SD) composition of isoflavone glucoside conjugates from textured vegetable protein. Textured vegetable protein was hydrated with boiling water and either baked for 30 or 60 min at 170 °C or fried for 5 or 7.5 min over medium heat. For each set of bars, from left to right, the bars represent β-glucosides, 6''-O-malonyl-β-glucosides, 6''-O-acetyl-β-glucosides, and aglucones. $n = 2$.
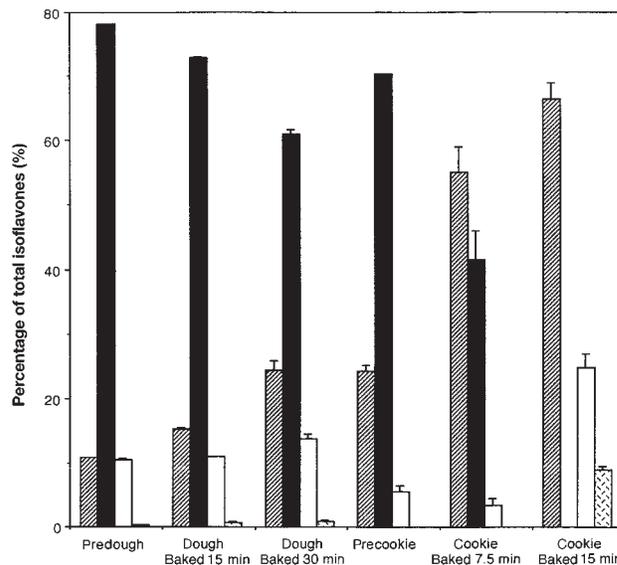
FIGURE 5. Effect of cooking times on the mean (±SD) composition of isoflavone glucoside conjugates in soy flour mixed as a dough with water and baked for 15 or 30 min at 190 °C or mixed as cookie dough with butter, sugar, and eggs and baked for 7.5 or 15 min at 190 °C. For each set of bars, from left to right, the bars represent β-glucosides, 6''-O-malonyl-β-glucosides, 6''-O-acetyl-β-glucosides, and aglucones. $n = 2$.

TABLE 3
Effect of low-fat processing technology on isoflavone concentrations in soymilk and tofu[1]

Food and isoflavone	6OMalGlc	β-Glc	6OAcGlc	Aglucone	Total
			μg/g		
Regular soymilk (mg/L)					
Daidzein	4.6 ± 0.1	34.3 ± 0.1	2.5 ± 0.1	ND	41.4
Genistein	8.9 ± 0.3	42.5 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	53.2
Glycitein	ND	1.4 ± 0.1	ND	ND	1.4
Low-fat soymilk (mg/L)					
Daidzein	1.7 ± 0.1	15.4 ± 0.2	ND	ND	17.1
Genistein	3.1 ± 0.1	21.1 ± 0.3	ND	ND	24.2
Glycitein	ND	0.6 ± 0.1	ND	ND	0.6
Non-fat soymilk (mg/L)					
Daidzein	0.7 ± 0.1	4.3 ± 0.1	ND	ND	5.1
Genistein	1.1 ± 0.1	5.4 ± 0.3	ND	ND	6.5
Glycitein	ND	ND	ND	ND	ND
Regular tofu (μg/g)					
Daidzein	14.6 ± 1.3	99.7 ± 3.4	7.9 ± 0.1	10.9 ± 0.3	133.1
Genistein	30.2 ± 0.6	122.7 ± 2.8	4.5 ± 0.1	11.6 ± 0.5	169.0
Glycitein	4.3 ± 2.0	16.6 ± 0.5	ND	ND	20.9
Low-fat tofu (μg/g)					
Daidzein	15.0 ± 0.1	50.8 ± 0.3	10.1 ± 0.2	23.0 ± 0.3	98.9
Genistein	32.0 ± 0.2	82.2 ± 1.0	6.1 ± 0.3	32.9 ± 0.3	153.2
Glycitein	4.1 ± 0.1	4.7 ± 0.4	ND	ND	8.8

[1] $\bar{x}$ ± SD. 6OMalGlc, 6″-0-malonyl-β-glucoside; βGlc, β-glucoside; 6OAcGlc, 6″-0-acetyl-β-glucoside; ND, none detected.

conjugates, but at a much slower rate. The 6OMalGlc conjugates were stable for up to 24 h at 4°C, but storage for several days in the cold also led to breakdown (Figure 3).

Isoflavones are found in soybeans as their 6OMalGlc conjugates. The glucoside composition of soyfoods is determined by the processing conditions. Grinding to produce flour and hexane extraction to remove fats does not alter glucoside conjugation. Dry heat, as in toasting of soy flour or extrusion used to produce TVP, caused a loss of carbon dioxide and led to the formation of substantial amounts of the 6OAcGlc conjugates. Hot aqueous extraction, used to produce tofu or soymilk, results almost entirely in the formation of β-glucoside conjugates. Fermentation, to produce miso and tempeh, causes a loss of the glucosides to form the aglucones (22). Ethanol extraction of soy flour to produce soy-protein concentrates removes the isoflavones (22). Processing leads to a wide range of isoflavone concentrations in soyfoods, particularly in soy products that have lowered fat contents and low isoflavone concentrations.

Cooking the soy products further altered the glucoside conjugates. The 6OMalGlc conjugates were most affected, being converted to β-glucoside or 6OAcGlc conjugates, depending on the mode of cooking and other ingredients present. Baked food products resulted in mostly β-glucoside conjugates, whereas fried products contained more 6OAcGlc conjugates. This mimics what is seen in processing, because moist heat produces conversion to the β-glucoside conjugates and dry heat results in the formation of 6OAcGlc conjugates. The total isoflavone concentration in the food was not reduced under normal cooking conditions. As food was burned, however, an increase in aglucones and a decrease in total isoflavones was observed.

The composition of the glucoside conjugates may have sig-

nificant effects on the bioavailability and pharmacokinetics of the isoflavones. Although most studies on isoflavones carried out in vitro or in animal models have used the aglucones, soyfoods are the source of isoflavones in most clinical trials and these contain almost exclusively glucoside conjugates. The present study showed that the composition of the isoflavone glucoside conjugates can vary from one food to another. Furthermore, cooking procedures may influence which glucoside conjugates are actually consumed by trial subjects. Because the composition of the isoflavone glucoside conjugates will alter the rate of absorption and possibly the degree of further metabolism, these alterations in the chemistry of isoflavones need to be taken in account when interpreting data from clinical trials.

REFERENCES

1. Messina M, Persky V, Setchell KDR, Barnes S. Soy intake and cancer risk: a review of the in vitro and in vivo data. Nutr Cancer 1994;21:113–31.
2. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 1995;333:276–82.
3. Hamilton RMG, Carroll KK. Plasma cholesterol levels in rabbits fed low fat, low cholesterol diets. Effects of dietary proteins, carbohydrates and fibre from different sources. Atherosclerosis 1976;24:47–62.
4. Sirtori CR, Agradi E, Conti F, Mantero O, Gatti E. Soybean-protein diet in the treatment of type-II hyperlipoproteinemia. Lancet 1997;1:275–7.
5. Barnes S, Grubbs C, Setchell KDR, Carlson J. Soybeans inhibit breast tumor in models of breast cancer. In: Pariza MW,

Aeschbacher H-U, Felton JS, Sato S, eds. Mutagens and carcinogens in the diet. New York: Wiley-Liss, 1990:239–53.

6. Hawrylewicz EJ, Huang HH, Blair WH. Dietary soybean isolate and methionine supplementation affect mammary tumor progression in rats. J Nutr 1991;121:1693–8.

7. Barnes S, Peterson TG, Grubbs C, Setchell KDR. Potential role of dietary isoflavones in the prevention of cancer. In: Jacobs M, ed. Diet and cancer: markers, prevention and treatment. New York: Plenum Press, 1994:135–48.

8. Hawrylewicz EJ, Zapata JJ, Blair WH. Soy and experimental cancer: animal studies. J Nutr 1995;125:698S–708S.

9. Lamartiniere CA, Moore JB, Brown NA, Thompson RA, Hardin MJ, Barnes S. Genistein suppresses mammary cancer in rats. Carcinogenesis 1995;16:2833–40.

10. Murrill WB, Brown NM, Zhang J-X, Manzolillo PA, Barnes S, Lamartiniere CA. Prepubertal genistein exposure suppresses mammary cancer and enhances gland differentiation in rats. Carcinogenesis 1996;17:1451–7.

11. Bakhit RM, Klein BP, Essex-Sorlie D, Ham JO, Erdman JW, Potter SM. Intake of 25 g soy protein reduces plasma cholesterol in men with elevated cholesterol concentrations. J Nutr 1994;124:213–22.

12. Potter SM, Bakhit RM, Essex-Sorlie DL, et al. Depression of plasma cholesterol in men by consumption of baked products containing soy protein. Am J Clin Nutr 1993;58:501–6.

13. Petrakis N, Barnes S, King EB, et al. Stimulatory influence of soy protein isolate on breast secretion in pre and postmenopausal women. Cancer Epidemiol Biomarkers Prev 1996;5:785–94.

14. Eldridge AC, Kwolek WF. Soybean isoflavones: effect of environment and variety on composition. J Agric Food Chem 1983;31:394–6.

15. Price KR, Fenwick GR. Naturally occurring oestrogens in foods—a review. Food Addit Contam 1985;2:73–106.

16. Kudou S, Fleury Y, Welti D, et al. Malonyl isoflavone glycosides in soybean seeds (Glycine max merrill). Agric Biol Chem 1991;55:2227–33.

17. Barnes S, Kirk M, Coward L. Isoflavones and their conjugates in soy foods: extraction and analysis by HPLC-mass spectrometry. J Agric Food Chem 1994;42:2464–74.

18. Wang H-J, Murphy PA. Isoflavone content in commercial soybean foods. J Agric Food Chem 1994;42:1666–73.

19. Farmakalidis E, Murphy, PA. Isolation of 6″-O-acetyidaidzein and 6″-O acetylgenistein from toasted defatted soy flakes. J Agric Food Chem 1985;33:385–9.

20. Barnes S, Sfakianos J, Coward L, Kirk M, Peterson TG. Soy isoflavonoids and cancer prevention: underlying biochemical and pharmacological issues. In: Butrum R, ed. Dietary phytochemicals and cancer prevention. New York: Plenum, 1996:87–100.

21. Walter ED. Genistin (an isoflavone glucoside) and its aglucone, genistein, from soybeans. J Am Chem Soc 1941;63:3273–6.

22. Coward L, Barnes N, Setchell KDR, Barnes S. Genistein and daidzein and their β-glucoside conjugates: anti-tumor isoflavones in soybean foods from American and Asian diets. J Agric Food Chem 1993;41:1961–7.