Isoflavone Levels in Soy Foods Consumed by Multiethnic Populations in Singapore and Hawaii

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Concentrations and glucosidic conjugation patterns of isoflavones were determined in soy foods consumed by multiethnic populations in Singapore and Hawaii. Six raw and 11 cooked food groups traditionally consumed in Singapore and 8 food groups consumed in Hawaii were analyzed by reversed-phase high-pressure liquid chromatography with diode array detection. Mean total isoflavone levels varied between 35 and 7500 ppm, with the lowest values found in soy milk and burgers and the highest levels observed in soybean and its seeds and in supplements. Total isoflavone levels and conjugation patterns varied as a function of soybean variety, storage conditions, and food processing. A large contribution to the differences in total isoflavone content between food groups was due to the water content in foods and to leaching of polar analytes into the water phase during boiling. Soy protein drinks and traditional soy foods were found to possess very similar isoflavone amounts considering usual serving sizes.

Keywords: *Isoflavones; genistein; daidzein; glycitein; isoflavone conjugates; soy foods; supplements; high-pressure liquid chromatography; diode array detection*

INTRODUCTION

Soy consumption has been implicated in the prevention of cancer and other chronic conditions due to the low disease risk of Asian populations with high soy intake (Adlercreutz and Mazur, 1997; Lee et al., 1991; Barnes et al., 1994b). This hypothesis is supported by the observation that Asians experience an increase in incidence of these chronic conditions after migrating to the West, suggesting a causal role of environmental factors (Nomura et al., 1978; Ziegler et al., 1993). The strongest protective effect of soy intake was reported for breast, prostate, and colon cancer (Messina et al., 1994; Adlercreutz, 1995; Kurzer and Xu, 1997) and potentially also endometrial cancer (Goodman et al., 1997). Recent case-control studies observed lower endogenous estrogen levels in Japanese women with high soy intake (Nagata et al., 1997) and a 15% reduction in breast cancer risk with every serving of tofu consumed (Wu et al., 1996). The prominent phytochemicals that are believed to contribute to the biological effects observed after soy consumption include isoflavones (Barnes et al., 1994b) and also phytate, saponins, sterols, and protease inhibitors (Messina et al., 1994). The cancer protective effects of isoflavones may be based on their antioxidant (Wei et al., 1995), antiestrogenic (Mousavi and Adlercreutz, 1993; Adlercreutz et al., 1993; Makela et al., 1995; Cassidy et al., 1994), antimutagenic and antiproliferative (Hirano et al., 1994; Peterson and Barnes, 1993), transformation-inhibiting (Franke et al., 1998a), differentiation-inducing (Constantinou and Huberman, 1995), angiogenesis-inhibiting (Fotsis et al., 1995), and apoptosis-inducing (Kyle et al., 1997) effects. The anticarcinogenic activity of isoflavones is supported by the prevention of mammary, prostate, and leukemic cancers in various animal models (Barnes et al., 1990; Lamartiniere et al., 1995; Murrill et al., 1996; Pollard and Luckert, 1997; Zhang et al., 1997; Uckun et al., 1995). Humans are exposed to isoflavones mainly through soybeans, including black soybeans (Franke et al., 1995) and its food products (Franke et al., 1994, 1998b; Adlercreutz and Mazur, 1997).

Gas and liquid chromatographic techniques with photodiode array, electrochemical, fluorometric, and mass spectrometric detection have been applied previously to determine isoflavone levels in soybeans and its products (Franke and Custer, 1994; Barnes et al., 1994a; Coward et al., 1993; Adlercreutz and Mazur, 1997; Pettersson and Kiessling, 1984). Daidzein, genistein, and glycitein are the major isoflavones found in these foods, occurring predominantly as glucosides and malonyl glucosides and to a minor extent as acetyl glucosides and "free" aglycons (Franke et al., 1998b; Kudou et al., 1991; Wang and Murphy, 1994a; Barnes et al., 1994a). In soybean seeds, total isoflavone levels average at 2000 ppm relative to dry weigt, but these concentrations vary depending on a variety of factors such as environmental, genetic, harvesting, and processing conditions (Wang and Murphy, 1994b; Tsukamoto et al., 1995; Barnes et al., 1994a; Coward et al., 1993). More recently, isoflavone levels in soy-based infant formula were added to the growing list of isoflavone-containing

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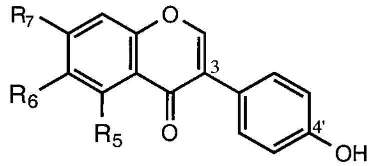


Aglycons	code	R5	R6	R7
Daidzein	DE	H	H	OH
Genistein	GE	OH	H	OH
Glycitein	GLYE	H	OCH_3	OH

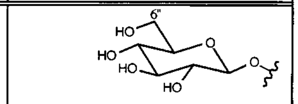
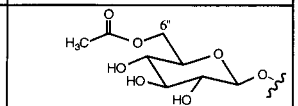
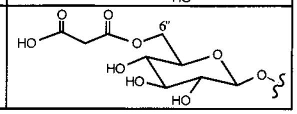
Conjugates	code	R7
7-O-glucoside of daidzein of genistein of glycitein	 D G GLY	
7-O-(6''-O-acetyl)-glucoside of daidzein of genistein of glycitein	 D-Ac G-Ac GLY-Ac	
7-O-(6''-O-malonyl)-glucoside of daidzein of genistein of glycitein	 D-Mal G-Mal GLY-Mal	

Figure 1. Soy isoflavone structures and codes assigned.

foods (Franke et al., 1998c; Setchell et al., 1987, 1997; Murphy et al., 1997).

Here we report concentrations of isoflavones, including data on conjugated and unconjugated analytes (Figure 1), in little investigated foods commonly consumed by Asians and Americans in Singapore and Hawaii. These results will support future epidemiologic studies exploring the role of soy consumption in disease prevention.

MATERIALS AND METHODS

Apparatus. HPLC analyses were carried out on a system Gold chromatograph with an autosampler model 507, a dual channel diode array detector model 168 (all units from Beckman, Fullerton, CA), and a Coulochem II-5200 electrochemical detector (ESA, Bedford, MA) using a 5011 coulometric cell. Absorbance readings were obtained from a DU-62 spectrophotometer (Beckman). Evaporation was performed with a Savant AS 160 Speed-Vac (Farmingdale, NY) at room temperature.

Chemicals. Methanol, acetic acid, 96% ethanol, dimethyl sulfoxide (DMSO), ethyl acetate, and all solvents used for HPLC and absorbance readings were of analytical grade or HPLC grade from Fisher Scientific (Fair Lawn, NJ). Butylated hydroxytoluene (BHT), sodium acetate, and genistein were purchased from Sigma Chemical Co. (St. Louis, MO). Daidzein and genistein were obtained from ICN (Costa Mesa, CA), flavone was from Aldrich (Milwaukee, WI), and coumestrol was from Serva (New York, NY).

Food Items. Soy foods from Singapore and Hawaii were obtained from open markets or from local stores: Garcinia-max diet shake, Rainbow Light Nutritional Systems, Santa Cruz, CA; Light and Fit Energy Shake, Earthrise Co., Petaluma, CA; Slim and Trim diet shake, Nature's Way Products Inc., Springville, UT; soy protein, Down to Earth, Honolulu, HI; Spiru-tein cappuccino high-protein meal, Nature's Plus, Melville, NY; soy flour, Arrowhead Mills, Hereford, TX; soy flour, Miller Co., St. Louis, MO; genistein food supplement, Source Naturals, Inc., Scotts Valley, CA; Soy Super Complex, Rainbow Light Nutritional Systems, Santa Cruz, CA; vegetarian enzyme complex, Futurebiotics, Brattleboro, VT; fat-free Soya Kaas jalapeno Monterey Jack style, American Natural Snacks, St. Augustine, FL; natto prepared cultured soybean, Aloha Tofu Factory, Inc., Honolulu, HI; Veggy Singles, Swiss alternative, Soyco Foods, Orlando, FL; Nu Tofu mozzarella cheese alternative and Nu Tofu Cheddar cheese alternative, Cemac Foods Corp., Philadelphia, PA; Veggy Singles, American alternative, Soy Power Co., Inc., Los Angeles, CA; White Wave dairyless vanilla, White Wave, Boulder, CO; Nancy's soy yogurt, Springfield Creamer, Eugene, OR; Garden Veggie, Wholesome and Hearty Foods, Inc., Portland, OR; Natural Touch vegan burger, Worthington Foods, Inc., Worthington, OH; Boca Burger, Boca Burger Co., Ft. Lauderdale, FL; Soy singles, American alternative, Soymage, Orlando, FL; Super Green Pro-96, Nature's Life, Golden Grove, CA; TakeCare plain, Protein Technologies International, St. Louis, MO; soy chocolate milk, Trader Joe, South Pasadena, CA; white soy milk, Pacific Foods of Oregon, Inc., Tualatin, OR; soybean seeds, Health Foods, San Diego, CA; roasted soybean seeds, Health Foods, Little Rock, AR.

Fresh items were frozen or cooked immediately after purchase followed by freeze-drying prior to extraction and analysis. Items of the same food group were collected on the same day from different sources/markets. Cooking was performed exclusively by boiling. All items from Singapore were boiled for 5 min except for soybeans (10 min). Boiling time for items from Hawaii was 3 min for soy sprouts, 5 min for tau pok (fried pressed tofu), 7 min for soybeans, 10 min for foo jook (soybean curd sticks), and 60 min for dry soybean seeds.

Soy Protein Drink Test. Eight soy protein drinks were tested in 33 volunteers regarding aroma, texture, color, flavor, acceptance, and willingness to consume again as criteria. A score scaled from 1 (worst) to 5 (best) was given to each criterion of all eight items after being consumed by the volunteers. An item was considered qualified for use in intervention studies when the mean score of each criterion exceeded a value of 3.1.

Extraction of Isoflavones from Soy Foods. Soy foods were freeze-dried and homogenized as described previously (Franke et al., 1998b), and 0.5 g of dry powder was extracted with 80% aqueous methanol (v/v) containing 20 ppm flavone as internal standard by sonication (10 min) and stirring (2 h) at room temperature, yielding isoflavone conjugates and originally present aglycons. After centrifugation, a clear aliquot was diluted 1:1 with 0.2 M acetate buffer (pH 4) and 20 μL was injected into the HPLC system. In a parallel experiment 20 μL of a 1:1 mixture of 80% aqueous methanol (v/v) containing 20 ppm flavone and 0.2 M acetate buffer (pH 4) was injected into the HPLC system in the same batch for internal standard recovery calculation purposes.

Standard Solutions, Calibration Curves, and Calculation of Food Levels. As detailed previously (Franke et al., 1994), we determined stock solution concentration of authentic standards with absorbance readings at the wavelength with maximum absorption (λ_{max}) using molar extinction coefficients (ϵ) (Ollis, 1962). Glycitein values (λ_{max} = 256 nm, ϵ = 22387) were used as previously determined (Kelly et al., 1993). Due to the lack of availability of authentic standards for certain conjugated analytes, λ_{max} and ϵ values of the respective aglycons were applied (Coward et al., 1993). We and others made this assumption because 7-O-substituents do not change the flavonoid chromophores significantly (Markham and Mabry, 1975; Markham et al., 1990) and because isoflavone response factors do not change with variable retention times when diode array detection is applied (Franke et al., 1998b). In addition, the identity of analytes was confirmed by using an external standard consisting of an extract from authentic toasted soy obtained from Dr. Stephen Barnes (University of Alabam), which has been analyzed by LC/MS (Barnes et al., 1994a).

Chromatographic Conditions. All HPLC analyses were carried out as reported previously (Franke et al., 1998b; Franke and Custer, 1994) on a NovaPak C18 (150 × 3.9 mm i.d.; 4 μm) reversed-phase column (Waters, Milford, MA) coupled to an Adsorbosphere C18 (7.5 × 4.6 mm i.d.; 5 μm) direct-connect guard column (Alltech, Deerfield, IL). Elution was performed at a flow rate of 0.8 mL/min with the following linear gradient: A = acetic acid/water (10:90 v/v), B = methanol/acetonitrile/dichloromethane (10:5:1 v/v/v); B in A (v/v), 5% for 5 min, from 5 to 45% in 20 min, from 45 to 70% in 6 min, and from 70 to 5% in 3 min with equilibration for 15 min before subsequent injection. Analytes were monitored by diode array detection at 260 and 280 nm and coulometrically at +500 mV during the entire HPLC run simultaneously. Observed peaks were scanned between 190 and 400 nm for identification purposes.

RESULTS AND DISCUSSION

Individual and total isoflavone levels and isoflavone conjugation and aglycon patterns in soy foods were determined for items typically consumed by Chinese people in Singapore (Table 1, 6 food groups), by Asian people in Hawaii (Table 2, 11 food groups), and by Western populations in Hawaii (Tables 3 and 4, 8 food groups). The results presented summarize and detail the dietary data used for recent epidemiologic studies on soy intake and urinary isoflavone excretion (Maskarinec et al., 1998; Seow et al., 1998). Each soy food group tested consisted of three to eight items, which were collected from different markets on the same day to account for variability within the same food type. The isoflavone concentrations were obtained by previously developed HPLC methods using diode array detection (Franke and Custer, 1994; Franke et al., 1998b) and are presented in aglycon units to account for differences in molecular weight of the various analytes. Most raw or unprocessed items such as raw foo jook (soybean curd sticks), raw tau kwa (pressed tofu), raw tau pok (fried pressed tofu), and raw soybeans are rarely consumed unprocessed, but data on these items (Franke et al., 1998b) were included here to explore differences due to processing (boiling). Treatment of soy extracts with acids or enzymes including glucuronidase/sulfatase mixtures (Franke and Custer, 1994; Setchell et al., 1987) leads to less complex chromatograms by hydrolyzing the conjugates into aglycons. However, conjugated and unconjugated isoflavones have different pharmacokinetic properties and biopotencies (Barnes et al., 1994b). Isoflavones are absorbed very quickly, with peaks in plasma levels reached 1 h and again 6−8 h after intake (Franke et al., 1997, 1998b), and are eliminated within 1−2 days, predominantly as glucuronide and sulfate conjugates through the kidney (Xu et al., 1994; Franke et al., 1994, 1998b; Franke and Custer, 1994; Setchell, 1985). Fermented items containing mainly aglycons were suggested to have higher isoflavone bioavailability compared to nonfermented soy items that contain mainly glucosides (Hutchins et al., 1995). We therefore decided to analyze conjugated and unconjugated isoflavones as occurring in soy foods by extraction with aqueous methanol (Barnes et al., 1994a).

Mean total isoflavone levels in the food groups investigated varied between 35 and 7500 ppm, with lowest values found in soy milk and burgers and highest levels found in soybean and its seeds and, of course, in supplements. Most foods we investigated varied between 100 and 800 ppm of total isoflavones, with low levels observed in items processed by mixing with nonsoy components (cheeses) and with high levels observed in "dry" items (soy protein drink powders). A large contribution to the variability in isoflavone content was found to be due to moisture. This occurred because most foods were found to have very similar isoflavone levels after correcting for water content, which is also apparent from the difference observed for raw (dry) and cooked (wet) foods, especially, in foo jook and soybeans (Table 1). Differences between raw and cooked foods that could not be explained by moisture were partly due to leaching of the polar isoflavone conjugates into the boiling water during processing. We determined isoflavone levels in the water used for boiling of some representative boiled items and found isoflavone amounts equaling almost exactly the difference between cooked and raw items after adjustment of moisture (data not shown). This was true not only for total isoflavones but, in particular, for the polar conjugates. Boiling for up to 1 h did not considerably change the conjugation or aglycon pattern. The temperature applied was probably too low and the period of boiling too short to lead to a sufficient hydrolysis and decarboxylation of the conjugates or degradation of the aglycons observed with dry heat such as roasting, toasting, or frying in oil (Coward et al., 1993; Barnes et al., 1994a; Franke et al., 1994). Within the soy milk group, the item with higher fat content showed significantly higher isoflavone levels, indicating that defatting removes isoflavones.

Similar to recent reports about isoflavones in various soy foods (Franke et al., 1994, 1998b; Kudou et al., 1991; Barnes et al., 1994a; Wang and Murphy, 1994a), we found typically a total isoflavone ratio of 45:45:10 for daidzein/genistein/glycitein, which did not change substantially between food groups or with processing. Supplements presented an exception; they had highly variable ratios of isoflavones. In contrast to total isoflavones, conjugation patterns in all soy foods were highly variable between and even within food groups. Glucosyl malonates are the predominant conjugate in the soybean seed (Kudou et al., 1991). We found the highest ratio of this conjugate (65−72%) in unprocessed soybean and its seeds, particularly when stored frozen, and in soy flours (Tables 1−3). Boiling soybeans for 1 h resulted in an 8% decrease in glucosyl malonates and a concomitant increase in 6% and each 1% of glucosides, acetylglucosides, and aglycons, respectively, whereas tau pok (fried and pressed tofu) showed the highest ratio of acetyl glucosides (24%). This can be explained by the heat applied during the processing of these items leading to conditions that cause hydrolysis in hot water and to decarboxylation by frying in oil, respectively (Barnes et al., 1994a).

Exclusively aglycons were detected in fermented tofu (Table 1), but miso, which is produced by incubation with *Aspergillus soja*, showed only incomplete hydrolysis of the isoflavone conjugates with a yield of ∼50% aglycons (Table 2). An even lower aglycon yield was observed for natto, which is fermented with *Bacillus natto* (Table 2). This is consistent with previous analyses of fermented products known to contain predominantly

Table 1. Mean Isoflavone Levels and Patterns in Soy Foods from Singapore[a]

	mg/kg																%[b]			
	D	GLY	G	D-Mal	GLY-Mal	G-Mal	D-Ac	GLY-Ac	G-Ac	DE	GLYE	GE	total DE	total GLYE	total GE	total isoflavones	glucosides	malonyl glucosides	acetyl glucosides	aglycons
raw tofu	**52**	**8**	**61**	**66**	**7**	**63**	**3**	**0**	**2**	**17**	**2**	**14**	**139**	**17**	**141**	**297**	**41**	**45**	**2**	**11**
range ($n^c = 4$)	48–55	6–10	39–85	33–108	3–9	34–97	3–5	0–2	1–4	10–33	0–5	7–34	107–177	15–19	124–164	256–319	31–60	29–59	2–3	6–23
interitem SD[d]	5	2	22	33	2	27	0	0	1	11	2	13	29	2	18	29	13	16	0	8
cooked tofu	**46**	**7**	**55**	**61**	**6**	**49**	**2**	**0**	**2**	**16**	**2**	**13**	**124**	**15**	**119**	**259**	**42**	**44**	**2**	**12**
range ($n = 4$)	44–50	6–8	38–73	40–94	3–8	39–47	0–4	0–2	1–3	10–26	1–4	8–27	103–150	36–145	92–149	231–287	32–55	33–60	32–55	32–55
interitem SD	3	1	20	24	3	11	2	0	0	8	1	9	19	2	24	28	0	0	0	0
raw tau kwa	**50**	**8**	**62**	**66**	**11**	**59**	**3**	**0**	**2**	**17**	**2**	**16**	**136**	**20**	**139**	**295**	**40**	**46**	**2**	**12**
range ($n = 4$)	33–60	5–9	38–75	54–77	8–13	44–68	2–4	-	2–3	13–26	1–4	11–22	116–155	16–24	105–159	237–333	32–43	45–47	1–2	9–2
interitem SD	12	2	17	9	2	12	1	0	0	6	1	4	17	3	26	45	6	0	0	6
cooked tau kwa	**52**	**8**	**64**	**64**	**11**	**59**	**2**	**0**	**2**	**19**	**2**	**15**	**137**	**21**	**141**	**299**	**41**	**45**	**2**	**13**
range ($n = 4$)	34–61	5–10	40–75	51–75	8–13	47–69	2–3		0–5	16–28	1–4	12–23	120–156	17–24	111–161	231–339	23–45	41–46	1–2	8–22
interitem SD	13	2	16	12	2	11	0	0	2	5	1	5	20	3	22	43	6	2	0	6
raw tau pok	**47**	**6**	**70**	**52**	**4**	**48**	**42**	**3**	**45**	**21**	**3**	**27**	**163**	**16**	**190**	**369**	**33**	**27**	**24**	**16**
range ($n = 4$)	23–72	2–9	29–108	22–83	1–7	19–76	16–62	1–5	21–65	12–28	3–4	21–36	87–244	7–25	99–285	193–554	28–36	21–30	19–29	11–31
interitem SD	20	3	33	25	2	23	20	2	21	7	0	7	65	7	78	149	3	4	4	10
cooked tau pok	**21**	**2**	**31**	**26**	**2**	**23**	**14**	**1**	**17**	**11**	**1**	**13**	**72**	**7**	**83**	**162**	**34**	**30**	**20**	**16**
range ($n = 4$)	19–24	2–3	22–35	14–29	1–3	14–27	6–20	0–1	9–22	8–15	1–2	10–15	56–89	5–9	61–104	123–202	31–37	22–39	12–27	13–25
interitem SD	3	0	7	11	1	10	6	0	6	3	0	2	17	2	20	39	3	7	6	6
raw foo jook	**723**	**135**	**926**	**71**	**2**	**57**	**35**	**6**	**26**	**331**	**41**	**308**	**1160**	**184**	**1317**	**2661**	**67**	**5**	**2**	**26**
range ($n = 4$)	531–971	100–187	703–1171	35–96	0–3	8–91	19–62	0–9	16–35	296–405	30–60	287–351	946–1496	140–259	1105–1625	2190–3380	61–72	2–9	2–3	24–28
interitem SD	193	39	208	26	2	35	20	4	8	50	13	29	244	53	226	522	5	3	0	2
cooked foo jook	**137**	**26**	**190**	**11**	**0**	**11**	**2**	**0**	**5**	**82**	**8**	**81**	**232**	**35**	**288**	**554**	**64**	**4**	**1**	**31**
range ($n = 4$)	112–143	20–34	164–240	4–19	0–2	4–18	0–5		35862	51–119	7–8	45–116	187–310	29–44	247–373	463–727	58–70	2–7	1–2	20–37
interitem SD	31	6	37	6	0	6	2	0	2	29	1	29	54	7	58	118	5	2	0	8
raw soybeans[f]	**257**	**35**	**251**	**604**	**79**	**481**	**0**	**0**	**3**	**52**	**0**	**28**	**913**	**114**	**763**	**1789**	**31**	**65**	**0**	**4**
range ($n = 4$)	227–295	31–41	234–282	506–806	46–129	414–566			0–5	42–62		19–37	797–1163	76–168	675–885	1548–2176	28–33	61–68	0	3–6
interitem CV (%)	11	17	9	23	48	16	0	0	116	22	0	30	19	38	12	15	9	5	118	24
interitem SD	28	6	22	137	38	77	0	0	3	12	0	8	169	43	93	274	3	3	0	1
cooked soybeans[f]	**227**	**33**	**218**	**382**	**57**	**293**	**2**	**0**	**10**	**38**	**2**	**21**	**649**	**92**	**541**	**1282**	**37**	**57**	**0**	**5**
range ($n = 4$)	179–298	23–38	175–289	314–446	34–114	241–393	0–8	-	7–13	31–52	0–2	16–25	577–803	61–152	436–720	1045–167	34–39	55–61		4–6
interitem SD	55	7	49	56	38	75	4	0	3	9	2	4	118	41	125	274	2	3	0	0
fermented tofu	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**250**	**49**	**288**	**250**	**49**	**288**	**587**	**0**	**0**	**0**	**100**
range ($n = 2$)										232–269		262–313	232–269		262–313	123–202				13–25
interitem SD										27	0	36	27	2	36	62	0	0	0	0
detection limit[g]	**0.5**	**0.5**	**0.2**	**0.5**	**0.5**	**0.2**	**0.5**	**0.5**	**0.2**	**0.5**	**0.5**	**0.2**	**1.8**	**2.1**	**0.9**	**3.8**	**1.2**	**1.2**	**1.2**	**1.2**
intra-assay CV[h] (%)	6	10	5	7	17	8	11	7	12	6	14	5	5	8	5	5	7	11	10	8

[a] Mean levels in foods expressed in aglycon units determined by duplicate analysis (give in boldface type); cooking time for all items was boiling for 5 min except for soybeans (10 min). Abbreviations are listed in Figure 1. [b] Rounding may lead to a total of more or less than 100%. [c] n = number of different food sources; 0 = below detection limit. [d] Interitem SD = standard deviation between different food items. [e] Tau kwa = pressed tofu; tau pok = fried tau kwa; foo jook = skimmed dry supernatant obtained by boiling soybean meal (soybean curd sticks). [f] Entire pod (seed + hull). [g] Detection limit given in mg/kg as determined with a signal-to-noise ratio of 3. [h] Mean intra-assay coefficient of variation for duplicate analysis of all 42 items.

Table 2. Mean Isoflavone Levels and Patterns in Traditional Asian Soy Foods from Hawaii[a]

	mg/kg																%[b]			
	D	GLY	G	D-Mal	GLY-Mal	G-Mal	D-Ac	GLY-Ac	G-Ac	DE	GLYE	GE	total DE	total GLYE	total GE	total isoflavones	glucosides	malonyl glucosides	acetyl glucosides	aglycons
firm tofu	**62**	**14**	**65**	**77**	**13**	**65**	**2**	**0**	**2**	**29**	**3**	**23**	**169**	**31**	**155**	**355**	**37**	**41**	**1**	**21**
range ($n^c = 3$)	20–106	6–24	19–101	24–121	4–24	20–97	0–3	0–1	1–3	26–3	1–4	15–27	73–256	14–54	67–217	154–497	30–44	31–46		9–39
interitem SD[d]	43	9	42	49	10	40	1	0	2	3	2	7	92	20	78	179	7	9	0	16
soft tofu	**51**	**14**	**53**	**67**	**13**	**60**	**2**	**0**	**2**	**18**	**3**	**14**	**137**	**30**	**129**	**297**	**40**	**44**	**1**	**15**
range ($n = 3$)	28–73	7–22	25–72	23–100	5–21	18–77	1–2	0–1	1–3	10–23	1–4	6–20	73–185	16–47	61–164	151–377	38–42	31–53	1–2	4–28
interitem SD	23	7	25	39	8	36	0	0	1	7	2	8	58	16	59	127	2	12	0	12
raw tau kwa[e]	**88**	**27**	**115**	**86**	**20**	**80**	**5**	**1**	**5**	**58**	**9**	**50**	**238**	**57**	**250**	**545**	**42**	**34**	**2**	**22**
range ($n = 3$)	75–105	14–39	110–124	69–113	18–23	65–108	5–6	1–2	4–6	54–65	6–13	39–62	203–259	39–77	230–276	523–574	39–43	30–42		18–25
interitem SD	15	13	8	24	3	24	0	0	0	6	3	11	30	19	24	26	3	7	0	4
cooked tau pok	**33**	**5**	**49**	**11**	**1**	**15**	**14**	**2**	**23**	**46**	**5**	**51**	**103**	**14**	**138**	**254**	**35**	**11**	**15**	**39**
range ($n = 3$)	19–44	3–6	23–62	7–13	1–2	9–21	8–17	1–2	11–31	18–69	3–8	22–81	52–134	18–	65–195	125–347	30–36	10–14	14–16	34–45
interitem SD	13	2	22	3	0	6	5	0	11	26	2	30	44	5	67	116	4	2	0	6
cooked foo jook	**46**	**8**	**69**	**8**	**1**	**8**	**3**	**0**	**4**	**44**	**6**	**25**	**101**	**16**	**105**	**223**	**55**	**7**	**3**	**34**
range ($n = 3$)	18–82	8–14	62–109	3–17	0–4	3–15	1–4	0–1	3–6	24–83	3–12	12–40	48–186	6–31	66–170	120–386	47–67	4–9	3–4	26–35
interitem SD	32	6	37	8	2	6	2	0	2	33	5	14	74	13	56	143	10	3	0	8
cooked soybeans	**233**	**33**	**253**	**148**	**24**	**135**	**17**	**1**	**18**	**20**	**3**	**20**	**418**	**61**	**425**	**904**	**57**	**34**	**4**	**5**
range ($n = 3$)	207–258	27–36	207–321	128–1651	23–26	99–166	15–23	1–2	15–24	15–25	2–3	15–25	396–438	56–64	364–536	827–1037	53–62	29–39	3–5	4–5
interitem SD	25	5	60	18	2	34	5	0	5	5	0	5	21	4	96	116	4	5	0	0
cooked green soybean seeds[f]	**16**	**13**	**20**	**57**	**30**	**48**	**2**	**1**	**2**	**2**	**2**	**1**	**76**	**46**	**71**	**193**	**24**	**70**	**2**	**3**
range ($n = 3$)	9–27	11–14	9–31	42–70	26–35	31–58	1–3	-	1–3	1–3	1–3	1–2	54–103	42–51	42–91	147–236	21–31	64–73	2–3	2–4
interitem SD	10	1	11	14	4	15	0	0	0	0	0	0	25	5	26	44	5	5	0	0
cooked soy sprouts	**11**	**1**	**19**	**38**	**5**	**46**	**0**	**1**	**0**	**0**	**0**	**0**	**50**	**8**	**67**	**125**	**24**	**72**	**2**	**1**
range ($n = 2$)	8–14		12–27	30–46	4–6	35–58			0–1				39–61	6–9	47–86	93–156	22–27	71–74		1–2
interitem SD	4	0	11	12	1	16	0	0	0	0	0	0	16	2	28	45	3	2	0	0
soy milk	**21**	**6**	**24**	**29**	**8**	**22**	**0**	**0**	**0**	**7**	**0**	**5**	**58**	**15**	**52**	**125**	**46**	**41**	**2**	**11**
range ($n = 3$)	15–29	3–8	15–34	3–55	1–15	2–38	0–1		0–1	5–9		2–6	40–75	5–23	22–58	87–153	23–75	6–70	1–3	5–16
interitem SD	7	3	10	26	7	18	0	0	0	2	0	2	17	10	9	34	27	33	1	5
miso	**23**	**2**	**36**	**11**	**2**	**11**	**4**	**0**	**4**	**60**	**12**	**64**	**98**	**16**	**115**	**230**	**30**	**12**	**3**	**55**
range ($n = 3$)	15–35	1–3	18–68	3-21	0–3	4–20	1–10		1–10	15–119	3–29	25–124	35–171	4–36	51–210	90–418	23–40	8–24	1–5	47–65
interitem SD	10	1	28	9	1	8	5	0	5	53	15	52	69	17	84	169	9	10	2	9
natto ($n = 1$)	**342**	**168**	**395**	**20**	**0**	**27**	**28**	**8**	**19**	**163**	**23**	**49**	**553**	**198**	**490**	**1241**	**73**	**4**	**4**	**19**
mean intra-assay CV[g] (%)	3	7	3	8	12	7	3	20	4	2	5	3	3	6	3	2	3	2	7	5

[a] Levels in foods as consumed and expressed in aglycon units determined by duplicate analysis (give in boldface type); cooking time was boiling for 3 min for soy sprouts, 5 min for tau pok, 7 min for soybeans, 10 min for foo jook, and 60 min for dry soybean seeds. Abbreviations are listed in Figure 1. [b] Rounding may lead to a total of more or less than 100%. [c] n = number of different food sources; 0 = below detection limit (see Table 1). [d] Interitem SD = standard deviation between different food items. [e] Tau kwa = pressed tofu; tau pok = fried tau kwa; foo jook = skimmed dry supernatant obtained by boiling soybean meal (soybean curd sticks). [f] Frozen raw material. [g] Mean intra-assay coefficient of varitaion for duplicate analysis of all 29 items.

Table 3. Isoflavone Levels and Patterns in Western Soy Foods from Hawaii[a]

	mg/kg																%[b]			
	D	GLY	G	D-Mal	GLY-Mal	G-Mal	D-Ac	GLY-Ac	G-Ac	DE	GLYE	GE	total DE	total GLYE	total GE	total isoflavones	glucosides	malonyl glucosides	acetyl glucosides	aglycons
soy protein drinks																				
Garcinia-max diet shake, chocolate	71	22	101	72	32	104	15	2	20	11	5	11	170	60	236	465	42	45	8	6
Light and Fit energy shake	207	32	367	100	40	177	23	4	26	43	30	46	373	106	617	1097	55	29	5	11
Slim and Trim diet shake	30	6	47	36	19	59	14	2	25	6	19	11	87	47	142	277	30	41	15	13
Down-to-Earth	114	23	177	167	84	324	50	16	62	40	19	33	371	143	596	1110	28	52	12	8
Spiru-tein, cappuccino	51	10	74	62	79	111	22	4	30	8	30	11	143	123	226	492	28	51	11	10
Spiru-tein, chocolate peanut butter	60	11	72	58	63	102	26	4	38	9	33	13	154	112	224	490	29	46	14	11
Super Green Pro-96	77	33	164	189	74	300	n.d.	12	51	33	2	32	299	121	547	967	28	58	7	7
TakeCare, plain	213	61	341	360	77	399	45	6	70	23	5	34	641	149	845	1634	38	51	7	4
mean ($n^c = 8$)	**103**	**25**	**168**	**131**	**59**	**197**	**28**	**6**	**40**	**22**	**18**	**24**	**280**	**108**	**429**	**816**	**35**	**47**	**10**	**9**
interitem SD[d]	146	43	251	295	33	256	23	5	33	16	3	20	417	50	500	921	11	10	3	1
soy milk																				
chocolate	23	5	28	1	0	2	0	0	0	0	0	0	24	5	31	60	94	5	0	0
Pacific	5	2	4	0	0	0	0	0	0	0	0	0	5	2	4	11	97	2	0	1
mean ($n = 2$)	**14**	**3**	**16**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**15**	**3**	**17**	**35**	**95**	**3**	**0**	**1**
interitem SD	5	1	4	0	0	0	0	0	0	0	0	0	5	1	4	11	2	1	0	0
soy cheeses																				
jalapeno Monterey Jack, fat free	23	3	35	43	11	39	9	1	12	2	6	1	78	21	88	188	33	50	12	5
Veggy Singles, Swiss alternative	12	5	12	3	0	31	0	4	0	3	7	1	18	17	44	79	36	43	6	15
Veggy Singles. American alternative	4	8	5	6	0	14	0	3	5	7	6	0	17	17	24	58	30	34	12	24
Nu Tofu mozzarella	10	13	12	9	2	0	0	0	3	3	6	3	21	21	19	61	58	17	5	20
Nu Tofu Cheddar	10	14	11	8	0	1	0	0	1	2	5	0	20	19	13	52	66	18	2	14
soy Gourmet Mozzarella style	0	0	3	2	0	0	0	0	0	0	3	0	3	3	3	9	42	27	0	30
Soy Singles	29	17	66	32	25	53	23	11	22	14	0	8	98	53	150	301	37	37	18	8
mean ($n = 7$)	**13**	**9**	**21**	**15**	**5**	**20**	**5**	**3**	**6**	**4**	**5**	**2**	**37**	**21**	**49**	**107**	**43**	**32**	**8**	**16**
interitem SD	23	12	73	35	44	57	43	15	29	15	0	11	98	37	161	286	12	14	14	4
soy yogurts																				
White Wave dairyless vanilla	115	45	146	124	41	131	52	3	4	18	5	17	309	94	298	701	44	42	8	6
Nancy's soy yogurt	88	22	112	57	15	54	10	2	8	76	4	67	230	43	240	513	43	24	4	29
mean ($n = 2$)	**101**	**34**	**129**	**90**	**28**	**92**	**31**	**3**	**6**	**47**	**4**	**42**	**270**	**69**	**269**	**607**	**43**	**33**	**6**	**17**
interitem CV (%)	19	47	19	52	67	59	94	40	39	87	13	84	21	53	15	22	0	38	53	94
vegetarian burgers																				
Garden Veggie	0	0	0	2	11	0	0	0	0	0	5	0	2	16	0	17	0	73	0	27
Natural Touch vegan burger	4	4	0	3	2	3	17	4	8	3	3	0	27	12	12	51	16	15	56	12
Boca Burger	8	14	12	5	0	6	10	0	12	0	2	0	23	17	30	70	49	16	33	3
mean ($n = 3$)	**4**	**6**	**4**	**3**	**4**	**3**	**9**	**2**	**7**	**1**	**3**	**0**	**17**	**15**	**14**	**46**	**22**	**35**	**30**	**14**
interitem SD	8	17	20	3	0	5	9	1	11	0	0	0	18	3	32	40	57	15	31	2
soy flours																				
Arrowhead	455	213	357	907	326	727	77	26	48	56	28	22	1496	593	1155	3243	32	60	5	3
DowntoEarth	204	103	239	488	194	621	12	6	9	12	3	6	715	306	876	1897	29	69	1	1
mean ($n = 2$)	**330**	**158**	**298**	**697**	**260**	**674**	**44**	**16**	**29**	**34**	**15**	**14**	**1106**	**449**	**1015**	**2570**	**30**	**65**	**3**	**2**
interitem SD	110	51	67	207	70	69	12	5	9	11	3	5	357	138	170	703	2	6	1	0

soybean seeds																				
raw	413	19	768	26	7	67	288	49	436	22	0	45	750	75	1317	2142	56	5	36	3
roasted	347	41	398	35	8	55	377	25	389	72	0	57	830	74	899	1803	44	5	44	7
mean (n = 2)	**380**	**30**	**583**	**31**	**8**	**61**	**332**	**37**	**413**	**47**	**0**	**51**	**790**	**75**	**1108**	**1973**	**50**	**5**	**40**	**5**
interitem SD	43	22	179	7	0	8	71	11	32	54	0	9	60	0	240	219	8	0	6	4
soy supplements																				
genistein food supplement	2061	2351	610	2069	1092	322	2328	1273	523	248	63	46	6705	4779	1502	12986	39	27	32	3
soy super complex	1201	1981	418	798	410	179	1858	1465	504	193	62	51	4050	3918	1151	9119	39	15	42	3
vegetarian enzyme complex	26	50	12	18	23	17	46	38	11	6	2	0	96	113	41	249	35	24	38	3
mean (n = 3)	**1096**	**1460**	**346**	**962**	**508**	**173**	**1411**	**926**	**346**	**149**	**42**	**33**	**3617**	**2936**	**898**	**7451**	**38**	**22**	**37**	**3**
interitem SD	25	42	10	20	24	15	39	32	10	5	1	0	88	95	35	219	2	6	5	0
mean intra-assay CV[e] (%)	7	17	9	9	13	3	9	23	20	11	30	17	4	13	5	5	3	5	2	6

[a] Levels in foods as consumed and expressed in aglycon units determined by duplicate analysis (given in boldface type); cooking time was boiling 3 min for soy sprouts, 5 min for tau pok, 7 min for soybeans, 10 min for foo jook, and 60 min for dry soybean seeds. [b] Rounding may lead to a total of more or less than 100%. [c] n = number of different food sources; 0 = below detection limit (see Table 1). [d] Interitem SD = standard deviation between different food items. [e] Mean intra-assay coefficient of varitaion for duplicate analysis of all 29 items.

isoflavone aglycons through bacterial hydrolysis of the conjugates (Wang and Murphy, 1994a; Coward et al., 1993; Franke et al., 1998b). Variable conjugate ratios were found in other food groups, which may be due to differential handling, processing, storage, and exposure to bacterial enzymes. Our observations are consistent with earlier findings on factors that influence the isoflavone conjugation pattern: the predominating glucosyl malonates in soy plants (Kudou et al., 1991) will be converted easily as a result of heat, water, and exposure to bacterial enzymes that form acetyl glucosides by decarboxylation of the malonyl moiety, glucosides by hydrolysis of the acyl group, and aglycons by hydrolysis of the sugar moiety, respectively (Barnes et al., 1994a; Wang and Murphy, 1994a; Franke and Custer, 1994).

There was no consistent pattern when mean isoflavone amounts were compared between the same foods groups from Singapore and Hawaii. Our data indicated that mean isoflavone levels of raw tau kwa (pressed tofu) and cooked tau pok (fried pressed tofu) were smaller by a factor of ~2 in Singapore items versus Hawaii items, and the opposite was true for cooked foo jook (soybean curd sticks) and fermented items, whereas other foods did not show any significant difference. A detailed analysis was very difficult in this respect due to the large variability within the food groups of each geographical area, and no conclusions could therefore be drawn regarding patterns of isoflavone contents as a function of geographical area. Isoflavone levels will depend ultimately on the individual processing and handling of the food item. The small intra-assay variability underscored the validity of the analytical procedure applied and could not explain the large difference we observed between or within food groups.

Soy protein drinks and soy supplements have become increasingly popular in North America in recent years and are a major source of isoflavone exposure in Caucasian populations. Although serving sizes were very similar, the composition of soy protein drinks varied in protein, carbohydrate, energy, and total isoflavone amount by factors of 4, 7, 2, and 5, respectively (Table 4). Three of 8 items analyzed contained fat in amounts of 1−3 g per serving, which may be of importance due to the presence of vitamin E in the lipophilic compartment. We found α-, γ-, and δ-tocopherol levels in fat-containing soy drinks ranging from 1 to 7 μg/g applying HPLC techniques developed for plasma micronutrient analysis (Franke et al., 1993). Tocopherol patterns in these items were observed to be typical for those in soy oils (Cooney et al., 1997), indicating that the lipids found in these drinks stem from the soy protein preparation rather than from additives. Most nonfat items were found to contain α-tocopherol acetate (60−985 μg/g) as an additive.

For compliance-related purposes, in future intervention trials soy protein drinks were tested regarding various criteria including acceptability, flavor, and willingness to consume again using a score scale from 1 (worst) to 5 (best) among 33 volunteers. We found large differences between the acceptance of these drinks (mean score = 1.4−3.5), with two items (Slim and Trim, TakeCare) being favored unanimously, whereas the other drinks did not qualify for use in intervention studies. According to our findings, one serving of soy protein drink results in total soy isoflavone exposure similar to a typical 4 oz serving size (Messina et al.,

Table 4. Composition of Protein Drinks[a] and Soy Supplements[b]

	serving size (g)	fat (g)	protein (g)	carbo-hydrate (g)	energy (cal)	total isoflavones mg	total isoflavones mg/g of protein	total isoflavones mg/kcal
soy potein drink (powder)								
Garcinia-max diet shake, chocolate	27	0	6	18	100	12.6	2.1	125.6
Light and Fit energy shake	28	0	14	8	90	30.7	2.2	341.2
Slim and Trim diet shake	28	3	15	30	207	9.1	0.6	44.1
Down-to-Earth	28	1	24	0	110	31.1	1.3	282.4
Spiru-tein, cappuccino	32	0	14	11	100	15.7	1.1	157.4
Spiru-tein, chocolate peanut butter	31	0	14	11	99	15.2	1.1	153.4
Super Green Pro-96	28	0	23	0	100	27.1	1.2	270.7
TakeCare, plain	29	1	20	4	100	47.4	2.4	473.9
soy supplements (pills)								
genistein food supplement						16973.3		
Soy Super Complex						9972.0		
vegetarian enzyme complex						204.5		

[a] Amount per serving. [b] Amount per pill.

1994) of traditional soy foods. Although total energy intake should be considered, this demonstrates the usefulness of soy drinks compared to traditional soy foods to be used in intervention trials.

In conclusion, the results presented summarize the isoflavone content and conjugation pattern of foods commonly consumed in Singapore and Hawaii. Our data are in very good agreement with recent reports on isoflavone content and conjugation patterns in soy foods consumed elsewhere (Coward et al., 1993; Barnes et al., 1994a; Wang and Murphy, 1994a; Franke et al., 1995). The original conjugation pattern of soy foods with predominating malonates (Kudou et al., 1991) changed as a function of treatment by heat and bacterial fermentation. Acetyl glucosides were formed in soy items when fried in oil. Boiling soybeans resulted in a decrease in glucosyl malonates and a concurrent increase in glucosides, with minor losses observed in total isoflavone content, probably by leaching of the polar analytes into the water. Fermented items contained predominantly aglycons. Although isoflavone levels in soy foods depend on environmental, genetic, harvesting, and processing conditions, it is important to note that isoflavone levels reported from the same food groups but from different laboratories may vary due to different analytical techniques applied. It has to be emphasized that instrument calibration, accurate determination of stock solution concentration by absorbance readings, and quality assurance using internal and external standards are urgently required for the production of reliable data (Song et al., 1998). A highly significant correlation between urinary isoflavone excretion and soy consumption was discovered in different ethnic groups, suggesting that urinary isoflavone measurements can serve as an excellent biomarker for soy intake (Seow et al., 1998; Maskarinec et al., 1998). Urinary isoflavone excretion was shown to be higher in populations with low breast cancer risk (Adlercreutz et al., 1991) and higher in controls relative to breast cancer cases in multiethnic (Ingram et al., 1997) and Asian (Zheng et al., 1998) populations, supporting the hypothesis of a cancer protective effect of soy and/or isoflavone exposure. Large variability of isoflavone content between and also within certain food groups needs to be considered when food composition databases are used in future epidemiologic studies evaluating the effects of isoflavone consumption.

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