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Solvent extraction selection in the determination of isoflavones in soy foods

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Abstract

Acetonitrile is superior to acetone, ethanol and methanol in extracting the 12 phytoestrogenic soy isoflavone forms found in foods. At 53% organic solvent in water, raw soy flour, tofu, tempeh, textured vegetable protein and soy germ were evaluated for isoflavone extraction efficiency. The efficiency of acetonitrile extraction was demonstrated in mass balance evaluations of toasting of soy flour and soymilk heating.

Keywords: Soy foods; Isoflavones

1. Introduction

Soy isoflavones are a phytochemical group of intense interest due to their associations with a variety of health protective effects including reducing the risk of cardiovascular disease, lowering rates of prostate, breast and colon cancers, and improving bone health among many other claims [1]. There are 12 chemical forms of isoflavones in soybeans and soy foods. Genistein, daidzein and glycitein are the aglucons with three possible glucoside forms, a β-glucoside, a 6″-*O*-malonyl-glucoside and a 6″-*O*-acetyl-glucoside, of the three aglucons [2]. The concentrations of these forms will vary in soy foods depending upon the type of processing that has occurred [3]. The evidence in the literature suggests that the biological effects of soy isoflavones do not depend upon the glucoside form [4]. The apparent bioavailability does differ with glycitein>daidzein>genistein [5]. One study did report higher absorption of aglucons in fasted rats suggesting absorption of aglucons may be faster in an empty stomach [6]. However in a food, this situation would rarely be the case.

In raw, unprocessed soybeans, the 6″-*O*-malonyl forms predominate. These malonyl forms will decarboxylate with time after extraction. We have reported a 0.2–0.3 mol% per h conversion of malonyl forms to β-glucosides at room temperature [2]. Maintenance of autosampler temperatures at 5 °C has been used to minimize this phenomenon [7]. This instability of the malonyl forms is one of the reasons the commercial availability of analytical standards of the malonyl forms has been difficult to achieve. This instability also necessitates prompt analysis of extracts within about 10 h of extraction to minimize artifact formation [2].

In soy foods that are processed with water, the native soy β-glucosidases will be active prior to any heat treatment generating the aglucons as in soaking

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of soybeans prior to processing into soymilk [3]. Heat treatment of moist soy foods, in soymilk and tofu production, will tend to generate β-glucosides at the expense of the malonyl forms. 6″-*O*-Acetyl forms are only observed in soy foods that have undergone a dry heat treatment such as in toasting of hexane-extracted soy flakes after soybean oil extraction and in extrusion of soy protein mixtures. Fermentation of soy foods to produce miso, natto and tempeh results in the production of aglucons from the microbial β-glucosidases. Therefore, accurate analytical methods that allow for quantification of all isoflavone forms will yield a precise picture of the food's processing history and in some cases, may be used for soybean variety identification.

Soy isoflavones have been analyzed by HPLC since the early 1980s. Although isocratic HPLC was attempted initially [8], the variation in the hydrophobicities of the isoflavone forms dictated gradient HPLC would be the predominant mode. Murphy [9] attempted to determine the optimum extraction protocol for the various isoflavone forms that were known at that time by comparing methanol, ethanol, acetone and acetonitrile (AcCN), with and without addition of hydrochloric acid. Most other researchers used 80% methanol as their preferred extraction solvent [10]. Farmakalidis and Murphy [11] demonstrated that 80% AcCN was superior to 80% methanol in extracting the acetyl-glucosides from defatted soy flakes. Recently, Griffith and Collison [12] have compared 80% methanol and 60% AcCN with and without acid for extraction of soy protein isolate and soy supplements and reported their results were similar to Murphy et al. [2]. Murphy et al. [2] extracted a variety of soy foods with acidified AcCN with varying amounts of water that were optimized for each food type. While most soyfoods were extracted efficiently with 53% ACN, these authors noted that each soy food type must be initially evaluated to determine proper ACN/water ratios. Recently, Klump et al. [13] have proposed an Association of Official Analytical Chemists' method for soy isoflavone analysis that requires only six standards. The extracted samples are treated with heat and alkaline conditions to convert all the 6′-*O*-acetyl- and 6″-*O*-malonyl-glucosides to β-glucosides. The six standards, three aglucons and three β-glucosides are commercially available. But this method does not allow quantitation of differences in the converted forms, the 6′-*O*-acetyl- and 6″-*O*-malonyl-glucosides.

There has not been a systematic evaluation of solvent extraction efficiencies for soy foods containing different distributions of the isoflavone forms. The intent of this study is to evaluate the efficiency in five soy foods and demonstrate the value of this technique in assessing the effects of processing on isoflavone distribution in soy foods.

2. Experimental

2.1. Solvent choices

Acetonitrile, acetone, ethanol and methanol were evaluated for their efficiency in extracting isoflavones from five different food matrices. Extractions were carried out with and without the addition of 0.1 N HCl. The solvents were used at 53% organic phase to water. This percentage organic phase was established using 10 ml organic solvent plus 2 ml water or 2 ml of 0.1 N HCl plus 7 ml of water. We have previously established that this additional 7-ml volume of water is the most efficient in maximizing the isoflavone extraction in the dry or freeze-dried soy food matrix for the foods evaluated here [2]. However, not all soy foods will require this much water while some foods will require more [2]. Soybean flour, texturized vegetable protein (TVP), tofu, tempeh (both purchased locally in retail stores) and soy germ (Schouten USA, Minneapolis, MN, USA) were used as representatives of soy isoflavone distributions found in typical retail soy foods. Two grams of soy food, except for soy germ where 0.2 g was used, were extracted with the appropriate solvent and processed in the same manner as in Murphy et al. [2]. Briefly, foods were extracted as 2-g samples, either as is, if food could be ground in a coffee mill to a free-flowing powder, or as a freeze-dried sample followed by grinding in a coffee mill, in 10 ml of acetonitrile, 2 ml of 0.1 N HCl or 2 ml of water and an additional 7 ml of water, the optimum for these food matrices, in a 125-ml screw-top Erlenmeyer flask with stirring for 2 h at room temperature. The extracts were filtered and taken to dryness on a rotary evaporator at $<30\,^{\circ}C$. The

residues were dissolved in 80% HPLC-grade methanol. An aliquot was filtered through a 0.45-μm PTFE filter (Alltech Associates, Deerfield, IL, USA) and analyzed by HPLC within 10 h of extraction to minimize malonylglucoside to glucoside interconversion. All organic solvents (HPLC grade) and acid (AR grade) were from Fisher Scientific (St Louis, MO, USA).

2.2. HPLC conditions

The HPLC analysis method of Murphy et al. [14] was used to separate and quantify the individual isoflavone moieties. Peak areas were evaluated using molar extinction coefficients we have previously reported for all 12 soy isoflavone forms [15] and shown in Table 1.

2.3. Soy flour toasting

To simulate extrusion heat conditions, soy flour was heated in a convection oven at 80 and 150 °C for 4 h. Duplicate samples at each temperature were removed periodically and analyzed for isoflavone distribution. The toasting experiment was repeated three times at the two temperatures.

2.4. Soy milk production

Soy milk was prepared as in Wang and Murphy [6] without heating. Immediately, the filtered soymilk was heated to 80 °C for 3 h. Duplicate samples were removed periodically, immediately freeze-dried and analyzed for isoflavone distribution. The soymilk heating experiments were replicated three times.

2.5. Statistics

All extraction experiments were performed in quadruplicate. All soy flour toasting and soy milk heating were replicated three times. ANOVA was carried out as a means to determine differences that were significant at $\alpha = 0.5$ using the SAS program (version 6.03, 1995, Cary, NC, USA).

3. Results and discussion

The food matrices selected for these experiments have very different isoflavone form distributions (Table 2). Soy flour contains predominantly the malonyl-β-glucosides equaling about 80% mol isoflavone mass, about 15% as the β-glucoside and

Table 1
Molecular masses, ultraviolet absorbance maxima and molar extinction coefficients of soybean isoflavones

Compound	Molecular mass	λ_{max} (nm)	Extinction coefficient (ε)
Daidzein	254	249[a]	31 563[a]
Daidzin	416	249[a]	26 830[a]
Acetyldaidzin	458	256[a]	29 007[a]
Malonyldaidzin	502	258[a]	26 830[a]
Genistein	270	263[a]	35 323[a]
Genistin	432	263[a]	30 895[a]
Acetylgenistin	474	261[b]	38 946[a]
Malonylgenistin	518	260[a]	29 895[a]
Glycitein	285	256[c]	25 388[a]
Glycitin	447	259[d]	26 713[a]
Acetylglycitin	489	260[e]	29 595[a]
Malonylglycitin	533	260[e]	26 313[a,e]

[a] Values Murphy has determined or used with isolated in-house standards.
[b] Ref. [19].
[c] Ref. [20].
[d] Ref. [21].
[e] Taken from Glycitein.

Table 2
Efficiency of solvent extraction of isoflavones from soy flour, tofu, tempeh, texturized vegetable protein (TVP), and soy germ (μmol/g)

Isoflavone	± Acid	AcCN	Acetone	EtOH	MeOH	LSD
Soy flour						
Din		0.500[a]	0.478[b]	0.526[ab]	0.517[ab]	0.047
Gin		0.558[a]	0.509[b]	0.574[a]	0.530[b]	0.042
Gly		0.036[b]	0.114[ab]	0.116[ab]	0.121[a]	0.036
MDin	−>+	2.982[a]	3.090[a]	2.725[b]	3.018[a]	0.114
MGin	−>+	2.710[a]	2.695[a]	2.376[c]	2.566[b]	0.106
MGly		0.238[b]	0.261[a]	0.227[b]	0.233[a]	0.013
ADin		0.0[c]	0.0[c]	0.007[b]	0.015[a]	0.007
AGin		0.059[a]	0.023[a]	0.023[a]	0.023[a]	0.002
AGly		0.0	0.0	0.0	0.0	0.0
Dein		0.063[b]	0.067[b]	0.063[b]	0.087[a]	0.008
Gein		0.056[bc]	0.056[c]	0.063[b]	0.104[a]	0.007
Glein		0.0[a]	0.007[a]	0.0[a]	0.0[a]	0.014
Total Dein	−>+	3.591[a]	3.630[a]	3.327[b]	3.638[a]	0.134
Total Gein	−>+	3.407[a]	3.281[ab]	3.037[c]	3.222[b]	0.093
Total Glein		0.316[b]	0.382[a]	0.340[ab]	0.372[a]	0.046
Total isoflavone	−>+	7.314[a]	7.293[b]	6.704[c]	7.232[b]	0.078
Tempeh						
Din		0.320[a]	0.286[a]	0.300[a]	0.293[a]	0.041
Gin		0.896[a]	0.817[b]	0.840[ab]	0.794[b]	0.069
Gly		0.067[b]	0.067[b]	0.072[a]	0.074[a]	0.002
MDin		0.837[ab]	0.863[a]	0.725[c]	0.769[bc]	0.084
MGin	−>+	1.442[a]	1.413[a]	1.156[b]	1.195[b]	0.139
MGly		0.068[a]	0.064[ab]	0.058[b]	0.062[ab]	0.008
ADin		0.017[b]	0.033[a]	0.031[ab]	0.033[a]	0.015
AGin		0.034[a]	0.034[a]	0.074[a]	0.074[a]	0.011
AGly	+>−	0.020[a]	0.0[b]	0.016[ab]	0.0[b]	0.016
Dein		1.177[b]	1.157[b]	1.138[ab]	1.087[b]	0.067
Gein		1.741[a]	1.693[a]	1.667[a]	1.533[b]	0.081
Glein		0.102[a]	0.102[a]	0.095[b]	0.098[ab]	0.007
Total Dein		2.350[a]	2.339[a]	2.185[a]	2.177[a]	0.185
Total Gein		4.156[a]	3.996[ab]	3.737[bc]	3.596[c]	0.270
Total Glein	−>+	0.256[a]	0.232[b]	0.239[ab]	0.232[b]	0.021
Total isoflavone	−>+	6.762[a]	6.567[ab]	6.161[bc]	6.005[bc]	0.067
Tofu						
Din		0.452[a]	0.413[ab]	0.425[ab]	0.389[c]	0.053
Gin		0.810[a]	0.741[b]	0.734[b]	0.620[c]	0.067
Gly		0.123[a]	0.112[b]	0.112[b]	0.101[c]	0.009
MDin	−>+	0.751[ab]	0.813[a]	0.693[bc]	0.651[c]	0.074
MGin	−>+	1.060[a]	1.039[a]	0.834[b]	0.697[c]	0.091
MGly	−>+	0.116[b]	0.120[a]	0.101[b]	0.098[b]	0.008
ADin		0.0[c]	0.011[ab]	0.011[ab]	0.020[a]	0.015
AGin		0.051[a]	0.046[b]	0.042[bc]	0.040[c]	0.004
AGly	+>−	0.041[a]	0.014[b]	0.016[b]	0.0[c]	0.014
Dein		0.709[a]	0.705[a]	0.657[b]	0.579[c]	0.039
Gein		1.104[a]	1.081[a]	0.970[b]	0.793[c]	0.067
Glein	−>+	0.007[a]	0.0[ab]	0.0[b]	0.0[c]	
Total Dein	−>+	1.913[a]	1.937[a]	1.787[ab]	1.638[b]	0.138
Total Gein	−>+	3.019[a]	2.904[a]	2.581[b]	2.152[c]	0.189
Total Glein		0.428[a]	0.375[b]	0.358[b]	0.332[c]	0.021
Total isoflavone	−>+	5.360[a]	5.216[ab]	4.726[b]	4.122[c]	0.044

Table 2. Continued

Isoflavone	± Acid	AcCN	Acetone	EtOH	MeOH	LSD
TVP						
Din		1.135[a]	0.983[b]	1.154[a]	0.981[b]	0.087
Gin		1.264[a]	1.063[b]	1.236[a]	0.954[c]	0.095
Gly		0.374[a]	0.336[b]	0.383[b]	0.329[b]	0.034
MDin		1.757[a]	1.633[ab]	1.671[ab]	1.536[b]	0.191
MGin		1.624[a]	1.434[ab]	1.392[b]	1.241[b]	0.195
MGly		0.381[a]	0.371[a]	0.319[ab]	0.298[b]	0.064
ADin	+>−	0.777[a]	0.555[c]	0.668[b]	0.498[c]	0.068
AGin		0.709[a]	0.605[b]	0.658[ab]	0.504[c]	0.055
AGly	+>	0.256[a]	0.162[ab]	0.155[ab]	0.047[b]	0.117
Dein		0.098[a]	0.091[b]	0.091[b]	0.079[c]	0.004
Gein		0.100[a]	0.078[b]	0.085[b]	0.067[c]	0.007
Glein		0.056[a]	0.053[ab]	0.049[b]	0.046[c]	0.004
Total Dein		3.764[a]	3.264[b]	3.583[a]	3.106[b]	0.307
Total Gein		3.693[a]	3.141[b]	3.407[ab]	2.767[c]	0.348
Total Glein	+>−	1.063[a]	0.919[b]	0.905[b]	0.905[c]	0.130
Total isoflavone	−>+	8.520[a]	7.324[c]	7.895[b]	6.778[d]	0.062
Soy germ						
Din		8.356[a]	8.065[a]	8.550[a]	8.820[a]	0.841
Gin		2.914[a]	2.838[a]	2.944[a]	3.005[a]	0.241
Gly		11.566[a]	11.226[a]	11.669[b]	11.924[a]	0.850
MDin		0.833[a]	0.735[b]	0.785[ab]	0.821[a]	0.078
MGin		0.280[a]	0.0[b]	0.288[a]	0.311[a]	0.048
MGly	+>−	1.109[b]	1.148[ab]	1.167[ab]	1.216[a]	0.103
ADin		6.417[a]	6.668[ab]	6.712[a]	6.511[ab]	0.290
AGin		2.392[ab]	1.960[a]	2.468[a]	2.380[b]	0.086
AGly		8.853[b]	9.231[a]	9.074[ab]	8.840[b]	0.356
Dein		1.101[a]	1.118[a]	1.118[a]	1.157[a]	0.091
Gein		0.493[ab]	0.511[a]	0.456[b]	0.478[ab]	0.044
Glein		2.572[a]	2.565[a]	2.614[a]	2.625[a]	0.137
Total Dein		16.709[a]	16.587[a]	17.161[a]	17.311[a]	0.766
Total Gein		6.078[a]	6.574[a]	6.156[a]	6.185[a]	0.333
Total Glein		24.070[a]	24.140[a]	24.495[a]	24.575[a]	1.109
Total isoflavone	−>+	46.857[a]	47.301[a]	47.812[a]	48.071[a]	0.739

Isoflavone concentrations with different superscripts are significantly different at $\alpha=0.05$ among solvents; $n=4$; +>− means extractions with added HCl are significantly greater than without HCl at $\alpha=0.05$; −>+ means extractions without added HCl are significantly greater than with HCl at $\alpha=0.05$. AcCN, acetonitrile; EtOH, ethanol; MeOH, methanol; LSD, least significant difference; Din, daidzin; Gin, genistin; Gly, glycitin; MDin, malonyldaidzin; MGin, malonylgenistin; MGly, malonylglycitin; ADin, acetyldaidzin; AGin, acetylgenistin; AGly, acetylglycitin; Dein, daidzein; Gein, genistein; Glein, glycitein; Total Dein, total daidzein; Total Gein, total genistein; Total Glein, total glycitein; Total isoflavone, sum of moles of all forms.

almost no detectable acetyl-β-glucosides and very small amounts of aglucons.

Tofu contains about 37% malonyl-β-glucoside, 25% β-glucoside and 37% aglucon. The aglucon fraction is generated due to the action of the soybean's native β-glucosidase during the 18-h soaking step in tofu and soymilk production. Modest decarboxylation of the malonyl forms occur due to the heating steps in soymilk and tofu production which follows mechanical disruption of the cotyledons.

Tempeh undergoes a different type of heat treatment compared to tofu and soymilk production. The resulting product contains higher concentrations of the aglucons compared to tofu and lower concentrations of the β-glucoside forms due to the action of probably both the native soybean β-glucosidase and

the fermentation organisms' β-glucosidases. The malonyl forms account for 35% of the isoflavone mol mass while the β-glucoside forms are reduced to about 17% compared to tofu. The aglucon forms account for 50% of the mol mass in tempeh. The intact, hydrated soybean seeds are autoclaved prior to inoculation with the tempeh fermentation organisms.

TVP undergoes drying to minimal moisture heat treatment and the profile of the isoflavone forms reflects this processing. Typically, a soy flour, soy concentrate or soy protein isolate will be used to produce a TVP. TVP contains significant concentrations of the acetyl forms probably due to the dry heat processing. The malonyl-β-glucosides account for 50% of the isoflavone forms with 32% β-glucoside forms and 20% acetyl-β-glucoside forms.

Soy germ represents a soy matrix containing isoflavone concentrations 6- to 10-fold higher than that found in other soy foods. The ratio of genistein/daidzein/glycitein forms is quite different from that found in soy foods derived principally from the cotyledons. The commercial soy germ product receives an intense dry toasting step. This heat processing step alters the isoflavone distribution even more than extrusion heating as observed in the TVP product. In toasted soy germ, the malonyl-β-glucosides are reduced to 5% of the total with the β-glucoside accounting for 50% of the distribution and the acetyl-β-glucoside representing 38% of the mol mass of isoflavones.

The data in Table 2 clearly indicate the four different solvents have different abilities to extract the different isoflavone forms. The hydrophobicity of the isoflavone forms is aglucon>acetyl-β-glucoside>malonyl-β-glucoside>β-glucoside based on their chromatographic behavior on reversed-phase columns in the presence of an acid in the mobile phase to protonate the malonyl forms [2]. AcCN clearly is the superior extraction solvent for these types of soy foods for most of the isoflavone forms. In soy flour, only methanol extraction yielded higher aglucon levels than AcCN but the aglucons represent only 3% of the total isoflavone mol mass. In the tofu, TVP and tempeh, with higher concentrations of the aglucons, AcCN was as efficient as the other solvents in aglucon yield. For these foods, 53% AcCN appears to be the best for evaluating total isoflavone concentrations and total daidzein, total genistein and total glycitein forms.

Acetone is almost as good as AcCN but not in all cases indicating the food matrix configuration may have an impact on the extractability of the isoflavone forms. Acetone is never better than AcCN, although it has a more hydrophobic ranking in the elutropic series on alumina [16]. In soy flour, tempeh, tofu and TVP, acetone extraction slightly underestimates the total individual isoflavone concentrations. Although not statistically significant, the total isoflavones extracted are always lower for acetone extraction compared AcCN. Acetone resulted in statistically significant lower extraction rates of almost all the isoflavone forms in TVP compared to AcCN suggesting an effect of the food matrix here rather than the isoflavone form.

Ethanol was a less efficient isoflavone extraction agent for these soy foods compared to AcCN and acetone. In ranking for total individual isoflavone and total isoflavone extraction, ethanol was third and fourth, respectively. In soy flour, ethanol was as efficient as AcCN and acetone in extracting the β-glucosides but less efficient in extracting the malonyl-β-glucosides compared to AcCN and acetone. Ethanol was less efficient in extracting the aglucons compared to methanol. For TVP, since ethanol was as efficient an extraction agent for the acetyl-β-glucosides as AcCN, the total isoflavone and total individual isoflavone extraction rates were greater for ethanol compared to acetone but were still lower than AcCN due to the lower rates of extraction of the other isoflavone forms.

Methanol was the least efficient solvent for extraction of different isoflavone forms found in these foods. For soy flour, methanol was as efficient as AcCN in extracting the isoflavones and was most efficient in extraction of the aglucons in this food. In the other three foods, methanol was significantly less efficient in extraction of the different isoflavone forms and total isoflavone yield. In tofu and TVP, methanol extraction underestimated the total isoflavone content because almost all the isoflavone forms were extracted at a lower rate than with the other solvents. In TVP, this lower extraction rate was most marked. Even the more hydrophobic aglucons were not extracted as well in TVP as they were by other solvents. The acetyl-β-glucosides were under-

estimated by 30–35% with methanol compared to AcCN. The β-glucoside and malonyl-β-glucosides were underestimated by about 25%. Overall, 53% methanol extraction underestimates the TVP isoflavone content by 20%.

The soy germ matrix appears as an outlier compared to the other soy foods evaluated here. Isoflavone concentration in soy germ is 6–10 times greater than in the other foods. Solvent extraction efficiencies were essentially not different for total isoflavone and total individual isoflavone forms as well as for most individual isoflavone species in this unique matrix. In contrast to the acetyl-β-glucosides in TVP that were poorly extracted, the same forms in soy germ are extracted with equal efficiency with methanol and AcCN but not as efficiently as with acetone.

Murphy et al. [2] recommended using 0.1 N HCl in the water phase of the isoflavone extraction media to maximize extraction. We have re-evaluated this recommendation with all four solvent combinations. It appears that addition of the acidifying agent has a mixed effect on improvement of extraction efficiency. For the majority of the individual isoflavone species and for total individual isoflavones in tempeh, TVP and soy germ, there is no statistical difference between using or not using 0.1 N HCl. Acetylgylcitin extraction in tempeh, tofu and tempeh and total isoflavones in tofu were greater with addition of the acid modifier. Extraction of malonyl-β-genistin in soy flour, tempeh, and tofu was greater without acid. Total daidzein and total genistein were greater in soy flour and tofu without added acid. Total isoflavones in TVP, tempeh, soy germ and soy flour were greater without added acid. The data present no systematic pattern for all foods nor for all isoflavone forms. Therefore to simplify the extraction protocol, it is probably better not to use acid in the extraction medium for these food matrices. However, other foods should be evaluated for the effect of acid addition to the extraction media in altering the isoflavone extraction.

To demonstrate the importance of selecting the proper extraction conditions for evaluating the effects of processing on soy isoflavone distribution, dry raw soy flour was heated at two temperatures to mimic effects of extrusion. Fig. 1 compares the effects of dry heat on soy flour isoflavones at 80 and 150 °C over a 4-h period. AcCN was used as the organic modifier in extraction of these samples. Total genistein (Fig. 1) represents the mathematical sum of moles of the individual forms of genistein found in this matrix. In order to show conservation of mass, the ability to accurately extract the different forms of genistein is critical. In the case of soy flour heated at 80 °C over 4 h (Fig. 1A), we observed very little change in isoflavone distribution. The distribution of the daidzein and glycitein forms was similar (data not shown). The total mass of isoflavones quantified remains the same over the heat-processing period. When the soy flour was heated at 150 °C (Fig. 1B), which may more closely approximate extrusion conditions, we observed major interconversion between the different forms of the genistein forms while total mol mass of genistein remained constant. 6″-*O*-Malonyl-genistin converts to genistin and 6″-*O*-acetyl-genistin in an equimole relationship. By 2 h, 2 μmol/g 6″-*O*-acetyl-genistin is formed while 2.5 μmol/g 6″-*O*-malonyl-genistin is lost. The β-glucoside, genistin, accounts for the other 0.5 μmol/g. A small mol fraction of genistein increases over the 4-h period. Over the time course of the reaction, the total mol mass of genistein is constant indicating the isoflavones are being extracted quantitatively. The daidzein and glycitein forms are transformed in a similar manner.

The effect of heat processing a liquid soy product on isoflavone distribution was evaluated in soy milk. Fig. 2 presents the data on the effect of moist heat on soy milk isoflavones held at 80 °C over 3 h. In this food matrix, the 6″-*O*-malonyl-genistin converts to the β-glucoside form (Fig. 3). Little 6″-*O*-acetyl-genistin is formed in this liquid matrix. There were very low (nmol) amounts of genistein formed in this liquid product suggesting 80 °C is sufficient to inactivate the native soybean glucosidases.

These data demonstrate a different mechanism for conversion of the isoflavone forms in liquid soy foods compared to the solid matrix. In the dry soy flour, we observed virtually no conversion of genistein forms at 80 °C. However at 150 °C in a dry food, the malonyl form appears to rapidly convert to the acetyl form. Although some authors have suggested that the malonyl forms convert directly to the acetyl forms and then to the β-glucosides during heat processing of food [17], no kinetic data have been

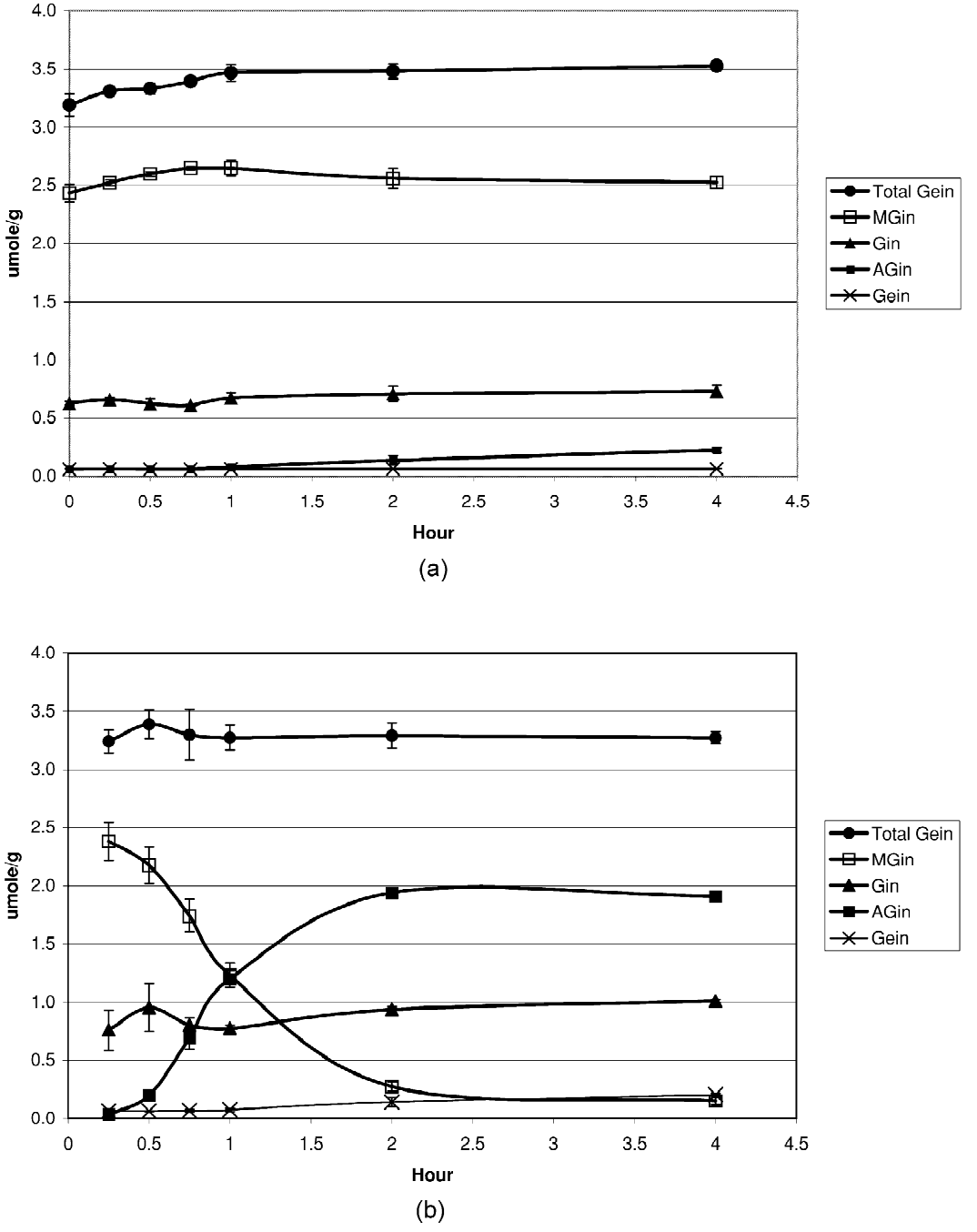


Fig. 1. (A) Effect of heating dry soy flour at 80 °C for 4 h on soy genistein distribution (μmol/g [as is basis]). Error bars are ±standard deviation. Total Gein=total mol genistein forms; MGin=malonylgenistin; Gin=genistin; AGin=acetylgenistin; Gein=genistein. (B) Effect of heating dry soy flour at 150 °C for 4 h on soy genistein distribution (μmol/g [as is basis]).

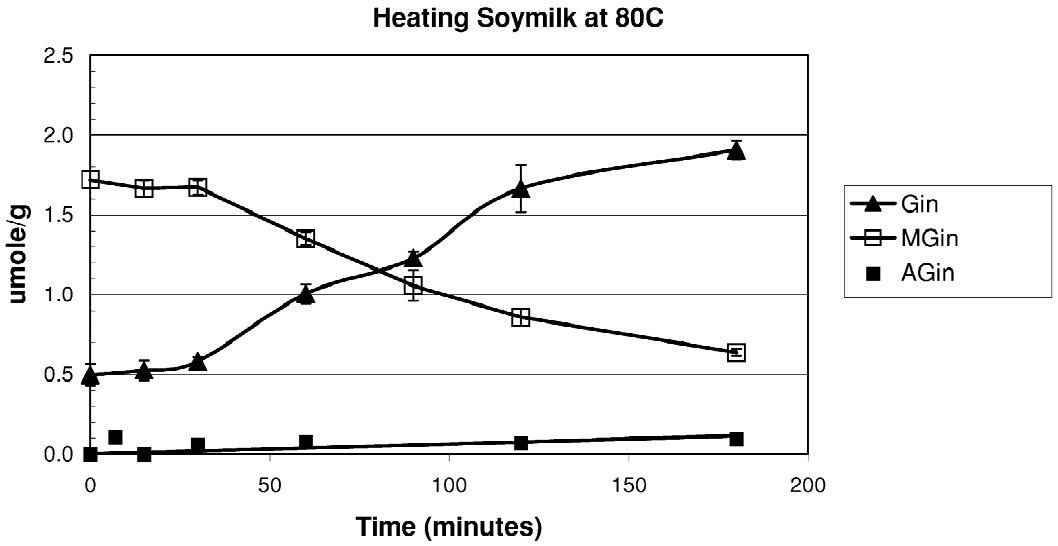
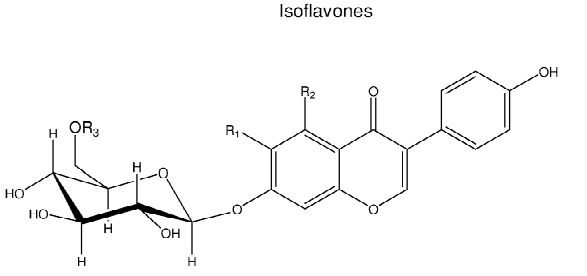


Fig. 2. Effect of heating soy milk at 80 °C for 3 h on soy genistein distribution (μmol/g [dry mass basis]).

reported to support this mechanism, only pre- and post-processing levels of the different isoflavone forms. This is the first report showing kinetic data that support different mechanisms for the interconversion of the isoflavone forms in liquid versus dry heat processing. Without using the proper extraction solvent, these observations could not be obtained. Mahungu et al. [18] attempted to evaluate the effect of extrusion on isoflavone form but could never account for total mass of isoflavone forms since they extracted their samples with 80% methanol. To study the mechanism of the effect of processing conditions on soy isoflavones in foods, care must be taken in using the best extraction system for the food matrix examined.



	R_1	R_2	R_3
Gin	H	OH	H
Gly	OCH_3	H	H
Din	H	H	H
MGin	H	OH	$OCCH_2COOH$
MGly	OCH_3	H	$OCCH_2COOH$
MDin	H	H	$OCCH_2COOH$
AGin	H	OH	OCH_3
AGly	OCH_3	H	OCH_3
ADin	H	H	OCH_3

Fig. 3. Structure of soy isoflavone glucosides.

3.1. Conclusions

Four solvent systems were evaluated for their efficiency to extract the 12 isoflavone forms found in five different soy matrices. AcCN appears to be the best choice for most foods. The importance in quantifying all isoflavone forms in a soy food is demonstrated by two studies on the effects of dry and moist heat treatment of soy.

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