Full Paper

Effects of Different Carriers on the Production of Isoflavone Powder from Soybean Cake

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Abstract: The objectives of this study were to use soybean cake as the raw material for the production of isoflavone powder and compare the effects of different carriers as well as drying methods on the powder quality. Results showed that with spray drying, a level of 40 % maltodextrin as carrier produced the highest yield (mass) of isoflavone powder, followed by 10 % gelatin and 1 % sodium alginate. However, a reversed trend was observed for the isoflavone content. With 1 % sodium alginate, freeze drying generated the greatest yield of isoflavone powder, followed by vacuum drying and spray drying. The isoflavone content also exhibited the same tendency. With poly-γ-glutamic acid (γ-PGA) as carrier, all six levels studied (0.57, 0.28, 0.14, 0.028, 0.014 and 0.003 %) were capable of forming powder containing high amounts of total isoflavone, which was comparable to that using 1% sodium alginate by freeze drying. Both high- and low-molecular-weight γ-PGA showed similar effects in terms of powder yield and isoflavone content.

Keywords: Isoflavone powder; soybean cake, γ-PGA, sodium alginate, maltodextrin.

Introduction

Isoflavones, a major class of flavonoids in soybeans, have received great attention in recent years because of their possible roles in the prevention of chronic diseases such as osteoporosis and hypercholesterolemia, as well as alleviation of postmenopausal syndromes [1-3]. Twelve isoflavones have been found in soybeans and are divided into four groups, i.e., malonylglucoside, glucoside,

acetylglucoside and aglycone, in which the glucoside forms are dominant [4, 5]. Soybean cake (defatted soybean meal), a by-product obtained during processing of soybean oil, was found to contain high amounts of isoflavone [6, 7]. Thus, it would be a great advantage to the health food industry if the isoflavone in soybean cake could be extracted and processed into powder.

Powder products are often processed by vacuum, freeze or spray drying, but the powder yield and quality can vary depending upon the drying methods and conditions used [8]. For instance, freeze drying is generally considered the most appropriate method to maintain high powder quality, but the capital cost is high. Conversely, spray drying is suitable for commercial powder production, however, this method requires control of several variables like both inlet and outlet temperatures, and the powder quality may be affected during high-temperature drying [9].

Different carriers like gelatin, gum arabic, maltodextrin, cellulose and glycerol monostearate have been used commercially to obtain nonsticky and free flowing powders [10, 11], but these carriers suffer a major drawback, namely, a large quantity has to be used for powder formation, which may increase production cost substantially.

Poly-γ-glutamic acid (γ-PGA), a nontoxic and biodegradable polymer consisting of many glutamic acid units linked by amide bonding between α-amino and γ-carboxylic acid groups, is produced from *Bacillus* sp. [12-14]. Both high-molecular-weight γ-PGA (H-γ-PGA) and low-molecular-weight γ-PGA (L-γ-PGA) ranging from 10,000 to 2 million Daltons have been commercialized and they are produced in different ionic forms, i.e., Ca, Na and H-forms. The applications of γ-PGA in removing heavy metals, toxic compounds and artificial dyes have been well documented [12-15], but the feasibility of using a low level of γ-PGA as carrier for powder formation remains unknown and requires further investigation. The objectives of this study were to isolate isoflavones from soybean cake and determine the effects of various drying methods and carriers, especially γ-PGA, on the yield of isoflavone powder product. The isoflavone content changes as affected by drying conditions were also investigated.
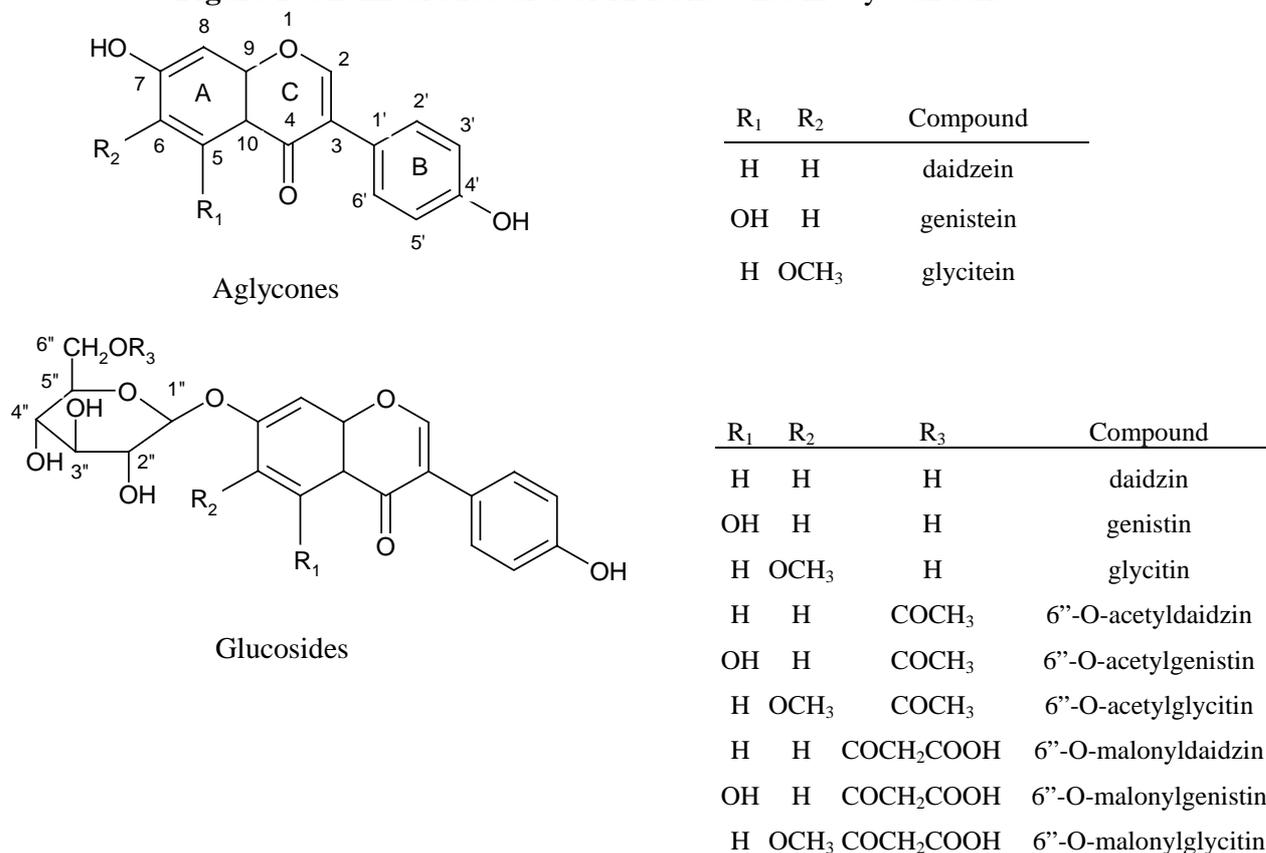
Results and Discussion

Isoflavone content in soybean cake

In a previous study we demonstrated that all 12 isoflavones in soybean cake plus the internal standard formononetin could be separated by HPLC within 15 min using a Vydac 201TP54 C_{18} column [7]. This method is much faster than those reported in the literature [6, 16, 17]. Figure 1 shows the chemical structures of the 12 isoflavones present in soybean cake. High r^2 values ranging from 0.9894~1 were observed for all the calibration curves of the 12 isoflavone standards. The linear regression equations of the 12 isoflavones were: y = 0.2049x–0.0016 (malonyldaidzin), y = 0.1686x+0.0218 (malonylglycitin), y = 0.4492x+0.0081 (malonylgenistin), y = 0.6777x+0.0018 (daidzin), y = 0.2729x–0.0164 (glycitin), y = 0.5138x+0.007 (genistin), y = 0.7899x+0.0048 (acetyldaidzin), y = 0.3716x+0.0087 (acetylglycitin), y = 0.9027x+0.022 (acetylgenistin), y = 0.8639x+0.0313 (daidzein), y = 0.4298x–0.0008 (glycitein) and y = 1.3841x+0.0679 (genistein), respectively. Malonylglucoside was the compound present in the largest amount in soybean cake (2,411 μg/g), followed by glucoside (2,184 μg/g), acetylglucoside (256 μg/g) and aglycone (159 μg/g) for a combined total of 5,010 μg/g [7]. These levels were much higher than those reported by Wang *et*

al. [18] or Coward *et al.* [19], who found malonylglucoside, glucoside, acetylglucoside and aglycone levels to be 1,013 and 1,340 µg/g, 475 and 249 µg/g, 0 and 207 µg/g, and 24 and 11 µg/g, respectively. These differences may be accounted for by variations in cultivar, growth environment, harvest time and storage conditions [6, 20].

Figure 1. Chemical structures of 12 isoflavones in soybean cake.`

Aglycones

R_1	R_2	Compound
H	H	daidzein
OH	H	genistein
H	OCH_3	glycitein

Glucosides

R_1	R_2	R_3	Compound
H	H	H	daidzin
OH	H	H	genistin
H	OCH_3	H	glycitin
H	H	$COCH_3$	6"-O-acetyldaidzin
OH	H	$COCH_3$	6"-O-acetylgenistin
H	OCH_3	$COCH_3$	6"-O-acetylglycitin
H	H	$COCH_2COOH$	6"-O-malonyldaidzin
OH	H	$COCH_2COOH$	6"-O-malonylgenistin
H	OCH_3	$COCH_2COOH$	6"-O-malonylglycitin

Processing of isoflavone powder by spray drying

With gelatin as carrier, it was shown a 5 % level would make spray drying difficult because of the resulting low solids contents. By raising the gelatin level to 15 or 20 %, the isoflavone powder could be processed by spray drying, but the isoflavone content was then too low. Thus, the optimum level of gelatin was chosen to be 10 %. With the inlet temperature set lower than 170 °C, the isoflavone homogenate stuck to the inner tube in the spray dryer and complete drying could only be attained when the inlet temperature reached 180 °C or above with the outlet temperature maintained at 120°. For feed rate, a high value (>10 %) resulted in incomplete drying, while a low one (<10 %) required a longer drying time. Therefore, a value of 10 % was selected as the most suitable feed rate. By choosing the most favorable conditions shown above, that is, gelatin level 10 %, inlet temperature 180 °C, outlet temperature 120 °C and feed rate 10 %, a total amount of 3.6 g powder (6.5% H_2O) containing 10,009 µg/g isoflavone was produced after drying.

With maltodextrin as carrier, a 10 % solution was prepared by dissolving 10 g of maltodextrin in 90 g of isoflavone extract and homogenizing for 10 min before spray drying, but the spray-dried powder was found to have a low solids content. By comparing maltodextrin concentrations of 20, 30 and 40 %, a level of 40 % was shown to be the most appropriate. Four feed rates of 10, 15, 20 and 25

% were also compared and the optimum level was found to be 20 %. By controlling the inlet temperature at 180 °C and outlet temperature at 120 °C, a total amount of 7.9 g powder (6.1% H_2O) containing 1,887 µg/g isoflavone was obtained after spray drying.

Because of its poor solubility in isoflavone extract, sodium alginate was first dissolved in deionized water and then mixed with isoflavone extract. A concentration of 4 % sodium alginate in water was found to be adequate to maintain a medium viscosity. A final concentration of 1 % sodium alginate in isoflavone extract was prepared by mixing 25 g of aqueous sodium alginate solution and 75 g of isoflavone extract. After homogenization for 10 min, the mixture was subjected to spray drying. By comparing four inlet temperatures (110, 130, 150 and 180 °C), it was shown that higher temperatures (150 and 180 °C) may cause degradation of the isoflavone powder particles, whereas a low temperature (110 °C) could result in inadequate drying. Therefore, the most favorable inlet and outlet temperatures were 130 and 100 °C, respectively. As for feed rate, a 7 % level was optimum as a higher feed rate ($\geq$10 %) led to incomplete drying. A total amount of 1.9 g powder (6.9 % H_2O) containing 23,847 µg/g isoflavone was thus generated after spray drying (Table 1).

Table 1. Effect of drying methods on isoflavone contents (μ g/g) [A] in a powder with 1 % sodium alginate as carrier [B].

Isoflavone	Spray drying	Vacuum drying	Freeze drying
Mdin	4,619±383[c]	6,745±135b	10,246±333a
Mglin	3,321±331[c]	3,788±261b	4,625±137a
Mgin	4,501±142[b]	6,739±22a	6,640±242a
Sub total	12,441±572[c]	17,272±657b	21,510±437a
Din	1,896±24[b]	2,689±82a	2,736±75a
Glin	1,844±41[b]	2,657±213a	2,765±65a
Gin	5,606±61[b]	10,075±113a	9,964±183a
Sub total	9,346±44[b]	15,421±408a	15,464±322a
Adin	285±4[b]	635±38a	638±42a
Aglin	232±10[c]	429±4b	502±21a
Agin	702±30[b]	1,192±74a	1,161±46a
Sub total	1,219±44[b]	2,255±208a	2,300±67a
Dein	428±44[c]	1,138±20[b]	1,255±10a
Glein	182±10[c]	946±18a	606±47b
Gein	230±59[b]	1,408±33a	1,288±128a
Sub total	840±5[c]	3,492±71a	3,148±92b
Total	23,847±577[c]	38,240±1,061b	42,423±734a

[A] Average of duplicate analyses±standard deviation. [B] Symbols bearing different letters (a-c) in the same row are significantly different (P<0.05). Mdin: malonyldaidzin; Mglin: malonylglycitin; Mgin: malonylgenistin; Din: daidzin; Glin: glycitin; Gin: genistin; Adin: acetyldaidzin; Aglin: acetylglycitin; Agin: acetylgenistin; Dein: daidzein; Glein: glycitein; Gein: genistein.

Evidently, the yield of isoflavone powder can be affected by both the type of carrier and spray-drying conditions. Fernandez-Perez *et al*. [21] studied the effects of inlet temperature (100-200 °C) and feed rate (2.5-10 mL/min) on the quality of spray-dried instant soup powder and found that the powder yield increased as the inlet temperature increased (<160 °C). However, a high inlet temperature (200 °C) would decrease the yield of powder, probably due to powder particle degradation. In addition, these authors pointed out that the faster the feed rate and the lower the outlet temperature, the lower the yield of powder. Goula and Adamopoulos [22] also reported that the lower the inlet temperature and the slower the hot air rate, the higher density of the powder was. Likewise, the higher the inlet temperature and the slower the hot air rate, the lower the moisture content of the powder was. Some other authors have suggested that a high feed rate may increase the volume of liquid droplets to thus produce a low yield of powder [23]. Krishnan *et al*. [24] further stated that carriers such as gelatin and sodium alginate possessed higher viscosity than maltodextrin, which was less efficient in terms of active material or core material encapsulation. Theoretically, the higher the solid content, the larger the yield of powder [21]. But, a high solids content would result in a low amount of active material being encapsulated into the powder. For example, in our study the highest yield of powder (7.9 g) was produced with 40 % maltodextrin as carrier, followed by 10 % gelatin (3.6 g) and 1 % sodium alginate (1.9 g). In contrast, the isoflavone content in the powder followed an increasing trend, with values of 1,887, 10,009 and 23,847 μg/g, respectively.

Production of isoflavone powder by vacuum drying

The results shown above clearly indicate that with 1 % sodium alginate as carrier, a powder product containing high level of isoflavone could be obtained by spray drying. Thus, we further investigated the possibility of processing isoflavone extract into powder with sodium alginate as carrier by vacuum drying. Of the five proportions studied, a powder product of 5.3 and 4.9 g was obtained for the isoflavone solutions containing 1 and 0.4 % sodium alginate, respectively, while the other three levels (0.2, 0.1 and 0.05 %) failed to form powder, probably because of low solids content. In contrast, powder products of 5.9, 5.6, 5.5 and 5.4 g resulted from the isoflavone solutions containing 1, 0.4, 0.2 and 0.1 % H-γ-PGA, respectively, whereas the level of 0.05 % H-γ-PGA failed to produce powder. The isoflavone content in the powder increased with the decreasing concentration of both carriers (Table 2). A concentration of 0.1% H-γ-PGA in the isoflavone extract produced the highest yield (51,564 μg/g) of total isoflavone, followed by the other three concentrations of 0.2, 0.4 and 1 %, which gave 49,900, 46,689 and 38,165 μg/g, respectively. Similarly, a level of 0.4 % sodium alginate in the isoflavone extract generated a greater yield (49,041 μg/g) of total isoflavone than that of 1% (38,661 μg/g). All four groups of isoflavones, namely, malonylglucosides, glucosides, acetylglucosides and aglycones showed the same trend.

The above results indicated the most appropriate concentrations for sodium alginate and H-γ-PGA in isoflavone extract were 0.4 and 0.1 %, respectively, with the proportion of carrier to isoflavone extract being 1:4 and 1:19 (w/w). Therefore, it is necessary to investigate the possibility of producing powder product by lowering the levels of both carriers. Four levels of 0.4, 0.2, 0.04 and 0.02 % of sodium alginate, and 0.1, 0.05, 0.01 and 0.005 % of both H-γ-PGA and L-γ-PGA, were each prepared in isoflavone extract as described above.

Table 2. Isoflavone contents (μ g/g) [A] in the vacuum-dried powder with sodium alginate and H-γ-PGA (Na) [B] as carrier [C].

Isoflavones	Carriers					
	Sodium alginate		H-γ-PGA (Na)[B]			
	1 %	0.4 %	1 %	0.4 %	0.2 %	0.1 %
Mdin	8,345±57[c]	9,857±91[b]	8,170±264[c]	9,986±270[b]	10,855±255[a]	11,315±197[a]
Mglin	2,853±40[e]	3,696±166[c]	2,865±23[e]	3,137±2[d]	4,357±196[b]	4,793±32[a]
Mgin	5,017±148[c]	6,410±101[a]	4,912±3[c]	5,791±117[b]	6,507±88[a]	6,385±112[a]
Sub total	16,214±246[e]	19,963±358[c]	15,947±286[e]	18,914±389[d]	21,719±29[b]	22,492±341[a]
Din	3,407±12[c]	4,480±113[a]	3,327±12[c]	4,290±24[b]	4,345±30[ab]	4,338±3[ab]
Glin	4,217±21[d]	5,401±119[ab]	3,983±45[d]	4,830±96[c]	5,277±121[b]	5,657±170[a]
Gin	9,315±42[c]	12,356±416[a]	9,417±79[c]	12,147±165[ab]	11,743±126[b]	12,087±15[ab]
Sub total	16,939±51[c]	22,238±410[a]	16,728±135[c]	21,266±237[b]	21,365±35[b]	22,083±153[a]
Adin	509±4[b]	665±26[a]	501±8[b]	652±13[a]	651±11[a]	679±2[a]
Aglin	482±5[d]	787±9[a]	466±10[d]	567±17[c]	734±49[ab]	689±17[b]
Agin	898±99[b]	1,098±46[a]	873±20[b]	1,123±23[a]	1,101±29[a]	1,124±22[a]
Sub total	1,889±98[c]	2,550±64[a]	1,840±19[c]	2,342±7[b]	2,486±67[a]	2,493±37[a]
Dein	546±5[c]	729±13[ab]	537±6[c]	765±6[a]	741±33[ab]	714±27[b]
Glein	365±6[c]	580±43[a]	357±3[c]	461±0[b]	445±51[b]	479±19[b]
Gein	2,708±64[d]	2,983±82[c]	2,757±6[d]	2,941±26[c]	3,144±31[b]	3,304±3[a]
Sub total	3,618±53[d]	4,291±52[c]	3,651±3[d]	4,167±32[c]	4,330±115[b]	4,497±22[a]
Total	38,661±448[d]	49,041±168[b]	38,165±172[d]	46,689±652[c]	49,900±117[b]	51,564±478[a]

[A] Average of duplicate analyses±standard deviation. [B] γ-PGA (Na) with high molecular weight.
[C] Symbols bearing different letters (a-e) in the same row are significantly different (P<0.05).
Mdin: malonyldaidzin; Mglin: malonylglycitin; Mgin: malonylgenistin; Din: daidzin; Glin: glycitin; Gin: genistin; Adin: acetyldaidzin; Aglin: acetylglycitin; Agin: acetylgenistin; Dein: daidzein; Glein: glycitein; Gein: genistein.

A powder product of 4.9, 4.7 and 4.6 g was formed for alginate levels of 0.4, 0.2 and 0.04 %, respectively. For both H-γ-PGA and L-γ-PGA levels at 0.1, 0.05 and 0.01 %, a powder product of 5.4, 5.2 and 5.2 g was generated for the former and 5.6, 5.3 and 5.3 g for the latter. Levels of 0.02 % sodium alginate and 0.005 % of H-γ-PGA or L-γ-PGA both failed to form powder, which may be due to the presence of a low solids content. The highest amount of malonylglucoside was found for 0.01 % H-γ-PGA and L-γ-PGA, which equaled 27,052 and 26,868 µg/g, respectively, followed by 0.05 % H-γ-PGA and L-γ-PGA (25,498 and 25,059 µg/g), and 0.04 and 0.2 % sodium alginate (24,764 and 21,978 µg/g). The same trend also occurred for both acetylglucoside and aglycone. However, only minor differences were shown for glucoside (Table 3). For total isoflavone, it also followed a similar trend as the four groups of isoflavones.

Table 3. Isoflavone contents (μg/g) [A] in the vacuum-dried powder with H-γ-PGA (Na) [B] and L-γ-PGA (Na) [C] or sodium alginate as carrier [D].

Isoflavones	Carriers					
	Sodium alginate		H-γ-PGA (Na)[B]		L-γ-PGA (Na)[C]	
	0.20%	0.04%	0.05%	0.01%	0.05%	0.01%
Mdin	10,915±47e	12,459±110c	12,934±247b	13,613±187a	12,971±283b	13,693±74a
Mglin	5,287±39a	5,377±187a	5,389±110a	5,558±26a	5,323±64a	5,444±135a
Mgin	5,776±407d	6,928±21b	7,176±171b	7,882±77a	6,765±187bc	7,731±248a
Sub total	21,978±415c	24,764±318b	25,498±529b	27,052±290a	25,059±534b	26,868±309a
Din	3,992±235b	4,365±115a	4,471±128a	4,390±175a	4,419±24a	4,448±144a
Glin	5,073±294b	5,099±366b	5,104±64b	5,182±80ab	5,073±19a	5,148±33a
Gin	1,2051±58b	12,731±352a	12,798±217a	12,772±93a	12,513±186a	12,669±214a
Sub total	21,117±587b	22,195±101a	18,777±326a	22,344±348a	22,005±182ab	22,266±324a
Adin	849±44b	951±24a	759±28c	887±29ab	778±28c	868±2b
Aglin	680±28c	717±20bc	667±8c	856±64a	657±14d	880±31a
Agin	1,121±22b	1,211±71ab	954±32c	1,313±61a	968±15d	1,360±6a
Sub total	2,650±6b	2,879±114a	2,380±53c	3,056±154a	2,403±57d	3,109±27a
Dein	637±51c	688±6bc	636±20c	806±27a	652±16bc	816±40a
Glein	558±1b	595±27ab	632±15ab	664±47a	623±15a	614±51a
Gein	3,287±35b	3,397±59ab	3,402±156ab	3,518±95a	3,355±9ab	3,458±56a
Sub total	4,482±86bc	4,679±91b	4,670±151b	4,989±169a	4,630±8b	4,888±67a
Total	50,227±92d	54,517±625b	54,922±150b	57,441±962a	54,098±765b	57,130±673a

[A] Average of duplicate analyses±standard deviation. [B] γ-PGA (Na) with high molecular weight. [C] γ-PGA (Na) with low molecular weight. [D] Symbols bearing different letters (a-f) in the same row are significantly different (P<0.05). Mdin: malonyldaidzin; Mglin: malonylglycitin; Mgin: malonylgenistin; Din: daidzin; Glin: glycitin; Gin: genistin; Adin: acetyldaidzin; Aglin: acetylglycitin; Agin: acetylgenistin; Dein: daidzein; Glein: glycitein; Gein: genistein.

Processing of isoflavone powder by freeze drying

Following the same approach, a mixture (100 g) containing sodium alginate and isoflavone extract was freeze dried at –40° under a 60 millitorr vacuum and with a drying time of 24 h. A powder product of 6.6 g (9.1 % H_2O) was obtained which contained the highest level of malonylglucoside, glucoside, acetylglucoside and aglycone (Table 1). Of the various dried products, freeze-dried powder contained the highest content of total isoflavone, followed by vacuum-dried and spray-dried powder (Table 1). However, for aglycone, vacuum drying was shown to produce a higher level than freeze drying, probably caused by conversion from glucose-containing isoflavone during extensive vacuum drying at 60 °C. In a study dealing with the conversion and degradation of isoflavone during heating, Chien *et al.* [5] demonstrated that aglycone can be formed from malonylglucoside, glucoside or acetylglucoside depending on heating temperature and time. Compared to vacuum drying, freeze drying showed a

higher level of malonylglucoside, but no significant difference was found in the contents of acetylglucoside (Table 1). Obviously, the highest yield of total isoflavone was accomplished by freeze drying, since both conversion and degradation of isoflavones were minimized. Nevertheless, vacuum drying generated a higher yield of powder (7.2 g) than either freeze drying (6.6 g) or spray drying (1.9 g).

A similar outcome was reported by Monsoor [25], who evaluated the effects of several drying methods on the functional properties of soy hull pectin, the lowest yield of powder being observed for spray drying, whereas there is no significant difference between freeze drying and vacuum drying. In our study, freeze-dried powder contained a higher amount of moisture (9.1 %) than vacuum dried (7.2 %) and spray dried (6.9 %), which may be explained by formation of porous structures in the powder during sublimation, which should be more prone to moisture absorption after drying [26]. The same phenomenon was reported by Tsami *et al.* [27], who found that freeze-dried powder could absorb moisture more readily than vacuum-dried powder, which can be attributed to the former being capable of forming a large number of pores with much smaller diameter.

By comparing the results shown above, freeze drying was proven to be the best method for production of powder with high-levels of isoflavone. Thus, we further investigated the effects of H-γ-PGA and L-γ-PGA on the production of isoflavone powder by freeze drying. Because of the high moisture absorption capacity and poor solubility in isoflavone extract, both H-γ-PGA and L-γ-PGA were dissolved separately in water to obtain a medium viscosity and a concentration of 2 %. In the beginning a mixture containing 25 g of H-γ-PGA or L-γ-PGA solution and 75 g of isoflavone extract was placed in a freezer (–30°) for 24 h. However, this mixture failed to freeze, mainly due to the presence of ethanol in the isoflavone extract. After various studies, a mixture containing 40 g of H-γ-PGA or L-γ-PGA solution and 100 g of isoflavone extract (0.28 % H-γ-PGA or L-γ-PGA in isoflavone extract) was frozen after 24 h at –30 °C. Six concentrations of 0.57, 0.28, 0.14, 0.028, 0.014 and 0.003 % H-γ-PGA or L-γ-PGA in isoflavone extract were prepared separately and placed in a freezer until frozen, after which each mixture was freeze dried at –40 °C under 60 millitorr for 24 h, and powder products of 8.4, 7.8, 7.9, 7.9, 7.6 and 7.5 g were produced, respectively, for H-γ-PGA, whereas for L-γ-PGA, a powder product of 8.4, 8.1, 7.7, 7.1, 7.5 and 7.7 g was formed. Of the four groups of isoflavones, both malonylglucosides and glucosides dominated for both powders containing H-γ-PGA and L-γ-PGA at all six levels (Tables 4 and 5). Only slight differences between acetylglucoside and aglycone were noted, probably because they are present in much smaller amounts in isoflavone extract. With 0.003 % H-γ-PGA or L-γ-PGA, the contents of total isoflavone in the freeze dried powder were 48,144 and 48,155 µg/g, respectively (Tables 4 and 5). Only minor differences were observed for 0.014 % H-γ-PGA and L-γ-PGA, which gave 48,204 and 47,245 µg/g, respectively. The same phenomenon also applied to H-γ-PGA and L-γ-PGA with levels at 0.28 and 0.57 %. No significant difference was found between 1 % sodium alginate and 0.57% H-γ-PGA or L-γ-PGA (Tables 4 and 5) for total isoflavone in the freeze dried powder. This result further demonstrated that a high yield of powder containing a large amount of isoflavone could be attained by using an extremely low level of 0.003 % H-γ-PGA or L-γ-PGA, and was comparable to that using 1 % sodium alginate as carrier. As mentioned before, with γ-PGA as carrier the powder production cost could be reduced greatly.

Table 4. Isoflavone contents (μg/g) [A] in the freeze-dried powder with H-γ-PGA (Na) [B] or sodium alginate as carrier [C].

Isoflavones	1 % sodium alginate	Carriers					
		H-γ-PGA (Na)[B]					
		0.57 %	0.28 %	0.14 %	0.028 %	0.014 %	0.003 %
Mdin	$9,160\pm92^d$	$9,562\pm258^d$	$10,527\pm166^c$	$10,768\pm13^c$	$12,476\pm273^{ab}$	$12,802\pm454^a$	$12,055\pm195^b$
Mglin	$3,118\pm141^{ef}$	$3,620\pm442^{bcd}$	$3,930\pm10^b$	$3,848\pm1^{bc}$	$3,436\pm365^{cde}$	$3,868\pm43^{bc}$	$3,982\pm39^b$
Mgin	$7,176\pm25^d$	$7,404\pm162^d$	$7,732\pm311^c$	$7,791\pm45^{bc}$	$7,958\pm75^{abc}$	$7,867\pm122^{bc}$	$8,056\pm29^{ab}$
Sub total	$19,455\pm258^e$	$20,586\pm538^d$	$22,189\pm466^c$	$2,2407\pm30^c$	$23,870\pm563^b$	$24,537\pm534^{ab}$	$24,093\pm205^{ab}$
Din	$3,579\pm18^a$	$3,707\pm27^d$	$3,902\pm64^{bc}$	$3,950\pm3^{abc}$	$4,030\pm85^a$	$3,896\pm41^{bc}$	$3,984\pm16^{ab}$
Glin	$4,071\pm21^a$	$3,917\pm131^a$	$4,106\pm135^a$	$4,241\pm52^a$	$4,253\pm67^a$	$4,159\pm184^a$	$4,323\pm10^a$
Gin	$10,437\pm77^e$	$11,094\pm157^{def}$	$11,770\pm332^a$	$11,648\pm336^{ab}$	$11,441\pm3^{abcde}$	$11,174\pm102^{cdef}$	$11,315\pm37^{bcde}$
Sub total	$18,088\pm116^{cd}$	$18,719\pm1^{cde}$	$19,779\pm531^a$	$19,838\pm385^a$	$19,724\pm156^a$	$19,230\pm327^{abc}$	$19,622\pm43^{ab}$
Adin	651 ± 8^{ab}	696 ± 22^{abcd}	716 ± 46^{ab}	731 ± 20^a	699 ± 24^{abcd}	679 ± 14^{abcd}	726 ± 6^a
Aglin	641 ± 5^d	672 ± 68^{ab}	643 ± 5^{ab}	667 ± 23^{ab}	719 ± 119^{ab}	795 ± 130^a	735 ± 50^{ab}
Agin	$1,078\pm2^b$	$1,148\pm38^{bcd}$	$1,183\pm41^{abc}$	$1,192\pm5^{abc}$	$1,218\pm2^{ab}$	$1,184\pm67^{abc}$	$1,193\pm1^{abc}$
Sub total	$2,370\pm11^a$	$2,516\pm127^{ab}$	$2,543\pm93^{ab}$	$2,590\pm3^{ab}$	$2,637\pm74^a$	$2,658\pm210^a$	$2,654\pm55^a$
Dein	715 ± 8^b	740 ± 46^a	768 ± 61^a	784 ± 18^a	727 ± 24^a	718 ± 43^a	764 ± 5^a
Glein	441 ± 9^{abc}	462 ± 40^b	474 ± 46^b	523 ± 51^{ab}	486 ± 45^b	470 ± 42^b	591 ± 12^a
Gein	539 ± 29^{abcde}	542 ± 28^{abc}	573 ± 14^a	554 ± 19^{ab}	429 ± 12^d	430 ± 5^d	419 ± 2^d
Sub total	$1,694\pm27^e$	$1,744\pm58^{abcde}$	$1,814\pm120^{abc}$	$1,860\pm52^a$	$1,642\pm81^{cde}$	$1,618\pm79^{de}$	$1,774\pm19^{abcd}$
Total	$41,607\pm126^e$	$43,565\pm352^d$	$46,325\pm1210^{bc}$	$46,695\pm306^{abc}$	$47,873\pm400^{ab}$	$48,204\pm923^a$	$48,144\pm198^a$

[A] Average of duplicate analyses±standard deviation. [B] γ-PGA (Na) with high molecular weight. [C] Symbols bearing different letters (a-f) in the same row are significantly different (P<0.05). Mdin: malonyldaidzin; Mglin: malonylglycitin; Mgin: malonylgenistin; Din: daidzin; Glin: glycitin; Gin: genistin; Adin: acetyldaidzin; Aglin: acetylglycitin; Agin: acetylgenistin; Dein: daidzein; Glein: glycitein; Gein: genistein.

Table 5. Isoflavone contents (μg/g)[A] in the freeze-dried powder with L-γ-PGA (Na)[B] or sodium alginate as carrier [C].

Isoflavones	1 % sodium alginate	Carriers					
		L-γ-PGA (Na)[B]					
		0.57 %	0.28 %	0.14 %	0.028 %	0.014 %	0.003 %
Mdin	$9,160\pm92^d$	9242 ± 125^d	10256 ± 320^c	10665 ± 66^c	12230 ± 75^b	11949 ± 637^b	12386 ± 570^{ab}
Mglin	$3,118\pm141^{ef}$	$2940\pm111f$	$3402\pm289de$	$3936\pm93b$	$3955\pm99b$	$4760\pm83a$	$4718\pm38a$
Mgin	$7,176\pm25^d$	7186 ± 58^d	$8200\pm127a$	$7891\pm92bc$	$7945\pm57abc$	$7455\pm160d$	$7946\pm23abc$
Sub total	$19,455\pm258^e$	19368 ± 179^e	$21857\pm736c$	$22491\pm251c$	$24130\pm231ab$	$24164\pm880ab$	$25050\pm632a$
Din	$3,579\pm18^a$	3606 ± 42^e	$3864\pm29c$	$3958\pm5abc$	$4048\pm50a$	$3857\pm88c$	$3864\pm40c$
Glin	$4,071\pm21^a$	4080 ± 441^a	$4075\pm53a$	$4114\pm70a$	$4125\pm207a$	$4052\pm108a$	$4132\pm144a$
Gin	$10,437\pm77^e$	10796 ± 30^{fg}	$11508\pm199bcd$	$11590\pm19abc$	$11550\pm109abc$	$11039\pm285ef$	$11123\pm63def$
Sub total	$18,088\pm116^{cd}$	18481 ± 453^{de}	$19447\pm222ab$	$19661\pm94ab$	$19723\pm148a$	$18948\pm480cde$	$19119\pm247abcd$
Adin	651 ± 8^{ab}	699 ± 46^{abcd}	$711\pm10abc$	$689\pm27abcd$	$696\pm35abcd$	$642\pm12d$	$659\pm23bcd$
Aglin	641 ± 5^d	648 ± 15^{ab}	$682\pm98ab$	$652\pm43ab$	$597\pm7b$	$767\pm25a$	$587\pm37b$
Agin	$1,078\pm2^b$	$1123\pm47cd$	$1188\pm1abc$	$1242\pm14a$	$1175\pm24abc$	$1124\pm24cd$	$1154\pm28bc$
Sub total	$2,370\pm11^a$	$2470\pm79ab$	$2581\pm109ab$	$2582\pm2ab$	$2468\pm65ab$	$2533\pm60ab$	$2399\pm89b$

Table 5. Cont.

Dein	715±8[b]	746±52a	783±38a	768±6a	712±23a	703±37a	711±29a
Glein	441±9[abc]	495±41ab	501±9ab	506±70ab	451±38b	472±44b	448±54b
Gein	539±29[abcde]	523±18bc	546±11abc	554±15ab	509±12c	424±19d	427±2d
Sub total	1,694±27[e]	1,764±111[abcde]	1830±58ab	1829±91ab	1672±28bcde	1599±100de	1586±85e
Total	41,607±126[e]	42083±465[de]	45715±347c	46564±435abc	47993±46ab	47245±1521[abc]	48155±1052a

[A] Average of duplicate analyses±standard deviation. [B] γ-PGA (Na) with low molecular weight.[C] Symbols bearing different letters (a-g) in the same row are significantly different (P<0.05). Mdin: malonyldaidzin; Mglin: malonylglycitin; Mgin: malonylgenistin; Din: daidzin; Glin: glycitin; Gin: genistin; Adin: acetyldaidzin; Aglin: acetylglycitin; Agin: acetylgenistin; Dein: daidzein; Glein: glycitein; Gein: genistein.

Table 6 shows the characteristics of various isoflavone powders made by different drying methods. Both spray-dried and freeze-dried powder possessed a slightly yellow appearance, while vacuum-dried powder was deep yellow, which may be due to long-time drying at 60 °C.

Table 6. Characteristics of the various isoflavone powder made by different drying methods.

Drying method	Carrier	Carrier content (%)	Color	Powder weight (g)	Water content (%)	Isoflavone content (µg/g)
Spray-drying	Gelatin	10	Light yellow	3.6	6.5	10,009
	Maltodextrin	40	Light yellow	7.9	6.1	1,887
	Sodium Alginate	1	Light yellow	1.9	6.9	23,847
Freeze-drying	Sodium Alginate	1	Yellow to white	6.6	9.1	43,423
	H-γ-PGA (Na) [A]	0.570	Light yellow	8.4	14.0	43,565
	H-γ-PGA (Na)	0.280	Light yellow	7.8	14.8	46,325
	H-γ-PGA (Na)	0.140	Light yellow	7.9	16.1	46,695
	H-γ-PGA (Na)	0.028	Light yellow	7.9	17.5	47,873
	H-γ-PGA (Na)	0.014	Light yellow	7.6	16.7	48,204
	H-γ-PGA (Na)	0.003	Light yellow	7.5	17.9	48,144
	L-γ-PGA (Na)[B]	0.570	Light yellow	8.4	13.2	42,083
	L-γ-PGA (Na)	0.280	Light yellow	8.1	14.7	45,715
	L-γ-PGA (Na)	0.140	Light yellow	7.7	15.9	46,564
	L-γ-PGA (Na)	0.028	Light yellow	7.1	17.1	47,993
	L-γ-PGA (Na)	0.014	Light yellow	7.5	17.4	47,245
	L-γ-PGA (Na)	0.003	Light yellow	7.7	17.9	48,155
Vacuum-drying	Sodium Alginate	1	Yellow	5.3	4.4	38,601
	Sodium Alginate	0.40	Yellow	4.9	4.5	49,041
	Sodium Alginate	0.20	Yellow	4.7	4.6	50,227
	Sodium Alginate	0.04	Yellow	4.6	4.9	54,517
	H-γ-PGA (Na)	0.10	Yellow	5.4	5.7	51,564
	H-γ-PGA (Na)	0.05	Yellow	5.2	5.8	54,922
	H-γ-PGA (Na)	0.01	Yellow	5.2	5.8	57,441
	L-γ-PGA (Na)	0.10	Yellow	5.6	5.4	52,542
	L-γ-PGA (Na)	0.05	Yellow	5.3	5.6	54,098
	L-γ-PGA (Na)	0.01	Yellow	5.3	5.5	57,130

[A] γ-PGA (Na) with high molecular weight. [B] γ-PGA (Na) with low molecular weight.

Tsami *et al*. [27] also reported that pectin-sugar powder made by vacuum drying exhibited deeper color than with freeze drying. With spray drying, the maltodextrin carrier produced the highest yield of powder, followed by gelatin and sodium alginate. With sodium alginate as carrier, freeze drying generated the greatest yield of powder, followed by vacuum drying and spray drying. It was also observed that the powder weight increased following a rise in carrier content, which resulted in a decrease of isoflavone level because of the dilution effect.

Conclusions

With spray drying, use of a level of 40 % maltodextrin as carrier results in a higher yield of isoflavone powder than with 10 % gelatin or 1 % sodium alginate as carriers. Freeze drying can generate a larger amount of isoflavone powder than vacuum drying and spray drying with 1 % sodium alginate as carrier. Compared to sodium alginate, an extremely low level of γ-PGA (0.003 %) as carrier can be used to produce powder containing high amount of total isoflavone. The results of this study may provide a basis for possible commercial production of isoflavone powder in the food industry. Further research is necessary to study the stability of isoflavone powder during storage.

Experimental Section

Materials

Soybean cake (50 kg) was purchased from Chung-Lian Oil Co. (Taichung, Taiwan), ground into powder and stored at -20° for use. Twelve isoflavone standards were obtained from LC Laboratories (Woburn, MA, USA), Sigma (St. Louis, MO, USA) and Nacalai (Kyoto, Japan). Formononetin (internal standard) was from Sigma. HPLC-grade acetonitrile was from Merck (Darmstadt, Germany). Analytical-grade ethanol (95%) was from Taiwan Tobacco and Wine Co. (Tainan, Taiwan). Deionized water was made using a Milli-Q water purification system (Millipore Co., Bedford, MA, USA). Gelatin, maltodextrin and sodium alginate were from Cheng-Fang Co. (Taipei, Taiwan). Both high-molecular-weight-γ-PGA (H-γ-PGA, MW 800 kDa) and low-molecular-weight-γ-PGA (L-γ-PGA, MW 200-400 kDa) in Na form were provided by Vedan Enterprise Corp. (Taichung, Taiwan).

Instrumentation

The HPLC instrument was composed of a Rheodyne 7161 injector (CA, USA), two Jasco pumps (PU 980 and PU 1980) and a Jasco MD-915 photodiode-array detector (Tokyo, Japan). Borwin computer software was used to process data. A Vydac 201TP54 C_{18} column (250 x 4.6 mm I.D., 5 μm) was used to separate the 12 isoflavones in soybean cake and powder. The Büchi Mini B290 spray dryer was from Büchi Co. (Flawil, Switzerland). The FD24 freeze dryer was from Ching-Ming Co. (Taipei, Taiwan). The KVED-1 vacuum dryer was from Tong-Yuan Co. (Taipei, Taiwan). The Sorvall RC5C high-speed centrifuge was from Du Pont Co. (Wilmington, Delaware, USA). The PT-MR 3000 homogenizer was from Kinematica Co. (Switzerland).

Extraction of isoflavone from soybean cake

Soybean cake (3 kg) was mixed with deionized water-ethanol (1:1, v/v, 9-L) in a flask, and the mixture was shaken for 2 h. Then the crude extract was divided into 10 portions and each subjected to centrifugation at 6,000 rpm for 20 min (25 °C), after which all the supernatants were collected and filtered through a glass filter paper. All the filtrates were combined to give approximately 4500-mL of filtrate that was used as raw material for processing the powder.

Processing of isoflavone powder by spray drying

Because of differences in solubility of the carriers in isoflavone extract, various proportions of gelatin, maltodextrin or sodium alginate to isoflavone extract have to be tried to find the optimum concentration. Four concentrations of 5, 10, 15 and 20 % gelatin in isoflavone extract were prepared by mixing 5, 10, 15 and 20 g of gelatin with 95, 90, 85 and 80 g of isoflavone extract, respectively. Similarly, four concentrations of 10, 20, 30 and 40 % maltodextrin in isoflavone extract were prepared by mixing 10, 20, 30 and 40 g of maltodextrin with 90, 80, 70 and 60 g of isoflavone extract, respectively. For sodium alginate, it was first dissolved in deionized water for a concentration of 4 % because of its poor solubility in isoflavone extract. Then a sodium alginate solution (25-g) was mixed with isoflavone extract (75-g) to obtain a final concentration of 1 %. Each carrier-containing isoflavone solution was subjected to homogenization for 10 min and then spray dried under the following conditions: feed rate 5, 10, 15 and 20 % and inlet temperatures 150, 160, 170 and 180 °C for gelatin solution; feed rates 10, 15, 20 and 25 % and inlet temperatures 150, 160, 170 and 180 °C for maltodextrin solution; feed rates 5, 7 and 10 %, and inlet temperatures 110, 130, 150 and 180 °C for sodium alginate solution. These conditions were chosen based on the different characteristics of carrier solutions like viscosity and solid content. After processing into powder, a sample (0.05 g) of either gelatin- and sodium alginate-containing powder or a sample (0.25 g) of maltodextrin-containing powder was separately dissolved in deionized water (5-mL). Then each solution (500 µL) was mixed with formononetin internal standard (200 µg/mL, 100 µL), followed by addition of deionized water (400-µL) and filtration through a 0.2-µm membrane filter. A 20-µL sample was injected for HPLC analysis.

Processing of isoflavone powder by vacuum drying

As the powder formation during vacuum drying can be affected by solids content, it is necessary to find out the optimum proportion of carrier to isoflavone extract. A concentration of 2 % sodium alginate and H-γ-PGA in deionized water was prepared separately, following by collecting 50, 20, 10, 5 and 2.5 g and mixing with 50, 80, 90, 95 and 97.5 g of isoflavone extracts, respectively. Proportions of carrier (sodium alginate or H-γ-PGA) to isoflavone extract at 1:1, 1:4, 1:9, 1:19 and 1:39 (w/w) were thus obtained with final concentrations of carrier of 1.0, 0.4, 0.2, 0.1 and 0.05%, respectively. After homogenization for 10 min, each solution was vacuum dried at 60 °C under 4 cm Hg for 48 h and then ground into powder. A powder sample (0.05 g) was dissolved in deionized water (5-mL), of which an aliquot (500-µL) was collected and mixed with formononetin internal standard (200 µg/mL,

100 μL) and diluted with deionized water (400 μL). The solution was filtered through a 0.2-μm membrane filter and 20 μL was injected for HPLC analysis.

To investigate the possibility of using a low level of carrier for production of isoflavone powder, four concentrations of 2, 1, 0.2 and 0.1 % of aqueous sodium alginate solution were prepared, and 20-g of each was collected and mixed with 80 g of isoflavone extract to obtain final concentrations of 0.4, 0.2, 0.04 and 0.02 %, respectively. Each solution was homogenized for 10 min and then vacuum dried at 60 °C under 4 cm Hg for 36 h. Likewise, four concentrations of 2, 1, 0.2 and 0.1 % aqueous solutions of both H-γ-PGA and L-γ-PGA were prepared, in which 5-g of each was collected and mixed with 95-g of isoflavone extract for the final concentrations at 0.1, 0.05, 0.01 and 0.005 %, respectively, and each solution was subjected to homogenization and vacuum drying. A 0.05-g of sample powder was collected for HPLC analysis following the same procedure described above.

Processing of isoflavone powder by freeze drying

For freeze drying, both sodium alginate and γ-PGA were not directly dissolved in isoflavone extract containing 50 % ethanol because of poor solubility. Instead, they were dissolved in deionized water to make freezing possible. Six concentrations of 4, 2, 0.4, 0.2, 0.04 and 0.02 % of sodium alginate in water were prepared, and 25-g of each was mixed with 75-g of isoflavone extract for the final concentrations of 1, 0.5, 0.1, 0.05, 0.01 and 0.005 %, respectively. After homogenization for 10 min, each solution was placed in a freezer (–30 °C) for 24 h for freezing. Similarly, six concentrations of 2, 1, 0.5, 0.1, 0.05 and 0.01 % of γ-PGA dissolved in water were prepared, and 40-g of each was mixed with 100-g of isoflavone extract giving final concentrations of 0.57, 0.28, 0.14, 0.028, 0.014 and 0.003 %, respectively. Each solution was homogenized for 10 min and then placed in a freezer (–30 °C) for 24 h. Next, all the sodium alginate and γ-PGA solutions were freeze dried at –40° under 60 millitorr for 36 h and subjected to grinding for powder production. A powder sample (0.05-g) was dissolved in deionized water (5-mL), of which 500 μL was collected and mixed with 100-μL internal standard formononetin (200 μg/mL) and 400-μL deionized water. The solution was filtered through a 0.2-μm membrane filter and a 20-μL sample was injected for HPLC analysis.

Identification and quantitation of isoflavone

The various isoflavones in soybean cake and powder were identified by comparing retention times and absorption spectra of unknown peaks with reference standards and co-chromatography with added standards. For quantitation, each isoflavone standard was dissolved in methanol for a concentration of 200 or 2000 μg/mL, while the internal standard (formononetin) was dissolved in methanol for a concentration of 200 μg/mL. Three standard concentrations of 1, 5 and 10 μg/mL of the 12 isoflavones were each prepared by mixing 25, 125 and 250 μL (from 200 μg/mL), respectively, with 500 μL internal standard (from 200 μg/mL) and diluting to 5 mL with methanol. Likewise, the other three standard concentrations of 20, 50 and 100 μg/mL for the 12 isoflavones were each prepared by mixing 50, 125 and 250 μL (from 2000 μg/mL), respectively, with 500 μL internal standard (from 200 μg/mL) and diluting to 5 mL with methanol. The final concentration of internal standard in each standard solution was 20 μg/mL. A 20-μL isoflavone standard solution of each was injected and 12 standard

curves were prepared by plotting concentration ratio against area ratio, and the linear regression equation and correlation coefficient (r^2) of each standard curve was calculated. A gradient mobile phase developed by Hsieh *et al*. [28] was used to separate the 12 isoflavones: a mixture of acetonitrile (A) and deionized water (B) (8:92, v/v) was used initially, increased to 10% A in 2 min, 12% A in 3 min, 22% A in 10 min, 23% A in 11 min, 35% A in 12 min, 50% A in 13 min, maintained for 3 min and returned to 8% A in 20 min. All 12 isoflavones were adequately resolved within 15 min with a flow rate of 2.0 mL/min and a column temperature of 35 °C and detection wavelength at 262 nm. Each isoflavone was quantified using a formula as described by Kao and Chen [6]. Duplicate analyses were performed, and the data were processed using SAS [29] and subjected to analysis of variance and Duncan's multiple range test for comparison ($\alpha=0.05$).

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Sample availability: Available from the authors.