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Plant Lignans in Soy-Based Health Supplements

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The presence of plant lignans in 14 different soy-based health supplements is reported here for the first time together with the analysis of the isoflavone content, for which these products are commercialized. Six plant lignans, i.e., secoisolariciresinol, matairesinol, syringaresinol, lariciresinol, isolariciresinol, and pinoresinol, have been identified and quantified by gas chromatography−mass spectrometry, and a positive correlation has been found between the levels of plant lignans and the levels of isoflavones in the different products. Additional quantification of plant lignans and isoflavones in soybeans has been carried out, and results are provided to allow the comparison of the average levels in soybeans and soy-based supplements.

KEYWORDS: Plant lignans; isoflavones; phytoestrogens; soy; supplements

INTRODUCTION

Isoflavones, lignans, and coumestans are broadly considered as the representatives of the phytoestrogens, a group of compounds that have been shown to act as modulators of the mammalian hormonal system (*1*). There exist a large variety of lignans. The mammalian lignans, represented by enterolactone and enterodiol, appear in the human body after metabolism of their dietary precursors, also named as plant lignans, in the human gut. Secoisolariciresinol and matairesinol were first identified as dietary precursors of mammalian lignans (*2*), but at present, new precursors have been described; among them, the plant lignans pinoresinol and lariciresinol have been found to be converted to mammalian lignans in high proportion (*3*). The importance of these findings is based on epidemiological studies that positively correlated the presence of enterolactone in human plasma and urine with a lower incidence of certain types of cancer (*4*, *5*) and cardiovascular disease (*6*, *7*).

Plant lignans can be found in a wide variety of plants, but their appearance in grains and whole grain products in relatively large amounts is especially relevant as they constitute the basis of most of the diets worldwide. Lignans are also present in some legume pulses (*8*), including soybeans and soy-derived products (*9*), a range of products that have experienced an enormous increase in their distribution in western countries (*10*).

Recently, the FDA Health Claim linking the consumption of soy protein with the prevention of cardiovascular diseases (*11*), and the evidence of the estrogenic action of soy isoflavones (*12*), promoted the appearance of a number of second generation soy products in which soy extracts are present among the ingredient list of food items as commonly consumed as bread. In this context, and regarding the problem of both side effects and long-term effects that hormone replacement therapy presents, many postmenopausal women have opted for alternative solutions. Soy extracts or soy isoflavone extracts in the form of tablets or capsules are widely commercialized as an alternative therapy for alleviating menopausal discomforts, and in some cases, they are advertised for the prevention of menopause-related diseases such as osteoporosis.

The aim of the present investigation was to determine the levels of plant lignans in soy-based health products, as well as the levels of isoflavones to completely characterize the phytoestrogen content of these products.

MATERIALS AND METHODS

Samples. Different soy-based preparations in the form of tablets or capsules were selected among those available at local stores in Helsinki (Finland) during the year 2002. Selected products contained soy as a main component and were advertised as beneficial for the consumer health and/or specifically labeled to alleviate or prevent menopausal-related symptoms or diseases. The type of product and specification of dosage are described in **Table 1**. The tablets or capsules were removed from their packages, and 10−20 pieces were used for the analysis. Single pieces were weighed to check the uniformity of the samples. Tablets were ground to a fine powder in a mortar, and the content of the capsules, after removal of the hulls, was pooled into

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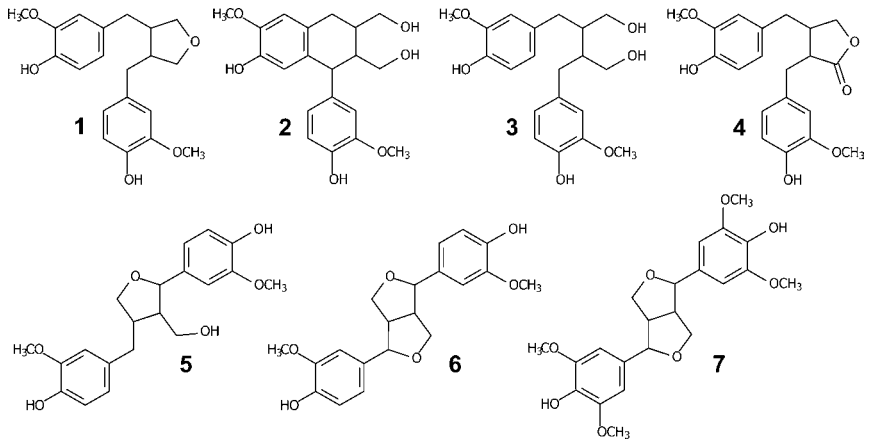

Figure 1. Structures of the plant lignans anhydrosecoisolariciresinol (**1**), isolariciresinol (**2**), secoisolariciresinol (**3**), matairesinol (**4**), lariciresinol (**5**), pinoresinol (**6**), and syringaresinol (**7**).

Table 1. Identification of the Soy-Based Products Involved in the Study

item	type[a]	country	dosage/day[b]	
1	I	Israel	2	capsules
2	I	Finland	2	tablets
3	I	United States	1–4	capsules
4	I	Finland	2	tablets
5	I	Finland	2	tablets
6	I	United States		capsules
7	I	Finland		tablets
8	S	United States	2	capsules
9	S	United States	2	tablets
10	S	France	2	capsules
11	S	Finland		tablets
12	S	Netherlands		capsules
13	S	Norway	3	tablets
14	S	Finland		capsules

[a] Refers to the type of formulation: I = isoflavone concentrate, S = soy protein-based product; see text for details. [b] Stated by the manufacturer.

plastic vials and stored at −20 °C until analyzed. Additionally, five different commercial brands of soybeans (*Glycine max* L.) were also purchased from local stores. After the beans were ground, the moisture was determined and the samples were stored at −20 °C until analyzed. All samples were analyzed in triplicate, and results are provided on a wet basis.

Standards. Daidzin, genistin, and glycitein were purchased from Apin Chemicals Ltd. (Abingdon, U.K.). Daidzein and genistein were obtained from Carl Roth GmbH & Co. (Karlsruhe, Germany), and glycitin was from Fujicco Co Ltd. Nacalai Tesque Inc. (Kobe, Japan). Plant-isolated standards of pinoresinol, syringaresinol, and lariciresinol were provided by Prof. Nishibe (Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Hokkaido, Japan). Matairesinol, secoisolariciresinol, and anhydrosecoisolariciresinol were synthesized as previously described (*13*, *14*), and isolariciresinol was prepared as reported (*15*). Anhydrosecoisolariciresinol is formed from secoisolariciresinol during the acid hydrolysis in the sample pretreatment (*16*) and, therefore, has to be included within the standards to accurately quantify the levels of secoisolariciresinol. Deuterated anhydrosecoisolariciresinol, secoisolariciresinol, and matairesinol, used as internal standards, were synthesized and kindly provided by Prof. Wähälä (Laboratory of Organic Chemistry, University of Helsinki, Finland). Chemical structures of the lignans included in the study are presented in **Figure 1**. All of the standard solutions used through the study were made in methanol.

High-Performance Liquid Chromatography (HPLC) Analyses of Isoflavones. Total soy isoflavones as a sum of individual daidzein, glycitein, and genistein expressed in aglycone equivalents were quantified by HPLC with coulometric electrode array detection, following a procedure recently described (*17*). Briefly, ∼20 mg of sample was incubated with 5 mL of acidified aqueous ethanol (1 M HCl 80% EtOH, 1 h at 80 °C) to hydrolyze the ester bond of the acylated isoflavone glucosides. After the samples were extracted with aqueous ethanol, the extracts were diluted with mobile phase (30% B, see composition below) and injected into the chromatograph under the following conditions: HPLC coupled with a Coularray detector (CEAD) (ESA Inc., Chelmsford, MA) with eight detection channels set between 200 and 700 mV. The analytical column, 150 mm × 3 mm i.d., 3 μm Inertsil ODS-3 (GL Sciences Inc., Japan), and guard column 10 mm × 3 mm i.d., 5 μm Quick Release C_{18} (Upchurch Scientific Inc., WA), were kept at 37 °C in a thermal chamber together with the detector cells. The mobile phase consisted of two eluents applied in gradient: (A) 50 mM sodium acetate buffer, pH 5/MeOH (80:20 v/v) and (B) 50 mM sodium acetate buffer, pH 5/MeOH/acetonitrile (40:20:20 v/v/v). All reagents were from major suppliers and were HPLC grade.

Gas Chromatography−Mass Spectrometry (GC-MS) Analyses of Plant Lignans. The plant lignan content was determined using a modification of the isotope dilution GC-MS method developed by Mazur and co-workers (*16*). Deuterated internal standards were not available for all of the plant lignans included in the study; therefore, quantification was carried out using $^2H_6$-matairesinol for the analysis of lariciresinol, pinoresinol, and syringaresinol and $^2H_6$-secoisolariciresinol for analyses of isolariciresinol. The sample pretreatment method is briefly described as follows: 100 mg of sample was hydrated, and the internal standards were added and incubated overnight with a hydrolytic reagent containing *Helix pomatia* juice extract (BioSepra S. A., Cergy-Saint-Christophe, France) and further extracted with diethyl ether. The organic phase was stored, and the water layer underwent acid hydrolysis (0.6 M HCl, 2 h at 70 °C) and extraction with diethyl ether:ethyl acetate (1:1, v/v). After the organic phase was removed and stored, a second stronger hydrolysis (1.5 M HCl, 1 h at 100 °C) was performed on the water residue to completely release the aglycone from the glucoside forms. After a final ether extraction with diethyl ether: ethyl acetate, the pooled organic extracts from the three different hydrolytic steps were purified by means of three consecutive chromatographies: methanolic extracts of the sample were applied to Lipidex 5000 (PerkinElmer, Boston, MA) following previously published conditions (*3*) and further purified by two ion exchange chromatographies as reported (*16*). Finally, the purified extracts were derivatized with 100 μL of QSM (pyridine/HMDS/TMCS, 9:3:1), redissolved in hexane, and injected into the gas chromatograph under the following conditions: Fisons GC 8000 (Fisons Instrumentation, Inc., Milan, Italy) coupled with a Fisons MD 1000 quadrupole mass spectrometer (Fisons Instrumentation, Inc., Manchester, U.K.), equipped with a 12 m × 0.22 mm i.d., 0.25 μm BP-1 capillary column (SGE International Pty Ltd., Ringwood, Australia); flow rate of helium carrier gas, 1 mL/min; oven temperature program, 150 °C (for 1 min) increased at 40 °C/min to 240 °C, increased at 3 °C/min to 280 °C kept for 1 min, and finally increased at 10 °C/min to 290 °C and kept for 8 min. The temperatures of the injection port, ion source, and interface were 280, 200, and 250 °C, respectively. The injection volume was 1 μL. If

Table 2. Levels of Daidzein, Glycitein, and Genistein and Total Isoflavone Content of the Soy-Based Health Products and Soybeans and Concordance with Declared Values

item	daidzein (mg/g)[a]	glycitein (mg/g)[a]	genistein (mg/g)[a]	actual total isoflavone content per piece[b] (mg)	stated total isoflavone content per piece[c] (mg)	difference between values (%)	daily intake[d] (mg)
1	12.1 (6)	1.28 (5)	18.5 (6)	13.7	12.5	+9.60	27.4
2	18.0 (5)	7.09 (2)	3.79 (1)	13.0	15.5	–16.1	26.0
3	3.37 (1)	2.06 (13)	1.35 (9)	4.27	7.78	–45.1	4.27–17.1
4	3.89 (6)	0.58 (7)	6.30 (6)	8.61	12.5	–31.1	17.2
5	4.10 (4)	0.56 (2)	6.62 (3)	12.4	12.5	–0.80	24.8
6	21.5(5)	2.18 (3)	22.1 (4)	28.4	24.9	–12.3	
7	15.0(2)	1.83 (3)	42.2 (3)	16.7	30.0	–44.3	
8	8.16 (3)	0.69(4)	13.9 (8)	16.4	17.5	–6.28	32.8
9	0.03 (3)		0.03 (3)	0.10			0.20
10	20.9(1)	6.71(1)	5.13 (1)	13.9	14.0	–0.71	27.8
11	12.8 (2)	0.83 (2)	54.1 (2)	30.5	31.1	–1.92	
12	3.66 (9)	1.51 (10)	0.93 (9)	3.35	5.61	–40.2	
13	0.74 (3)	0.10 (1)	0.32 (3)	0.22			0.66
14	1.74 (9)	0.37 (8)	0.32 (9)	1.26	5.20	–75.7	
soybean[e]	0.39, 0.19–0.59	0.04, 0.02–0.08	0.49, 0.28–0.70				

[a] Values are means (CV) of triplicate analyses. [b] Sum of isomers expressed in aglycone equivalents. Piece refers to tablet or capsule. [c] Value given by the producer and further converted to aglycone equivalents. [d] Following recommendations given by the producer (calculated from **Table 1**). [e] Values are means, 95% CI of five different samples of commercial soybeans, triplicate analyses.

not mentioned, all reagents were from major suppliers and with the highest purity available.

Statistics. Data treatment was performed by SPSS for Windows 11.0 (SPSS, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Individual values for daidzein, glycitein, and genistein expressed as aglycone equivalents and total isoflavone levels found in the 14 soy-based preparations are presented in **Table 2** together with the stated amount given by the producer and the calculated discrepancies between the values. Calculating the average of the single isoflavones in each of the products (95% CI) results in higher levels of genistein (3.76−21.3) than daidzein (5.02−12.9) and glycitein (0.79−3.17). The total isoflavone levels of the different products varied from 0.10 mg/piece for item 9 to 30.5 mg/piece for item 11 (6.60−16.6). The calculated daily intakes among the products commercialized with dosage recommendations varied from 0.20 mg/day for item 9 to 32.8 mg/day for item 8.

A total of seven plant lignans were identified in all of the samples using reference compounds. A representative multiple ion monitoring chromatogram corresponding to item 1 is presented in **Figure 2**. Matairesinol was not detected in items 9, 12, 13, and 14, and similarly, lariciresinol and pinoresinol were not identified in items 9, 13, and 14, respectively. Quantification of the levels in the different samples gave syringaresinol as the most abundant lignan in 50% of the samples followed by isolariciresinol (21% of the samples). When the average levels (μg/g) were calculated among the different preparations, syringaresinol appears at the highest concentrations (14.5−42.0) followed by pinoresinol (8.44−37.1), secoisolariciresinol (5.12−25.4), isolariciresinol (4.21−22.9), lariciresinol (7.62−20.1), and matairesinol (0.04−0.18). The total lignan concentrations varied between 0.58 μg/g for item 9 and 327 μg/g for item 6 (41.3−137). Results are collected in **Table 3** together with the calculated daily dosage for comparison between preparations.

The presence of plant lignans in commercially available soy-based health supplements is reported here for the first time together with the isoflavone values. Information about the products was found in the packages, in leaflets, or on the web page of the manufacturers/distributors. If not explicitly declared, data for the total isoflavone content were calculated from the ingredient list statement and expressed as aglycone equivalents. Nature, nomenclature, and composition vary widely within the different preparations; they are described as containing isoflavone concentrates, soy protein isolates, soy protein concentrates, soy germ extracts, or fermented concentrates of soy protein and are commercialized as natural products, dietary supplements, or herbal extracts. For this study, and in order to simplify all of the nomenclature, the supplements were divided into soy protein-based products (S) or isoflavone concentrates (I), depending on whether the product was advertised to contain whole soybean or soy protein concentrates or isolates or whether isoflavone concentrates were added to the formulation (**Table 1**). In contrast to the composition, the alleged health benefits after consumption of these products are more homogeneous. All of the products, except items 3 and 7 for which no specific health benefit is attributed to their consumption, and item 4 which is commercialized to improve men's health, have some mention in their advertisement leaflets about the ability of isoflavones to improve menopausal symptoms and/or related diseases by acting as a natural alternative to hormone (estrogen) replacement therapy and the general maintenance of perimenopausal women's health. Recent studies have shown that the consumption of a minimum of 30 mg of isoflavone per day both in the form of soy protein or isoflavone isolate form reduces hot flushes frequency in a modest 10−20% once the placebo effect is subtracted (*18*). The North American Menopause Society (NAMS) has recently stated that the results achieved so far from clinical trials are insufficient to either support or refute efficacy for soy foods or isoflavone supplements in the treatment of menopause-associated vasomotor symptoms (*19*).

As it has been shown previously (*20*−*22*), differences between the declared amount and the actual concentrations of isoflavones are very large in some cases (**Table 2**), but these differences were not statistically significant (paired samples *t*-test, $p = 0.09$) in our study. Only for items 5 (Finland), 8

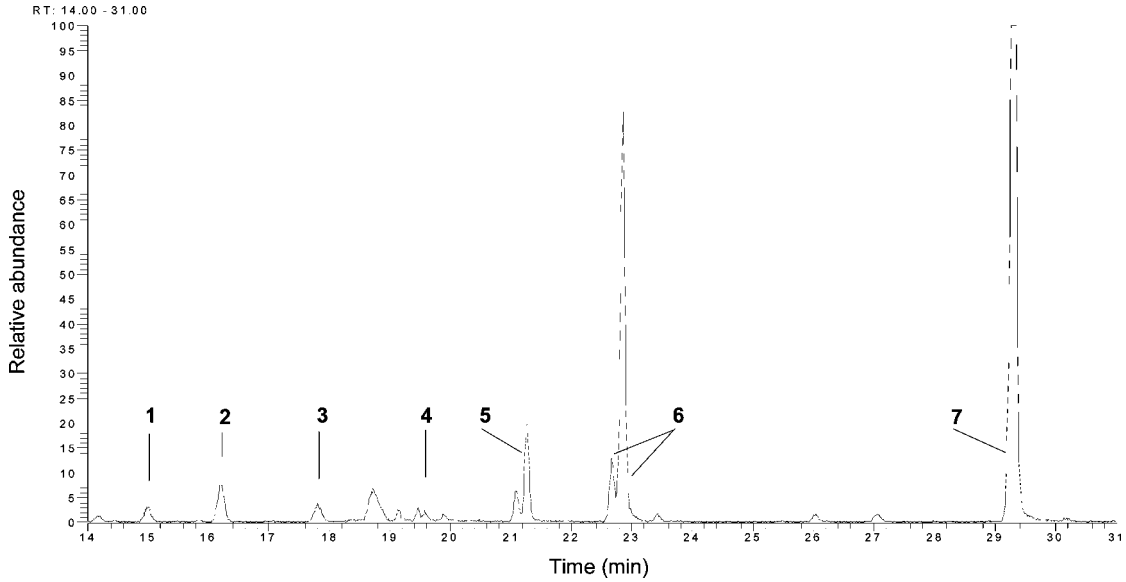

Figure 2. Multiple ion monitoring chromatogram of item 1. Identification of plant lignans **1** (*m*/*z* 488, 14.9 min); **2** (*m*/*z* 558, 16.1 min); **3** (*m*/*z* 560, 17.8min); **4** (*m*/*z* 502, 19.6 min); **5** (*m*/*z* 576, 21.3 min); **6** (*m*/*z* 502, 22.8 min); and **7** (*m*/*z* 562, 29.3 min).

Table 3. Plant Lignan Levels of the Soy-Based Health Products and Soybeans

	individual lignans (μg/g)[a]						total lignans		
item	secoisolariciresinol	matairesinol	isolariciresinol	lariciresinol	pinoresinol	syringaresinol	μg/g	μg/piece	daily intake[b] (mg)
1	15.8 (2)	0.08 (8)	19.8 (10)	14.4 (3)	19.8 (1)	48.3 (1)	118	50.8	0.10
2	18.9 (1)	0.10 (7)	8.99 (10)	16.7 (3)	8.81 (1)	50.7 (1)	104	46.9	0.09
3	5.21 (2)	0.01 (7)	2.50 (5)	7.01 (2)	3.68 (6)	17.9 (2)	36.4	22.9	0.09
4	12.0 (1)	0.06 (6)	22.3 (1)	12.3 (1)	20.3 (1)	20.2 (1)	87.2	69.8	0.14
5	11.1 (1)	0.06 (8)	23.7 (10)	12.2 (16)	21.1 (1)	19.3 (10)	87.6	96.4	0.19
6	74.2 (2)	0.32 (1)	68.8 (9)	41.7 (1)	63.3 (7)	78.3 (1)	326	202	
7	32.2 (7)	0.46 (11)	3.82 (7)	33.9 (6)	92.1 (10)	74.8 (9)	237	66.9	
8	8.93 (2)	0.03 (15)	14.8 (1)	13.6 (1)	15.8 (1)	16.5 (1)	69.7	50.2	0.10
9	0.06 (5)		0.22 (12)		0.10 (9)	0.19 (9)	0.58	0.95	0.002
10	22.9 (3)	0.08 (8)	12.0 (4)	14.7 (2)	9.91 (2)	43.4 (1)	103	43.9	0.09
11	4.46 (11)	0.16 (11)	8.08 (10)	2.41 (9)	16.1 (4)	10.7 (10)	42.0	18.9	
12	3.24 (3)		2.43 (5)	4.59 (2)	2.05 (3)	10.2 (5)	22.5	12.4	
13	1.86 (2)		1.15 (6)	3.04 (1)		2.40 (8)	8.45	1.61	0.005
14	2.33 (1)		1.25 (3)	3.21 (2)		2.67 (1)	9.47	5.30	
soybean[c]	3.34, 1.93–4.75	0.02, 0–0.04	6.10, 3.33–8.87	2.87, 1.23–4.51	4.46, 2.87–6.05	3.82, 3.07–4.57	20.6		

[a] Values are means (CV) of triplicate analyses. [b] Following recommendations given by the producer (calculated from **Table 1**). [c] Values are means, 95% CI of five different samples of commercial soybeans, triplicate analyses.

(United States), 10 (France), and 11 (Finland), the values obtained closely matched with those provided by the manufacturer.

The profile of plant lignans in soy-based health products reflects that from the original soybean (**Table 3**). Among the identified lignans, syringaresinol was the most abundant plant lignan in the majority of the samples, whereas matairesinol appears at the lowest concentration in all of the samples. Secoisolariciresinol and matairesinol have been quantified in a number of foods including cereals, oilseeds, nuts, beans, vegetables, and beverages such as coffee, tea, and wine (*23*−*26*). These results have been collected into phytoestrogen databases (*27*−*29*). Only with the exception of flaxseed that contains predominantly, but not only (*30*) secoisolariciresinol, the total lignan content in foods does not correspond to the sum of secoisolariciresinol and matairesinol. Phytoestrogen databases largely underestimate the total lignan content of foods other than flaxseed; therefore, the predicted levels of the mammalian lignans enterolactone and enterodiol that can be achieved after a certain meal are, in most situations, lower than the actual levels (*31*). As new plant lignans are being identified as precursors (*3*), it is necessary to quantify them and update the databases. Although new precursors have been recently identified in flax and pumpkin seeds (*30*, *32*), quantified in rye and wheat bread-based animal diets (*33*), and even analyzed in selected wines (*34*), no studies have reported so far the levels of new plant lignan values in human foods. Therefore, the comparison of the amount of lignans provided by the soy-based health products, with the lignan levels present in food sources, is not possible to any satisfactory extent.

Roughly compared, the average total lignan concentration of the 14 soy-based health products (89.5 μg/g, 41.3−137) is four times higher than the average total lignan concentration found in five different samples of soybeans (20.6 μg/g, 18.3−22.8) (**Table 3**). This difference describes the concentration of phytochemicals that may take place during the manufacturing process. There is, however, a remarkable variation in the individual and total lignan values found between the different products.

To estimate the association between the type of formulation and the total lignan content (in μg/g) of the health products, nonparametric analysis of variance (Shaphiro−Wilk statistic

0.820, $p = 0.009$) was used. Concentrations were significantly ($p = 0.013$) higher in samples classified as isoflavone concentrates (I) than in those classified as soy protein-based products (S). The amount of total lignans in the different preparations varies largely and in a similar way as do the isoflavone levels. This association was further estimated to be positively correlated (nonnormally distributed data) both in normal (Spearman's ρ (r_s) = 0.629, $p < 0.01$) and logaritmic scale ($r_s = 0.807$, $p < 0.01$). Furthermore, excluding item 11 in which the total isoflavone:lignan ratio differs from the rest of the compounds, a better correlation could be achieved, although explanations for this deviation cannot be inferred from the product information. Therefore, we can conclude that the amount of soy lignans is related to the amount of soy isoflavones in the soy-based health products and that they are more abundant in the products labeled as isoflavone concentrates than in products containing whole soy extracts.

Mammalian lignans have been reported to be minor metabolites of syringaresinol, and isolariciresinol is not converted to enterodiol or enterolactone (*3*). Nevertheless, almost half of the plant lignan content in soy-based health supplements corresponds to dietary precursors of mammalian lignans; therefore, the appearance of enterolactone and enterodiol after their consumption should be taken into account when health benefits are attributed to these preparations. Regular consumption of soy-based health products might increase the levels of mammalian lignans in plasma and urine to those related with a lower risk of developing chronic diseases. Unfortunately, this cannot be generally stated for all of the products in the present study because of the considerable variation in the levels, the average value being 49 μg/piece (21.7−76.8).

In conclusion, results showed a large variation of lignan concentrations among the products included in the study. This variation was positively correlated with the total isoflavone content for which these products are commercialized. Additionally, higher lignan concentrations can be found in those products labeled as isoflavone isolates as compared with those labeled as soy protein-based products; therefore, we can conclude that during the manufacturing process of isoflavone concentrates or soy protein-based products, lignans are also extracted and that they are present in the final product in considerable amounts. The lignan content of the soy-based health supplements should be taken into consideration when discussing and evaluating the possible health effects of the supplements.

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