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Content of phytoestrogens in soy-based dietary supplements

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ABSTRACT

The isoflavone content of 14 soy-based dietary supplements intended to help alleviate perimenopausal and menopausal symptoms on sale in Italy were analysed using HPLC with UV detection. The aim was to quantify soy isoflavones after hydrolysis as aglycones, which are the bioactive part of isoflavone molecules. In the examined products, the amounts of isoflavones were frequently expressed ambiguously, and none of the products stated whether the isoflavone content of the product was expressed as aglycones or as conjugates. Each product revealed a different aglycone concentration profile. These supplements have different "fingerprints", probably due to different sources of raw materials and to methods used in processing and preparation of extracts. In more than half the supplements tested, the actual values contained were below those stated and below those expected to help alleviate perimenopausal and menopausal symptoms.

1. Introduction

Until a few years ago, hormone replacement therapy (HRT) was commonly prescribed for the relief of menopausal symptoms. However, reports from recent studies into the long-term effects of HRT and its possible relation to increased risk of endometrial and breast cancer and arterial and venous disease, have put some women off this type of treatment (Beral, Bull, & Reeves, 2005; Rossouw et al., 2002). Not surprisingly, increasing numbers of women are looking to complementary therapies, such as dietary supplements and herbal preparations, for the management of menopausal symptoms.

The main ingredients of the supplements on sale are phytoestrogens. The term phytoestrogens refers to any plant substance or metabolite that induces biological responses in vertebrates and can mimic or modulate the actions of endogenous estrogens, usually by binding to estrogen receptors. Substances that are classified as phytoestrogens include, in particular, isoflavones and lignans. Isoflavones belong to the polyphenol group and are present in significant concentrations in a variety of seeds and other parts of many plant species belonging to the *Leguminosae* family. Soybeans, alfalfa, clover and chickpeas have all been found to contain a range of isoflavones, and a large number of isoflavone dietary supplements contain extracts from soy as their principal ingredients.

In soy products, the principal isoflavones genistein, daidzein and glycitein are present in differing ratios and may be found as glucosides or as etherified glucosides or even in their free form as aglycones, depending on the methods used in the processing and preparation of extracts (Delmonte & Rader, 2006; Li, 2000). The phytoestrogen content in soy-based supplements is frequently declared by the manufacturer as the sum of the three isoflavones; in some cases quantitative information on the content of individual isoflavones is also given, but in others it is not clear whether the stated content refers to the sugar conjugate or to the free isoflavone aglycone. If any health benefit is to be expected as a result of taking soy supplements, these relate exclusively to the active part of the isoflavone molecule, which is the aglycone without sugar moiety (Eisenbrand, 2007; Setchell et al., 2001; Wuttke, Jarry, & Seidlová-Wuttke, 2007). This study analysed the isoflavone content of 14 soy-based dietary supplements intended to help alleviate perimenopausal and menopausal symptoms on sale in Italy, using HPLC with UV detection; the aim was to quantify the amounts of soy isoflavones after hydrolysis as aglycones, which are the bioactive part of the isoflavone molecules.

2. Materials and method

2.1. Standards, reagents and samples

The phytoestrogen standards of genistein, daidzein and glycitein were purchased from Sigma (St. Louis, Mo., USA), and genistin and daidzin were purchased from Fluka (Buchs SG, Swiss). All reagents, solvents and chemicals were of analytical or HPLC grade, and were obtained from Sigma or Serva (Heidelberg, Germany). Ultrapure water was prepared using a Milli-Q System (Millipore

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S.A., Molshein, France). Fourteen soy-based dietary supplements were selected from amongst the most representative brands, and only one lot of each product was purchased at a local supermarket. The supplements also contain the following, in different amounts and combinations: vitamins (B_6, C, D_3 and E), lactobacillus, fish oil, glucosamine, chondroitin sulphate and other botanicals containing antioxidants or phytoestrogens with lower estrogenic activity. The majority of supplements declared "soy isoflavones"; only three products labelled isoflavones content generically or from *Pueraria lobata*.

2.2. Determination of genistein, daidzein and glycitein

The HPLC method used in this study to separate and quantify genistein, daidzein and glycitein in soy supplements was based on the work of César et al. (2006) with soy dry extracts.

Commercial samples were ground to a fine powder and 100 mg were extracted with 100 ml of 3 M HCl ethanolic solution instead of the 50 mg in the original report. After sonication for 5 min, the solution was put into a steam bath for 40 min. The sample solution was then diluted 1:5 with the mobile phase, composed of 0.1% acetic acid and methanol (52:48).

As regards standard solutions, 10 mg of each reference substance were prepared in 10 ml of methanol; 1 ml from each stock solution was subsequently transferred to a volumetric flask (25 ml), which was filled with the mobile phase.

The HPLC analyses were performed with a Perkin Elmer Series 200 system equipped with a gradient pump, thermal chamber for the column, UV detector (at 254 nm) and Turbochrom Navigator version 6 software (Perkin Elmer, Shelton, USA). The injection volume was 20 μl. The column used was a C18 endcapped Lichrospher (5 μm, 250 × 4.6 mm) from Grace (Deerfield, Il, USA) at 40 °C.

2.3. Quality control of the method

The linearity of response was estimated in triplicate at six concentration levels within the range of 0.25–20 μg/ml, and the correlation coefficients (r^2) were 0.9999 for genistein, 0.9996 for daidzein, and 0.9998 for glycitein. Six replicates of each sample were analysed at the same time to assess the repeatability, and RDSs were 3.3% for genistein, 2.8% for daidzein and 5.3 for glycitein. The recoveries were obtained after the addition of standard compounds: 94% was the value for genistein and daidzein, glycitein was found at 100%. The lower standard solution concentration, which produced peak areas with RDS below 2.0%, was used as the limit of quantification. This quantification limit corresponds to an amount of 0.12 g/100 g in the sample for all isoflavones.

3. Results and discussion

The soy isoflavones in dietary supplements were quantified after hydrolysis as aglycones because these are the bioactive part of the isoflavone molecule. Fig. 1 shows the chromatograms of a standard solution containing the soy isoflavones, both in aglycone (genistein, daidzein, glycitein) and in glycone (genistin and daidzin) form, and of a sample after acid hydrolysis. To evaluate the efficacy of hydrolysis described in our reference method, one sample was analysed with and without treatment with hydrochloric solution. In the sample analysed without hydrolysis the amount of glycones plus aglycones (expressed as aglycone equivalents

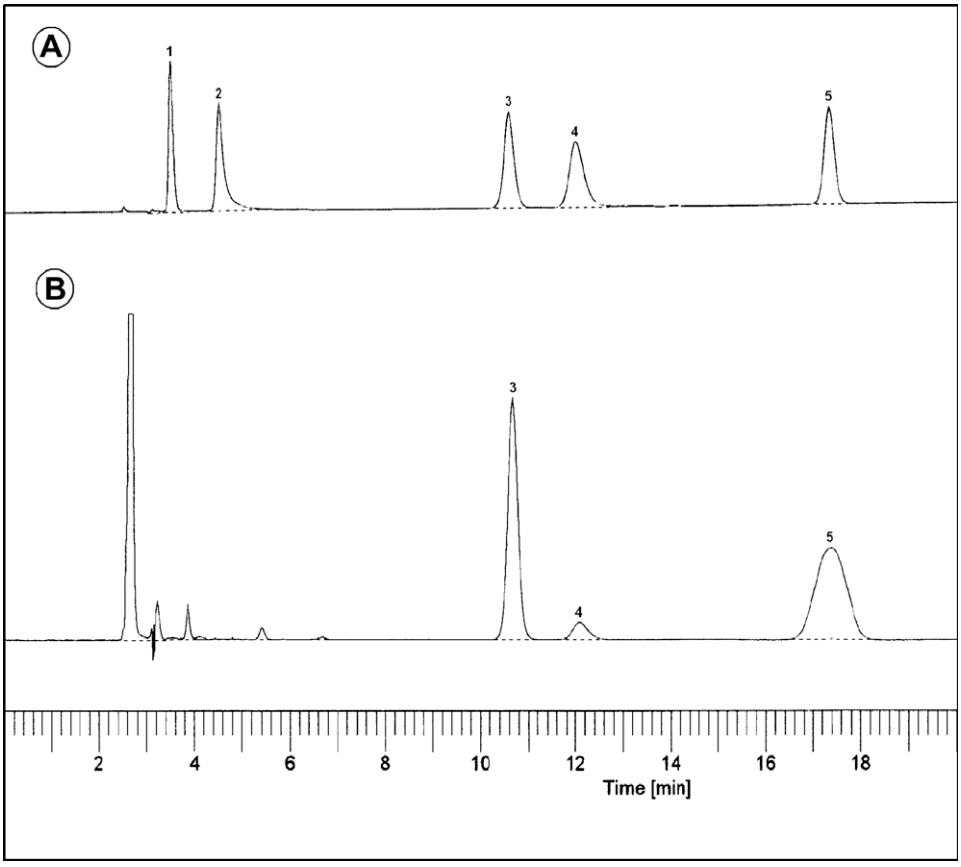

Fig. 1. HPLC chromatogram of isoflavones in standard mixture (A) and in soy-based dietary supplements after hydrolysis (B). Numbers of peaks are as follows: (1) daidzin, (2) genistin; (3) daidzein; (4) glycitein and (5) genistein.

Table 1
Isoflavone contents of tested products (g/100 g of sample).

Product	Genistein	%	Daidzein	%	Glycitein	%	Total isoflavones as aglycones	Total isoflavones stated on label	Percentage difference from label (%)
1	2.00	51	1.79	46	0.14	3	3.93	4.82	−18
2	1.72	43	2.10	53	0.16	4	3.98	7.00	−43
3	2.30	43	2.27	43	0.72	14	5.29	7.00	−24
4	1.49	53	1.16	41	0.14	6	2.79	11.03	−75
5	0.21	13	0.92	58	0.46	29	1.59	14.70	−91
6	2.41	61	1.41	36	0.12	3	3.93	10.67	−63
7	8.53	95	0.42	5	n.d.	0	8.95	6.67	+34
8	12.15	86	1.02	7	0.96	7	14.13	11.94	+18
9	0.26	21	0.69	55	0.30	24	1.25	1.25	0
10	0.48	42	0.67	58	n.d	0	1.15	13.3	−91
11	9.46	58	6.14	38	0.66	4	16.26	17.78	−9
12	0.73	12	4.07	67	1.32	22	6.11	8.20	−25
13	3.72	39	4.77	51	0.95	10	9.43	8.00	+18
14	n.d	0	n.d	0	n.d	0	n.d	0.33	–

n.d < 0.12 g/100 g quantification limit.

using molecular weight) and the amount of aglycones obtained after hydrolysis did not differ in the range of repeatability of the method. In order to evaluate the stability of free isoflavones after hydrolysis, one sample containing only aglycones was also analysed, and again there was no difference before and after hydrolysis in the range of repeatability. (Data not shown.)

With regard to the 14 commercially phytoestrogen supplements analysed, Table 1 shows data expressed as g/100 g relating to the individual concentrations of aglycones, the total isoflavones as aglycones, and the respective levels of isoflavones stated by the manufacturer on the label, with percentage difference between assayed and declared levels. The percentages of genistein, daidzein and glycitein in relation to 100% of total isoflavones are also reported. Each of the 14 products revealed a different profile in aglycone concentration, with only genistein being effectively present in products 7, 8 (about 90%), while the amount of this substance is very low in samples 5, 12 (about 10%), and it is absent from supplement 14. Daidzein is the prevalent isoflavone in products 2, 5, 9, 10, 12, 13 (51–67%), but the amount is minimal in samples 7, 8 (⩽7%).

It was evident that these supplements, in line with other studies (Nurmi, Mazur, Heinonen, Kokkonem, & Adlercreutx, 2002; Stürtz, Lander, Schmid, & Winterhalter, 2008), have different "fingerprints", probably on account of different sources of the soy raw materials and the methods of processing and preparation of extracts. The variations in content and composition of isoflavones in soybeans have been investigated by several researchers (Franke et al., 1999; Nakamura, Tsuji, & Tonogai, 2000). Soy germ can provide a threefold increase in isoflavone content compared with the whole bean; the ratios between the three isoflavones daidzein, genistein and glycitein vary from 7:10:1 in soya flour to 4:1:3 in soy germ (Clarke & Lloyd, 2004). However, the ingredients stated on the labels of half of the products (1–3, 7–9 and 13) were specified as "extract of soy seed", while the remaining supplements declared "soy isoflavones"; the majority of supplements labelled the title of isoflavone extract, with the exception of products 10,12 and 14.

Table 2
The total contents of isoflavones of analysed supplements and values given by manufacturers (mg as aglycones/day).

Product	Total isoflavones as aglycones	Values given by the manufacturer
1	27.51	33.75
2	33.83	60.00
3	44.96	60.00
4	33.48	68.00
5	5.40	50.00
6	29.47	80.00
7	107.40	80.00
8	94.67	80.00
9	20.00	(Not given)
10	6.90	80.00
11	73.17	80.00
12	54.99	70.00
13	94.30	80.00
14	–	40.00

Samples 7, 8 and 13 contain higher levels of total isoflavones, as aglycones after hydrolysis, than stated by the manufacturer, while sample 9 contained the same quantity as that declared. In four products the levels of total isoflavones as aglycones ranged from −9% to −25% compared with the values stated by the manufacturer. The remaining products contained much less than the amounts stated on the labels, between −63% and −91%. A possible explanation for these discrepancies could be that the producer indicated isoflavones as conjugate form, and the weight of the glucose unit effectively accounts for approximately 40% of the total glucoside weight, in the malonyl and acetyl forms the weight of inactive sugar moiety increases to 50% (Clarke, Bailey, & Lloyd, 2008). Our findings are in accordance with the results obtained by Nurmi et al. (2002); they reported that the isoflavone contents in soy-based supplements (expressed, after hydrolysis, as aglycones) were mainly lower than the values given by the producers. On the contrary, Stürtz et al. (2008) quantified the intact isoflavones (glycosides forms) in different supplements and observed values higher than those declared on the label. These results point out the need for a common rule for the declaration of isoflavone contents in dietary supplements.

Soy is recognised as the major dietary source of phytoestrogens and soy-based products have been shown to contain significant quantities of total isoflavones, with soybeans and soy flour containing the highest quantities; fermented soy products, including miso and tempeh, also contain high concentrations of these compounds. In Western industrial countries, soy products are not traditional foods and the average daily intake is less than 2 mg isoflavones/day. In contrast, soy products are part of the traditional diet in Asian countries, and daily isoflavone intake is about 15–50 mg (Eisenbrand, 2007). Intake of soy and isoflavones in the Asian diet is often cited as a possible explanation of measurable health benefits, and clinical studies are conducted at similar levels of exposure.

As regards climacteric-associated complaints, one recent review of 31 placebo-controlled clinical studies of the effects of soy protein/isoflavones showed that beneficial effects are either very weak or barely noticeable, and that only nine double-blind placebo-controlled trials documented any significant relief of symptoms, with daily doses that varied from 34 to 150 mg (Wuttke et al., 2007).

The total amounts of isoflavones as aglycones for daily doses, i.e. tablets, capsules or grams are calculated on the basis of experimental results and reported in Table 2 together with the values stated by the manufacturers. The effective daily intake at the suggested dose is lower than the declared dose in more than half the products tested.

4. Conclusion

The amounts of isoflavones contained in the analysed products were frequently expressed in an ambiguous manner, and none of the products specified whether the isoflavone content stated on the label was expressed in aglycones or in conjugates. The isoflavone contents expressed as aglycones in analysed samples often do not coincide with the values stated on the labels. It is to be recommended that the contents be expressed in a common way, so that the amounts of the isoflavones can be easily evaluated and compared.

However, the isoflavone contents are extremely variable and about half of the supplements supply doses below the values (34–150 mg) that appear to have some effects in alleviating perimenopausal and menopausal symptoms. For this reason it is also important to standardise the amount of isoflavones present in these products.

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