Short Communication

Low aglycone content in commercial soy drink products

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The effectiveness of soy isoflavones to prevent bone loss in postmenopausal women is controversial. While consumption of soy in Vietnam is very high, we recently reported a prevalence of osteoporosis comparable to that of many Western populations. In the present study, we analyzed the isoflavone content of soy drink products commercially available in Vietnam and Sweden, and we also compared these products to "home-made" soy drink from beans of different origin. The amounts of the bioactive aglycones (daidzein, glycitein and genistein) and their glycoside isomers were quantified by high-pressure liquid chromatography. We found that the total isoflavone content was low in all preparations, around 70-100 mg/L and of this only 10% were bioactive aglycones. Of these, the Vietnamese products contained significantly lower levels of glycitein than the products from Sweden and "home-made" soy drink preparations. The results show that consumption of several liters of soy drink per day would be needed to achieve threshold levels for a protective effect on bone. There was no significant association between total protein and isoflavone content in different products. Accurate labeling of soy drink and other products eg of aglycone and glycoside content would allow health professionals and researchers to better explore the possible benefits of soy in dietary intervention studies.

Key Words: isoflavones, HPLC, soy drink, osteoporosis, Vietnam

INTRODUCTION

Osteoporosis and its consequence of fracture represent a global public health problem, associated with increased mortality, concomitant morbidity, and reduced quality of life. Plant derived phytoestrogens may offer a cheap and non pharmacological alternative for the prevention and treatment of bone loss.[1] The most important biologically active phytoestrogens (aglycones) recognized are daidzein, glycitein and genistein and their inactive glycosides daidzin, glycitin and genistin.[2] The chemical structure of these isoflavones is similar to that of estrogen.

It is often assumed that Asian populations have a lower rate of osteoporosis because of a high phytoestrogen content in their traditional diet.[3] In Vietnam it has been estimated that between 200-300 thousand tons of local soy bean are consumed every year in eg soy drink, tofu and soy sauce.[4] Still, the prevalence of osteoporosis in Vietnam is high, and comparable to that of many Western populations.[5]

The effectiveness of soy isoflavones to maintain bone in postmenopausal women is controversial, since inhibition of bone resorption, stimulation of bone formation and no significant effects have been reported.[6-7] The minimal threshold intake of dietary phytoestrogen to exert significant effects on bone is between 90 to 120 mg per day of aglycones.[8] In the present study, we analyzed the isoflavone content of soy drink products commercially available in Vietnam and Sweden.

METERIALS AND METHODS

Commercial soy drink products

Three different brands of soy drinks are commercially available in Vietnam (VN1-Vinamilk®; VN2- Vinasoy®; VN3-Unisoy®) and three from Sweden (S1-Provalmel®; S2-Gogreen®; S3-Alpro®) were purchased from local supermarkets. All products were manufactured within the last 30 days.

"Home-made" soy drink prepared in the laboratory

Soy drink was prepared from beans of different origin R1-2 (US); R3 (UK) and R4 (Vietnam). One hundred g of whole dry soybeans and 1000 mL of tap water were mixed in a common soy drink homogenizer (Soja-queen, Miko, GmbH, Frankfurt, Germany). The original processing time for homogenizing and heating at 80℃ was 18 min (R1). For preparations R2-4, heating was prolonged until 3 hours. Each soy drink preparation was freeze-dried in 10 mL aliquots until extraction.

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Isoflavone extraction from soy drink

Isoflavone calculation was based on 10 mL of soy drink. Samples were extracted in 50 mL of 80% methanol heated at 80℃ and simultaneously shaken at 100 rpm in water bath (Heto, Denmark) for 60 minutes.

All samples were immediately filtered (Munktell's Swedish paper No 8) into disposable tubes, and centrifuged at 300×g for 20 minutes. The solvent was evaporated under a steam of nitrogen at 55-60℃ to complete dryness. Dried samples were kept at -20℃ until analysis by reverse phase high-pressure liquid chromatography (HPLC).

Prior to injecting into the column, sample extracts were re-suspended in 1 mL of acetonitril 16%, vortex-mix and centrifuged at 13000 x g for 20 min. 500 µL of supernatant of each sample was transferred into a clean HPLC vial and analysed for their aglycone and glycoside concentrations.

HPLC quantification of isoflavones

HPLC was carried out on a 4 mm×150 mm C18 reverse phase column (ReproSil-Pur Column C18-AQ), 5 mm packing (150 mm×4 mm ID, 70306-21004, R15.AQ. S1504, Dr Maisch HPLC GmbH, Ammerbuch-Entringen, Germany) with an auto sampler (Basic Marathon type 816, Netherlands) to inject 50 µl of sample and flow rate of 0.8 mL/min. Mobile phase was prepared as follows: Solvent A, 0.1% (V/V) acetic acid in water; Solvent B, 0.1% (V/V) acetic acid in 90% HPLC-grade Acetonitrile. The solvents were filtered through a 0.45 µm filter. Detection was by UV absorbance at 250 nm. The peak areas were integrated for quantitation by CSW32 chromatographic station program for Windows. The retention times (min) of our standards were as follow: daidzin (12.18); glycitin (12.44); genistin (14.22); daidzein (18.74); glycitein (19.13); and genistein (24.23) (Figure 1). Isoflavones were identified by comparing spectral data retention times to those of standard references.

Isofavones standards

Daidzin ($C_{21}H_{20}0_9$) from Fluka; daidzein ($C_{15}H_{10}0_4$), genistin ($C_{21}H_{20}0_{10}$), genistein ($C_{15}H_{10}05$), glycitein ($C_{16}H_{12}0_5$) from Sigma; glycitin ($C_{22}H_{22}0_{10}$) from Fisher; The internal standards formononetin from Fluka. All these standards were purchased from Sigma-Aldrich Fine Chemicals, St.Louis, MO, USA.

Calculation of isoflavone levels

The amount of isoflavone present was calculated from the sample peak area calibrated against the peak area of standards (Figure. 1). Formononetin was used as an internal standard. The total isoflavone content was calculated as the sum of 6 isoflavones (daidzin, daidzein, glycitin, glycitein, genistin and genistein). All data were mean values from duplicate measurements. The intra-assay variation (n=35) was 2.1% and the inter-assay variation (n=35) was 3.1%.

The theoretical maximum yield of aglycone after total transformation of its glycoside was calculated as: amount of glycoside (mg/L)/glycoside MW×aglycone MW.

Statistical analysis

All data were expressed as mean from duplicate measurements. Differences between products from Vietnam, Sweden and "home-made" soy drink were analyzed by Kruskal-Wallis ANOVA followed by Mann-Whitney U-test. Correlations were assessed using Spearman's rank-order correlation. A p-value < 0.05 was considered statistically significant.

RESULTS

The content of isoflavones and proteins for each product are shown in Table 1. There were no significant differences between groups. There was also no significant correlation between the amount of total protein and the isoflavone content in the soy drink preparations (R=0.39).

The predominant isoflavones in all products were inactive glycosides, accounting for around 90% of the total isoflavone content. The two most abundant biologically active aglycones were glycitein and genistein (Table 1). The glycitein content in the Vietnamese products, was significantly lower than both the Swedish products and the "home-made" soy drink preparations (p<0.05, respectively).

In the "home-made" soy drink preparations, the amounts of isoflavones were markedly increased after prolonged heating in the R2 preparation compared to the R1 sample, which was prepared from the same US beans

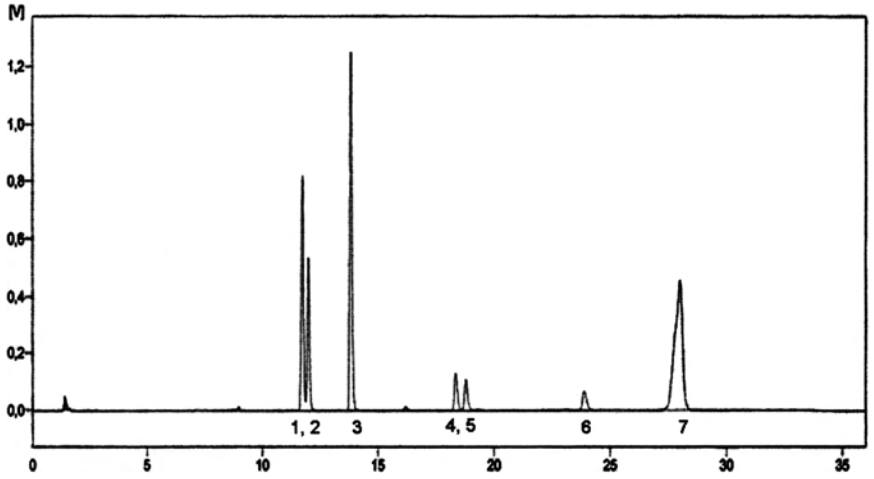

Figure 1: HPLC chromatogram of 6 different isoflavone standards and internal standard (Formononetin). Absorbance was detected at 250nm. (1) Daidzin; (2) Glycitin; (3) Genistin; (4) Daidzein; (5) Glycitein; (6) Genistein; (7) Formononetin.

Table 1. The content of separate glycosides (daidzin, glycitin, genistin) and aglycones (daidzein, glycitein, genistein) (mg/L) and total protein (g/L) of commercial soy drink products from Vietnam (VN1-3), Sweden (S1-3) and home-made soy drink prepared from beans of different origin (R1-4).

	Glycoside			Aglycone			Protein
Product	daidzin	glycitin	genistin	daidzein	glycitein	genistein	
VN1	18.34	18.29	17.32	0.84	2.65	3.77	21.42
VN2	19.19	26.08	20.10	0.81	2.60	2.17	16.03
VN3	22.89	26.89	21.45	0.65	2.54	3.10	20.79
S1	33.43	34.11	40.24	4.85	5.76	3.40	33.68
S2	21.04	22.92	20.21	1.62	3.37	4.78	29.39
S3	17.79	26.80	21.00	0.81	3.43	2.71	27.71
R1-US	15.78	38.13	27.38	1.52	5.10	3.62	23.15
R2-US	35.76	46.08	47.06	1.53	5.54	3.01	27.41
R3-UK	24.76	34.78	26.92	1.85	3.72	6.09	24.24
R4-VN	16.29	24.00	17.34	0.68	3.29	1.81	26.59

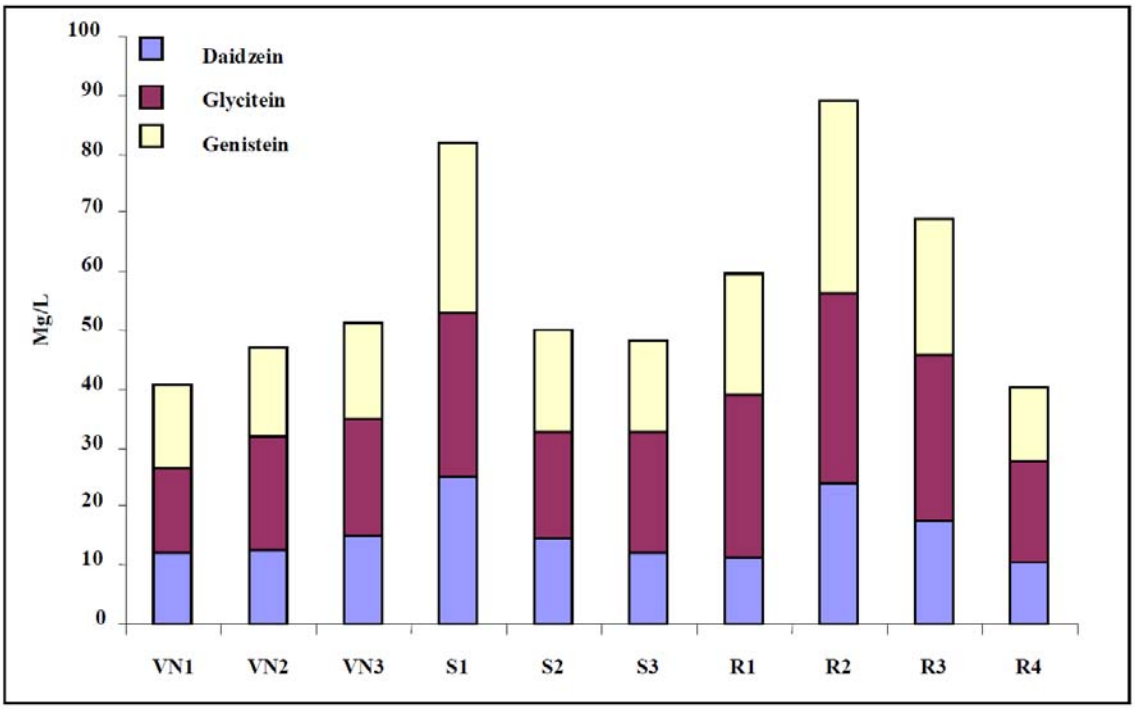

Figure 2. Calculated maximum yield of bioactive aglycones in different soy drink preparation. Calculation are based on the assumption that after ingestion there is transformation of all glycoside isomers into the bioactive compound. (VN1-3, commercial Vietnamese products; S1-3, commercial Swedish products; R1-4, "home-made" soy drink prepared at the lab from beans of different origin).

according to the standard procedure. The difference was mainly attributed to an increase in the glycoside content whereas levels of aglycones were quite similar (Table 1).

The theoretical maximum yield of aglycones after total transformation of its glycosides in all 10 investigated preparations, is shown in Figure 2**.** The "home-made" preparations had higher total isoflavone content than all commercial products, except for S1. The isoflavone distribution in the different preparations was quite similar.

DISCUSSION

While many scientific panels have now reached a consensus on the effect of phyto-estrogen on cardiovascular disease prevention,[9] the same conclusions have not been reached for prevention and treatment of osteoporosis.[10] There are conflicting data on the effectiveness of soy isoflavones on bone metabolism.[6-7] The inconsistent findings may reflect insufficient amounts of bioactive phytoestrogen ie aglycones and individual variations in sensitivity and isoflavone metabolism.[2]

In our study of ten different soy milk preparations, the mean total isoflavone content varied between 70-100 mg/L. All products contained only around 10% of the bioactive alycones, which can be absorbed directly through the intestine. Even an individual with a high capacity to metabolize the glycosides would need to consume several liters of soy drink per day in order to achieve a threshold of around 90-120 mg per day of aglycones, in order to be effective on bone.[8] Certainly, such consumption is not feasible in everyday life not even among high soy consumers as in Vietnam.

A large variety of factors may influence soy bean isoflavone content eg type of bean, crop year, growing temperature, location of agriculture, storage and processing.[11] The "home-made" soy drink (R1 and R2) was found to provide a higher total isoflavone content than most of the commercial products. For the "home-made" preparations,

the whole beans including the hull were used whereas in the industrial process VN1 was prepared after removing the hull (manufacturer personal communication). The hull of soy-bean itself has a very low isoflavone content but its removal may cause loss of the hypocotyl axis, which is a major source of soy aglycones.

We found prolonged heating for 3 hours to markedly enhance the β-glucoside content of the "home-made" soy drink preparations. In R2 as compared to R1, the total isoflavone content increased by 38%. Heating soy drink at 80℃ will convert the conjugated malonyl-glucosides into the non-conjugated β-glucosides, which have the potential for further transformation into active isomers. Also prolonged heating did not change the aglycone content, which is in agreement with previous data.[12]

The present study found considerable variation in total isoflavone content when soy drink was prepared from beans of different origins and the differences were mainly due to glycoside amounts. Previous studies have also shown significant variations in the content of active aglycones and their glycosides in different commercially available soy foods in the US, Australia and Korea.[13]

Given the increasing interest and consumption of soy products, there is an apparent need to establish universal guidelines and uniform industry standards for labeling of aglycone content. At present, consumers and health professionals are confused about what types and amounts of different soy consumables might confer any beneficial health effects. The food labeling claim for soy protein in the prevention of coronary heart disease has been approved by many health authorities e.g. in the US, UK, France, and Asian countries, but has also been seriously questioned.[14] Variations in the content of active aglycones and their bioavailable glycosides in different products could well account for many of the inconsistencies in reported outcomes from observational and clinical studies.

It is often suggested that the isoflavone content of a soy product can be deduced from information of total protein content. The present study showed that such assumptions may not be justified. We found no significant association between total protein and isoflavone content in the different commercial and "home-made" soy milk preparations.

Accurate labeling of soy foods in general, and soy drink in particular, would be a major step forward to support consumers world-wide. Such a measure would also allow health professionals and researchers to better explore the health benefits and risks of soy in dietary intervention studies.

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AUTHOR DISCLOSURE STATEMENT

All the authors declare no competing financial interests.

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越南市售黃豆飲品之低異黃酮素含量

對於黃豆異黃酮預防停經後婦女骨質流失之效益仍具有爭議。雖然越南人民攝取高量黃豆製品，但骨質疏鬆盛行率卻與許多西方國家族群相當。本篇研究分析越南與瑞典兩國市售黃豆飲品之異黃酮含量，並選取不同來源黃豆，自製成飲品，以比較它們的異黃酮含量。異黃酮含量以高效液相層析法測量，包括具生物活性的醣苷配基(aglycones)形式的異黃酮素(木質素黃酮、黃豆素黃酮與金雀素黃酮)，以及其醣苷配體異構物。結果發現，總異黃酮素含量在所有製品中皆不如預期，約 70-100 mg/L，其中只有 10%醣苷配基具生物活性。同時也發現，越南國內製造的黃豆飲品，所含的黃豆素黃酮(glycitein)，顯著低於瑞典製品以及自製的不同來源之黃豆飲品。這樣的結果顯示，每天必須喝下數公升的黃豆飲品，才能達到可預防骨質流失的黃豆異黃酮量。不同的黃豆飲品之總蛋白質含量與異黃酮量並沒有顯著之相關性。黃豆相關飲料製品應具備更精確的異黃酮素含量標示，以便未來研究者在飲食介入研究中，能更容易闡明黃豆相關製品對人體健康之益處。

關鍵字：異黃酮素、高效液相層析、黃豆飲品、骨質疏鬆症、越南