Chapter 9

Possible Risks in Caucasians by Consumption of Isoflavones Extracts Based

Maria Graça Campos and Maria Luísa Costa

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/47837

1. Introduction

With the increase in human life expectancy many health concerns start to be important, which raises the need for more and different therapeutic solutions.

One of the emerging issues that attracted the attention of research teams looking for new and better medicines is prevention of hormonal dependent tumors in both genders and the relief of climacteric symptoms in post-menopausal women, as the prevention of cardiovascular diseases (CVD).

For both of the above situations testosterone, estrona and estradiol metabolism are fundamental for a possible cardiovascular effect or a desoxinucleic acid (DNA) damage in breast cancer. Phytoestrogens, as isoflavones, in a first approach, seems to be potentially useful solutions. These compounds have the ability to mimic estrogens and induce an estrogen-like effect dependent on their affinity for alpha and beta estrogen receptors (ERs) [1], and this is related to their chemical structures.

Phytoestrogens were discovered by Bennetts *et al*, who were trying to identify the cause of a specific sheep breeding problem in Western Australia. They implicated equol, a metabolite of daidzin that is an isoflavone existing in subterranean clover pastures [2]. Four decades later, in 1984, this compound was identified in human Urine by Setchell *et al* [3] and related to consumption of soy and further implicated in a possible prevention of hormonal dependent tumors, cardiovascular diseases and osteoporosis.

At that time, the world was focused on the *French Paradox* (data collected with French population) that claimed the ingestion of antioxidants, mainly from red wine as the alcohol itself, could be a panacea for cancer prevention and cardiovascular diseases. According to data from the world's largest study of heart disease, conducted by the World Health Organization (WHO), during the past decade in 21 countries with 10 million men and

women, French heart disease statistics appeared to have been under-estimated and the "*French Paradox*" overestimated. The French rate of heart disease was actually similar to that of the Italians, Spanish, and the Germans (mainly southern Germany), but still lower than many other countries.

Despite that, other researchers looking in a different way for the same reasons made epidemiological studies on Asian populations in the '90s that revealed a possible relationship between the ingestion of soy and the prevention of hormonal dependent tumors and also a lower incidence of climacteric symptoms in postmenopausal women. These results, known as the "*Japanese Paradox*" (data collected with Japonese population), induced Western people to consume soy and soy derivatives rich in isoflavones as genistein, daidzein, glycitein and the respective glucosidic forms (genistin, daidzin and glycitin). A large number of studies have attempted to demonstrate that soy consumption decreases the risk of developing several chronic diseases, in particular, cancer, osteoporosis [4, 5], cardiovascular diseases [6], and also the relief of climacteric symptoms [7]. Up until now however, the majority of these benefits have not been proven [for review see 9].

These two epidemiologic studies are mainly focus on prevention of cardiovascular diseases and cancer, using phenolic compounds as the targets for this bioactivity. The first one, *French paradox,* fail and leave behind an important side effect, cirrhosis, in people that increase the intake of wine all over this last decades. Related to the "*Japanese Paradox*", the scientific community starts to have data enough to think that this concept needs to be evaluated with caution to prevent a future failure.

Until now, in Europe isoflavones are considered as food compounds, nevertheless they are antinutrient compounds, and is the European Food Safety Authority (EFSA) that is responsible for the risk assessment evaluation of them. They recognize the potential importance for human health of the issue of isoflavones from food digestion, but should the alleged beneficial or detrimental health effects be scientifically proven. The Isoflavones ESCO working group evaluates the relevant scientific information available. This includes *inter alia*: to assess the potential of isolated isoflavones to trigger adverse human health effects; the possible human health benefits of the use of isolated isoflavones for the general population and particularly for women with complaints during and after the menopausal period; whether there is any scientific basis for differences concerning the hazard assessment of isolated isoflavones from soy and/or red clover in comparison with soy or red clover extracts. The final document will be public and available at EFSA web site soon (http://www.efsa.europa.eu/en/esco/escoisoflavones.htm).

For this reason in the present chapter we'll not focus an overview of the isoflavones, as that is the current work of, but we'll discuss other points of view that can contribute for understanding of the topic especially for cancer and relief of climacteric symptoms. In this last point, recently (2010) Rebbeck *et al* [12] presented a study were they evaluated whether genes involved in the metabolism of steroid hormones are associated with hormone levels or menopausal symptoms. They used a population-based prospective sample of 436 African American (AA) and European American (EA) women who were premenopausal at

enrollment and were followed longitudinally through menopause. Evaluation of the relationship between steroid hormone metabolism genotypes at catechol O-methyltransferase (COMT), cytochrome P450 (CYP) as the isoformes CYP1A2, CYP1B1, CYP3A4 and CYP19, Sulfotransferase 1A1 (SULT1A1), and SULT1E1 with hormone levels and menopausal features were carried out. The results show in EA women, SULT1E1 variant carriers had lower levels of dehydroepiandrosterone sulfate, and SULT1A1 variant carriers had lower levels of estradiol, dehydroepiandrosterone sulfate, and testosterone compared with women who did not carry these variant alleles. In AA women, CYP1B1*3 genotypes were associated with hot flashes (odds ratio [OR], 0.62; 95% CI, 0.40-0.95). Interactions of CYP1A2 genotypes were associated with hot flashes across menopausal stage ($P = 0.006$). Interactions of CYP1B1*3 ($P = 0.02$) and CYP1B1*4 ($P = 0.03$) with menopausal stage were associated with depressive symptoms. In EA women, SULT1A1*3 was associated with depressive symptoms (OR, 0.53; 95% CI, 0.41-0.68) and hot flashes (OR, 2.08; 95% CI, 1.64-2.63). There were significant interactions between SULT1A1*3 and hot flashes ($P < 0.001$) and between SULT1A1*2 and depressive symptoms ($P = 0.007$) on menopausal stage, and there were race-specific effects of SULT1A1*2, SULT1A1*3, CYP1B1*3, and CYP3A4*1B on menopause. These results suggest that genotypes are associated with the occurrence of menopause-related symptoms or the timing of the menopausal transition [10].

Isoflavones as we'll explain later in this chapter will interfere with most of these enzymes and Caucasians and Asians present polymorphic changes, in some of them, that can change the bioactive response to various situations, from compounds metabolism to cancer induction or prevention.

2. Chemistry, main food sources, bioactivity and possible toxicity of isoflavones related to ethnic differences

Isoflavones are biologically active heterocyclic phenolic compounds (subgroup of isoflavonoids) (Figure 1) that are absorbed by the intestine, circulate systemically, and are eliminated by the kidneys and liver [11]. In plants isoflavones occur predominantly as β-glucosides (genistin, daidzin, glycitin), or as acetyl-β-glucosides and malonyl-β-glucosides [12, 13]. Genistein is the more abundant isoflavone in the majority of soy products and also the most active of these compounds, and being able to interact with the estrogenic receptors [14].

Traditionally the main food sources of isoflavones are soy and other beans and pulses, and also fermented soy foods, where the glucosides have been transformed into aglycones which are absorbed more efficiently than glucosides.

In the past these have been more commonly consumed by Asian populations, but are growing in popularity in Europe. Similarly, in recent decades a new generation of soy products have entered the market (e.g. yogurts, cheeses, soy milk drinks, infant formula's) and commonly consumed food products incorporating soy flour (e.g. bakery products) and protein isolates (e.g. meat products and soy meatless products such as soy burgers). More recently, the development of nutritional supplements rich in isoflavones has targeted niche

markets in response to scientific research, that is still controversial, but that suggests a beneficial effect from these food components.

Isoflavones were first discovered in the 1930's, as a bioactive agent, following the disruption of estrogen action and increased infertility in sheep that had been grazing on red clover, thereby earning the often used name 'phytoestrogens'. Subsequently isoflavones have been shown to bind to, or indirectly interact with several key nuclear receptors, including hormonal (estrogen receptors alpha and beta [ERs], progesterone and androgen receptors), xenobiotic sensing receptors (Pregnane X receptors [PXR] and Peroxisome proliferator activated receptors [PPARs], and steroidogenic and hypothalamus-pituitary-thyroid (HPT) axis pathways. Isoflavones are structurally similar to the endogenous estrogen 17-beta estradiol, but much less potent on binding to the ERs, although genistein, the more active isoflavone, have a greater binding affinity for ER beta (43.9% related to 100% for estradiol [15]).

These compounds, as other phytoestrogens, are molecules with the ability to mimic estrogen pharmacological action through the linkage with ERs, and because of that are called *estrogen-like* molecules [15].

The benefits and the inherent risks associated to the ingestion of *estrogen-like* molecules are related to their binding affinity to beta-ERs and to alpha-ERs.

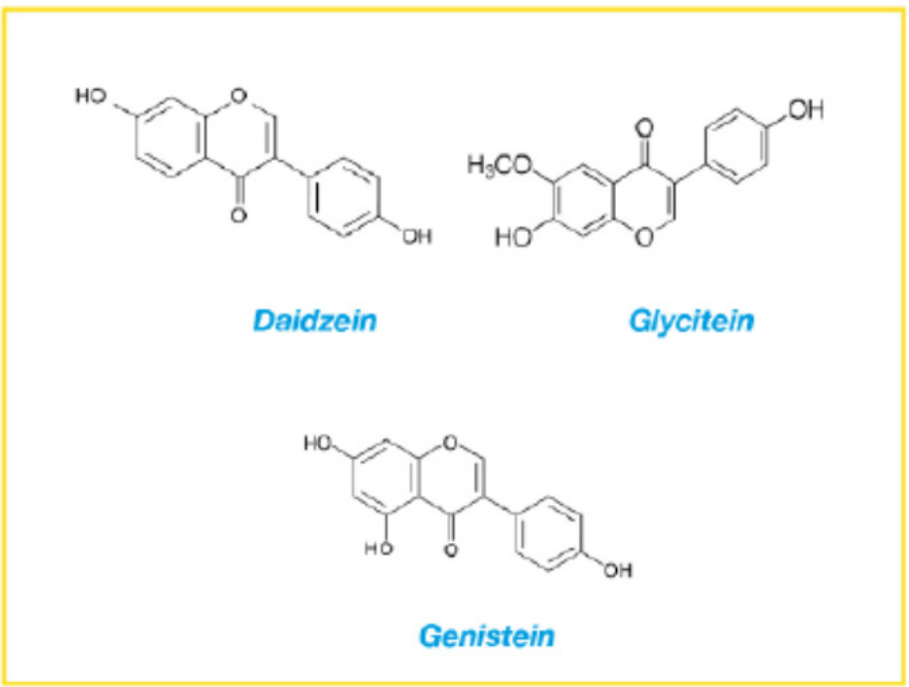

Figure 1. Chemical structures of iso-flavone equivalents

Beta-ERs are mainly located in bones, brain, thymus, bladder, and the cardiovascular system. Its activation by estrogenic compounds or estrogen-like compounds can probably improve or prevent conditions such as osteoporosis and cardiovascular diseases [16, 17]. In the other hand, alpha-ERs appear to predominate in breast, uterus and ovary; some authors

report that linkage between estrogen or estrogen-like molecules and alpha-ERs is potentially dangerous. In breast cancer, for example, it could promote proliferation of damaged DNA [18,19]; and it can also lead to endometrial hyperplasia, as concluded in a randomized, double-blind, placebo-controlled study evaluating the safety of a long-term treatment (up to 5 years) with soy isoflavones. Although no cases of malignancy were detected, the hyperplasia called into question the long-term safety of phytoestrogens with regard to the endometrium [8].

Another important issue indicates a possible relationship between low doses of isoflavones and an increase in tumor proliferation, as it was pointed out by some authors.The same authors also concluded that, genistein and daidzein could not only stimulate pre-existing tumors, but also antagonize the effects of the tamoxifen (antitumour agent). Thus, women with breast cancer or with a history of a previous breast cancer event should be made aware of the risks of potential tumor proliferation while taking soy products [20]. Previously, Hsieh *et al*, also pointed out the possibility that lower amounts of genistein could stimulate alpha – receptors and influence the proliferation of breast cancer cells [22].

It is furthermore of particular relevance the fact that genistein can be estrogenic and antiestrogenic in human body. Clarify these mechanisms, as it was done for tamoxifen, can be crucial to the understanding of a possible benefit/risk ratio. For example, although tamoxifen has been useful both in treating breast cancer patients and in decreasing the risk of getting breast cancer in women at high risk, it also has some serious side effects. These side effects arise from the fact that while tamoxifen acts as an antiestrogen that blocks the effects of estrogen on breast cells, it mimics the actions of estrogen in other tissues such as the uterus. Its estrogen-like effects on the uterus stimulate proliferation of the uterine endometrium and increase the risk of uterine cancer [23]. The same was refered before in the data from, Unfer *et al*, (2004) [8].

The relevance of ethnic metabolism will be crucial to clarify some misunderstandings. As an example of the ethnic importance of polymorphic enzymes in metabolic pathways a meta-analysis conducted combining the data with 34 published studies that included 11 962 cancer cases and 14 673 controls in diverse cancers was carried out. The SULT1A1*2 revealed contrasting risk association for UADT cancers (OR=1.62, 95% CI: 1.12, 2.34) and genitourinary cancers (OR=0.73, 95% CI: 0.58, 0.92). Furthermore, although SULT1A1*2 conferred significant increased risk of breast cancer to Asian women (OR=1.91, 95% CI: 1.08, 3.40), it did not confer increased risk to Caucasian women (OR=0.92, 95% CI: 0.71, 1.18). Thus risk for different cancers in distinct ethnic groups could be modulated by interaction between genetic variants and different endogenous and exogenous carcinogens [24].

Even all these results were carried out with Europeans (which don´t consume soy very often) and others with Asians (which included this food many years ago in their normal diet plan), it isn't frequent to discuss their metabolic pathways.

As it is known, populations vary genetically and differ in the occurrence and frequency of particular genetic polymorphism depends on the time when the mutation occurred in

relation to human migrations. If it occurred after the populations split, it will be unique to one population and its descendants. If it happened in the ancestor population, it is possible present in all descendent groups. A well-known example is the frequent ALDH2 deficiency in Asian individuals which is rarely found in Caucasians. As metabolizing enzymes are involved in the detoxification on endogenous and xenobiotic toxins, in the activation of procarcinogens, in the formation of reactive intermediates and in their neutralization, many studies have been undertaken concerning the correlation between genetic polymorphisms and metabolizing enzymes and cancer risk [25].

Ethnic differences in metabolism are a consequence of various factors as, for example, adaptation to different environments, differences in nutrition, behavior and cultural differences. This genetic polymorphism of metabolizing enzymes cause differences in effects and toxicity of the ingested compounds between individuals and all population. The so called "idiosyncratic drug interactions" are now explained on the basis of genetic polymorphisms. Beside the genetic variability differences can appear after enzyme induction or inhibition by the affected compound itself or by others or xenobiotics, e.g., from foods [25].

In Phase I metabolization, genistein, daidzein and equol (important metabolite of daidzein-7-*O*-glucoside) inhibit the Cytochrome P450 (CYP) 1A2, 1B2, 2E1, 3A4 and in phase II they induce UDP glucuronosyl transferase (UGT), Glutathione-S-Transferase (GST) and Quinone Redutase (QR); SULT1A1, SULT1A3 and SULT1E1 are inhibited for all of them [26].

CYP 1A2, for example, is important for estrogen metabolization (E1 and 2) in 2- or 4-OH E1 and 2 followed of the 4-OH E1 and 2 sulphate metabolite by SULT1A1*2 and *3, or to 2- and 4-OCH3 E1 and 2 by COMT. The inhibition of CYP 1A2 and SULT1A1, SULT1A3, probably can induce E1 and E2 to be metabolized by CYP 3A4 (*1B) to 16-alpha OH E1 and 2.

Rebbeck *et al*, also observed race-specific associations with CYP1B1, CYP1A2, and SULT1A1 on menopausal symptoms; race-specific effects of SULT1A1*2, SULT1A1*3, CYP1B1*3, and CYP3A4*1B on time to late premenopause, early menopausal transition, and menopause; and interactions of race with SULT1A1*2 and SULT1A1*3 on time to menopause [10].

The involvement of all these enzymes in the different ethnic groups constitutes a gap in the understanding of what can be used with benefit from other continents. The controversy persists regarding the role of a low ratio of 2-hydroxyestrone /16alpha-hydroxyestrone (2-OHE_1)/(16 -OHE_1) as a potential estrogen metabolism marker of increased risk for breast cancer [27]. Was suggested that soy consumption increases this ratio only in women who are equol producers given a possible protection against breast cancer [28].

The European American women that carry the CYP3A4*1B allele important in these last steps of metabolization of estrogens were indicated for a early menopause independent of the SULT1A1*2 or *3 type. The SULT1A1*2 (Arg213His) have a frequency of *ca* 30% for Caucasians and African Americans but only 8-17% for Asians [25].

GST present important genetic differences and is induce by isoflavones. The consequences of polymorphic GST isoenzymes are probably more relevant for carcinogenesis and for the

detoxication of toxic xenobiotics and of chemotherapeutics than for drug metabolism in general. For example, 44-64% of the Asians present a GSTT1*0 polymorphism against 10-36% in Caucasians with the consequent difference in the metabolizing behavior [25].

This is also important for possible interactions between those molecules and some medicines, special in polimedicated patients. Daidzein, one of the principal isoflavones in soybean, can inhibit CYP1A2 activity and alter the pharmacokinetics of theophylline in healthy volunteers. Theophylline is a bronchodilator with a narrow therapeutic index (5–20 mg/L), and it is primarily eliminated by hepatic metabolism mediated by CYP1A2. [26].

Steroid hormone metabolism genes are not generally responsible for interindividual variation in steroid hormone levels or with changes in these levels across the menopausal transition. However a better understanding of all these mechanisms will be important to prevent future damage if we ingest compounds that will change the natural equilibrium of ethnic groups.

If these associations are confirmed, they may provide information about the prediction of menopausal symptoms and allow clinicians to individualize and target hormone therapy in women experiencing menopausal symptoms. Because hormone exposures, genotypes involved in hormone metabolism, and the phenotypic manifestations of these factors on symptoms are all associated epidemiologically with risk of cancer and other diseases, a better understanding of the role of genotypes and intermediate phenotypes such as hormone levels may ultimately assist our understanding of steroid hormone– related disease etiology and prevention [10].

So although, until now, the risk assessment for soy products is not clear enough to consider their consumption safe, humans are increasingly being exposed to isoflavones in soy and soy derivatives (other products containing isoflavones from different sources aren't so rich). Thus better information about the safety of these soy phytoestrogens is urgently needed.

3. Examples of products available to be consumed by European population

In this section is provided an overview (not exhaustive but presenting some discuss points) of the potential benefit/risk impact of nutritional products and supplements, that include isoflavones in their composition and that are recommended mainly for climacteric relief symptoms in post-menopausal women and/or prostatic cancer prevention in man and CVD prevention.

The European Prospective Investigation into Cancer and Nutrition (EPIC) study evaluated in 2002 the consumption of soy products in 10 European countries [29]. Results from this study revealed that soy consumption at that time was low in Western Europe and that non-dairy substitutes were the most frequently consumed items.

In this section we'll demonstrate the wide variation of isoflavone levels that these products may provide to the consumer. Because there are not recommendations of the optimal

isoflavone consumption, the information displayed in these products should be clearly specified, especially in the case of health supplements. This is essential for accurate risk assessment studies, where knowing the exposure level of the population to these products and more specifically the exposition to each isoflavone is fundamental.

Nowadays, over the counter (OTC) tablet preparations [30, 31], nutraceuticals and various supplements with isoflavones extracted from soy [32] and other plants are sold in various countries. These products are often used by postmenopausal women to do hormonal therapy replacement as another option to allopathic medicines with oestrogen derivatives, and also with the claim of cancer prevention. However, the impact of that exposure in Caucasian women health is underestimated.

As a result of the promising scientific findings related to isoflavones, the soy-processing industry has grown worldwide from manufacturing alternative dairy products and milk-free infant formulas to provide a wider range of products embracing all types of tastes and consumer health concerns. These new products, sometimes named as second generation soy foods, are based in the inclusion of soy ingredients (soy pieces, soy powder, soy flour, soy protein, etc) among the ingredient list of food items commonly consumed by western populations.

Development of new soy products is especially important in Europe where the population does not accept the characteristic soybean flavor. Some years ago, only a few traditional soy foods were common items in European markets, and among those, soy nuts, soy sauce, tofu and soymilk were easily found, but the recent appearance of the so-called second-generation of soy foods; products made by adding soy ingredients (soy protein) to a wide variety of manufactured foods resulting in products which generally simulate familiar dishes [32].

A number of soy protein-based products are included, mainly meat substitutes (texturised soy protein) in a number of forms, as well as dairy-products substitutes. Same protein is added to enrich a number of foods commonly present in European diets: bread, cookies and soups or dietary supplements (powders) and energy bars used to complement sport diets. The latest version to use soy protein or isoflavone isolates appeared in the form of capsules or tablets directed, and labelled, to promote the claimed health benefits of soy isoflavones between the consumers [32].

It is well known that isoflavone levels in soy-based products depend primarily upon the soybean variety used. Isoflavone content within a single variety can differ three times from year to year [33]. In some studies, this variability has been attributed to climatic and environmental factors [34]. Moreover, soybean processing and storage conditions usually lead to a significant isoflavone loss in soy-based products [35]. In addition, isoflavone glucoside conjugates are easily alterable during extraction and cooking[36], and it has been even shown variations between products included in the same batch [32].

In some cases the presence of isoflavones is intended and therefore its concentration is known, but in the majority of the soy foods the levels of isoflavones need to be quantified in order to evaluate the possible health effect of these products.

Results from our previous work with some of the above cited products, selected soy-foods produced and distributed within Europe (Table 1), provide total isoflavone values for a possible evaluation of the potential exposure to these molecules [32].

Group	Designation	Examples	N
I	Traditional soy foods	Tofu, Soy beans, Soy nuts, Soy sauce	9
II	Non-dairy soy products	Soy-enriched plain yogurt, Soy milk yogurt, Soy milk dessert, Plain soy milk, Cream substitute	18
III	Meat analogues	Soy meat strips, Soy escallops, Meat pieces, Soy minced meat, Flavoured minced meat	5
IV	Second-generation soy foods	Pasta with broccoli and cheese, Energy bar with fruit and muesli, Powdered tomato soup, Soy-enriched orange cookies, Soy-enriched rice cookies, Crisp bread, Snack with almonds	24
V	Health supplements		59

Table 1. Groups of soy based foods and health supplements

In this work a total of 115 soy-based products were purchased at local retailers and natural health food stores in Finland, Spain, UK and Portugal during the years 2002-2005. Commercial availability and European manufacture were the only selection criteria. The selected items belong to different groups of soy foods and a classification was needed in order to allow the comparison between levels of isoflavones provided by the different products. Items were divided into four different groups: 1) Traditional soy foods, 2) Non-dairy soy products, 3) Meat analogues, 4) Second-generation soy foods and 5) Health supplements. Food samples were (when necessary) freeze-dried, homogenized and stored at -70 °C until analysis [32].

Soy isoflavones in samples were quantified as previously reported [30, 38, 39] using a HPLC systems equipped with a Coularray detector or diode array detection. Peaks corresponding to soy isoflavones were confirmed by LC/MS-MS as previously reported [39]. Synthetic standards were used for quantification through calibration curves, and control samples, were introduced between run to assure repeatability was acceptable (CV <15%) over the analysis time. All reagents were from major suppliers and HLPC-grade. Results are mean values of triplicate analyses, and were only accepted when coefficient of variation (CV) between the replicates was <15%. Only values above the limits of quantification of the method for each isoflavone (~1 ppm, depending on the analyte) are reported [39]. All values are expressed as aglycone equivalents.

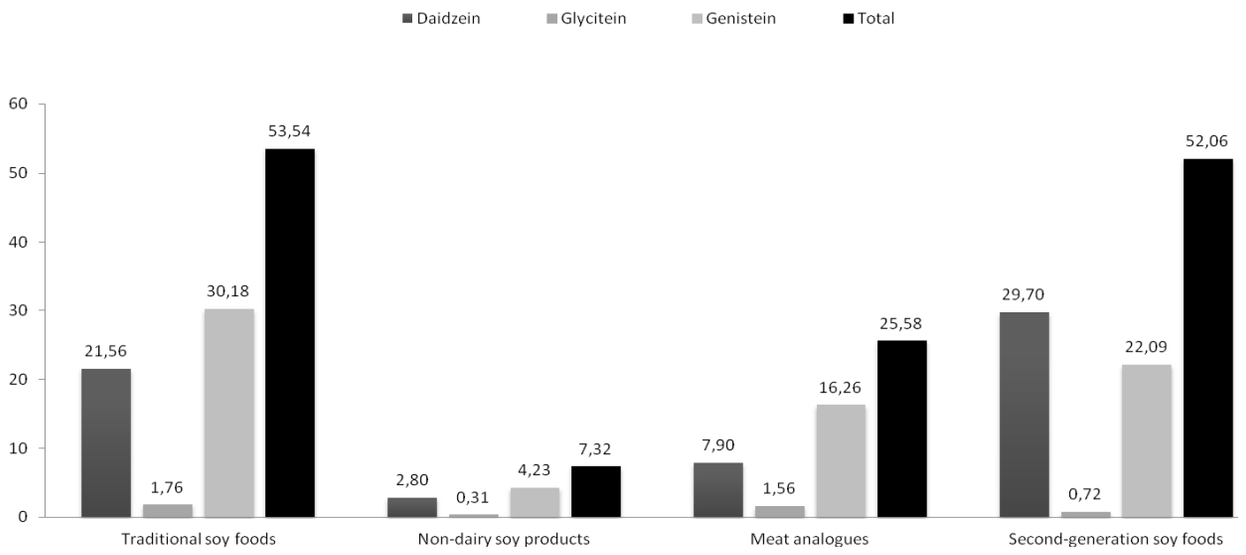

Figure 2. Isoflavone means in Traditional soy foods, no-dairy soy products and meat analogues analised as examples of European consume.

In Figure 2, from our previous data [32], genistein was found to be the most prevalent isoflavone in Traditional soy foods, no-dairy soy products and meat analogues. In second generation soy foods, daidzein was the most prevalent isoflavones. However differences between the various products were considerable.

Diverse studies have been conducted in different countries in order to analyze the isoflavone content in soy products. Chan *et al* determined the concentrations and distribution of isoflavones in 47 soy-based foods [40]. They also studied the conjugation pattern of isoflavones and found that varied within and between food groups as influenced by the types of soybeans and the processing or cooking techniques used. The authors found very high values in certain foods and concluded that might not be safe, supporting the relevance of the risk assessment for the recommendation of safe intake levels. Similar results were obtained by Boniglia et al [41].

Three years ago, Boniglia *et al*, achieved similar results. These authors presented a study of the isoflavone content of 14 soy-based Italian dietary supplements – indicated for the improvement of perimenopausal and menopausal symptoms. The objective was to quantify soy isoflavones fraction after hydrolysis into free aglycones. They noticed that, in the examined products, the amounts of isoflavones were frequently expressed ambiguously. None of the products stated whether the isoflavone content of the product was expressed as aglycones or as conjugates. Even worse, each product revealed a different aglycone concentration profile. They also concluded that this difference was probably related to the different sources of raw materials and methods used in the processing and preparation of extracts. They also observed that in more than half the supplements tested, the actual values contained were below those stated and below those expected to relief perimenopausal and menopausal symptoms [41].

Similarly, our study of a 59 selected soy health supplements shown a large variation in the composition, and a wide difference between the content of isoflavones analyzed and that reported in the product [32].

Our study demonstrates, as the others cited before, the wide variation of isoflavone levels that these products may provide to the consumer. Because there are not recommendations of the optimal isoflavone consumption, the information displayed in these products should be clearly specified, especially in the case of health supplements. This is essential for accurate risk assessment studies, where knowing the exposure level of the population to these products and more specifically the exposition to each isoflavone is fundamental.

Despite the predominant isoflavone forms in soybeans [38] and non-fermented soy products (like soy protein or soy milk derivates) are glucosides [42]; fermented soy foods (like soy sauce) contain mainly aglycones [43]. The form in which isoflavones are present in soy-based foods and health supplements is important to assess the bioavailability of these products [36], Considering that genistein is, in a theoretical point of view [15,44], about 1000 times more active that daidzein the heath benefit and/or the toxicity could be different in two apparently identical products.

For this confusing data our previous proposition was, and still is, applying the calculation of "Theoretical Efficacy (of isoflavones) Related to Estradiol (TERE) [44]. Like that it is possible evaluate the theoretical impact of exposure to estrogen-like activity of isoflavones in various countries from all the data of different studies already published [44]. This theoretical calculation estimates the "Theoretical Efficacy (TE)" of a mixture with different bioactive compounds in a way to obtain a "Theoretical Efficacy Related to Estradiol (TERE)". The theoretical calculation that was proposed for some of the authors of this chapter integrates different knowledge about this subject and sets methodological boundaries that can be used to analyse data already published. The outcome should set some consensus for new clinical trials using isoflavones (isolated or included in mixtures) that will be evaluated to assess their therapeutically activity.

To do the theoretical calculation [44] the amounts of isoflavones were multiply for the "ERs binding affinities" based on the values obtained in literature [15]. After applying the proposed model, "Theoretical efficiency related to estradiol (TERE), for each mixture of isoflavones, the" Theoretical Efficiency (TE) was estimated which can be used to compare the potential bioactivity [44].

Daidzein and genistein do not have the same binding affinity to alpha- and/or beta-ERs [15]. Also known and frequently mentioned by several authors is that the linkage of oestrogens or oestrogenic compounds to alpha-ERs could be dangerous with breast cancers because it could aid the proliferation of damaged DNA in tumours [45, 46]. The amounts theoretical linked from the different isoflavones to the alpha- oestrogen receptors as an add benefit (when added to the beta-receptors affinity) but also as a potential risk to be under evaluation.

However, it is also accepted that beta-ERs are mainly located in bones, brain, thymus, bladder, cardiovascular system and its activation by estrogenic compounds, or compounds with the ability to mimic estrogenic molecules, such as phytoestrogens, can improve and prevent conditions like osteoporosis and cardiovascular diseases [17, 47] and this will be used as the benefit parameter.

In global terms the total efficacy of the TERE will be determined adding these two values. However the amount linked to the alpha-receptors will be consider as a possible risk limitation, which that needs to be evaluated when the increased dose will induce an improved TERE but the risk assessment for the toxicity can be a handicap. Ultimately, different extracts could be compared, even when the relative amounts present in the extracts are very different.

The example of TERE calculation for samples show in Figure 2 is presented in Table 2, using "Second generation soy foods" data.

	Second generation soy foods	Receptor Type	
	Intake (mg/100g)	Alpha-ERs	Beta-ERs
daidzein	29,2	(29.2 x 0.031/100) 0.009	(29.2 x 0.020/100) 0.006
genistein	22,09	(22.09 x 0.86/100) 0.19	(25 x 43.9/100) 9.7
Total	51.79	0.199	9.706
TE	**9.9**	(= 0.199 + 9.706)	
TERE	(100 total linkage to ERs/9.9 TE) = **1/10.1** of the theoretical activity of the estradiol		
RISK		**~ 2%**	
BENEFIT			**~ 98%**

Daidzein binding affinity for alpha–ER is 0.031 and 0.020 for beta-ER [15];
Genistein binding affinity for alpha–ER is 0.86 and 43.9 for beta-ER [15].

Table 2. Example of TERE calculation with mean values for *Second generation soy foods* using the "Estrogen Receptor affinity binding values" with daidzein and genistein.

From the total values in Figure 3 and 4 is possible evaluate the amount of daidzein and genistein linked to both receptors and have an idea about the Theoretical Efficacy (TE) of these components if related to estradiol (TERE), Figure 5.

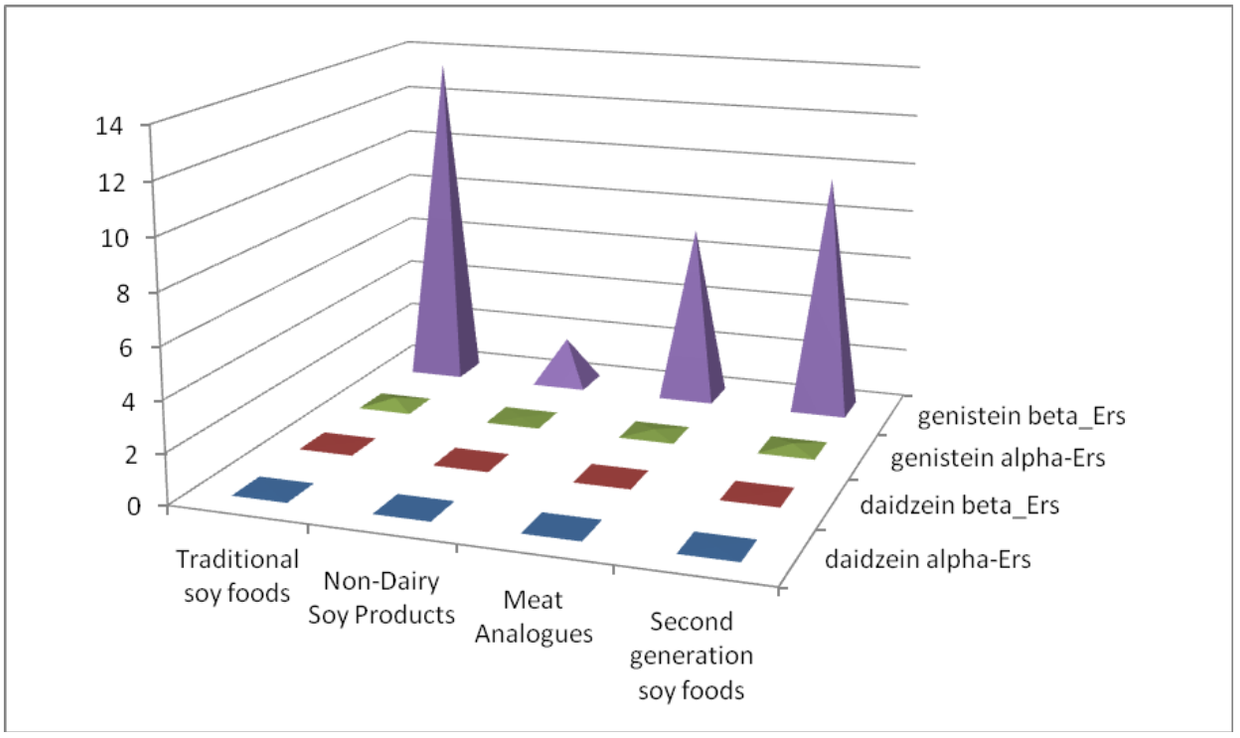

Figure 3. "Estrogen Receptor affinity binding values" for daidzein and genistein content in samples of Group I to IV.

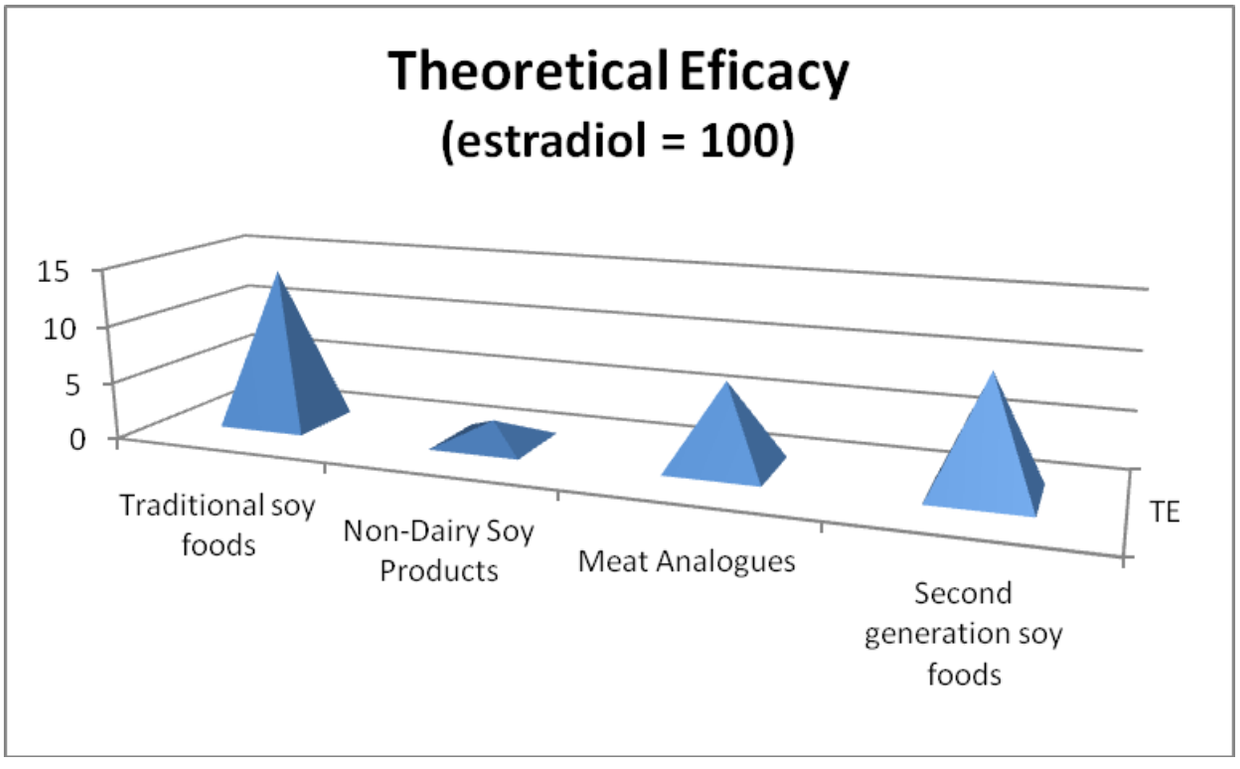

Figure 4. TE values for samples of Group I to IV.

With the exception of Non-dairy soy products that present very low bioactivity related to estradiol the other three groups have values between 7 and almost 14% of the estradiol bioactivity.

Nevertheless the apparent majority of benefice related to risk, as was pointed out above, the low amounts of isoflavones linked to alpha receptors can increase the possibility of proliferation in hormonal dependent tumours and the safe amounts were not evaluated yet.

If the same analysis is made with health supplements the values are absolutely different. In Figure 6 it is shown the TE for the highest values of daidzein and genistein found in our samples (daidzein 371.48 and genistein 172,47 mg/g of pill) [32]. The pill with the content of 172,47mg of genistein also had 208.41mg of daidzein. The TE for this product is almost 80% of the bioactivity of estradiol with a clear uncertainty of influence in the bioability of these compounds in human body.

The exposure risk of all those products is unknown and needs to be evaluated in a near future even no enough data for now make sense.

The data presented herein intend to demonstrate the wide range of isoflavone levels that different products may provide to the consumer, even when recommended for the same therapeutic effect and still if the product keeps the same isoflavones profile all the time. Its relevant to mention that the majority of the soy suppliers breed a wide range of soy cultivars, which results in a different mixture of compounds, and consequently in a variable final product composition and hazard [19, 38].

The Europeans, as can be seen in results presented recently by Konar *et al*, in 2012 consumed legumes with low levels of dietary isoflavones. In this study, 6 legumes (chickpea, red kidney bean, haricot bean, yellow lentil, red lentil and green lentil) were analysed to determine their contents for 10 different isoflavones (both free and conjugated). Methanolic extracts obtained by ultrasound-assisted extraction were analysed by triple quadrupole LC-MS/MS. Chickpeas were the best source of isoflavones (3078 372 mu g/kg total content), with a significant amount of biochanin A and its conjugated form, sissotrin. Kidney beans had the second highest concentration of isoflavones (1076 mu g/kg) and were particularly rich in genistin (946.4 +/- 228.5 mu g/kg). The total isoflavone concentrations of yellow split lentils, green lentils, red lentils and haricot beans were each below 200.0 mu g/kg. However it was determined that the legumes commonly consumed in Western diets (those analyzed in this study) are not so concentrated as soy and soy products as sources of Isoflavones [48].

As it was explained and discussed in the previous section the metabolic pathways and the genetic polymorphic enzymes involved in detoxication of the ingested compounds in our body can conduce to a different bioactivities and consequent risk impact in the various ethnic groups. Only a full risk assessment will prevent the danger or will help to understand the benefit of an increase in the isoflavone intake by Caucasians that for the moment is absolutely out of control.

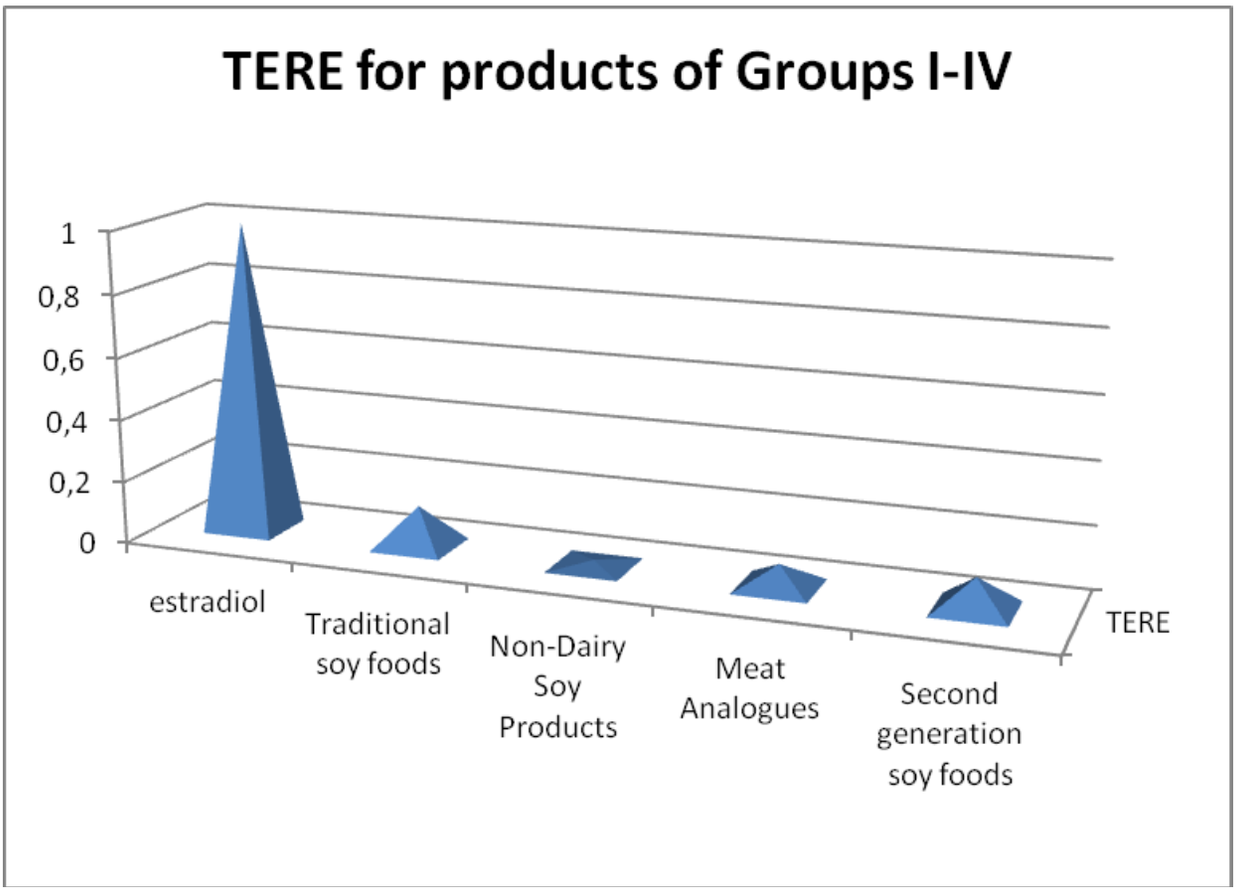

Figure 5. TERE values for samples of Group I to IV.

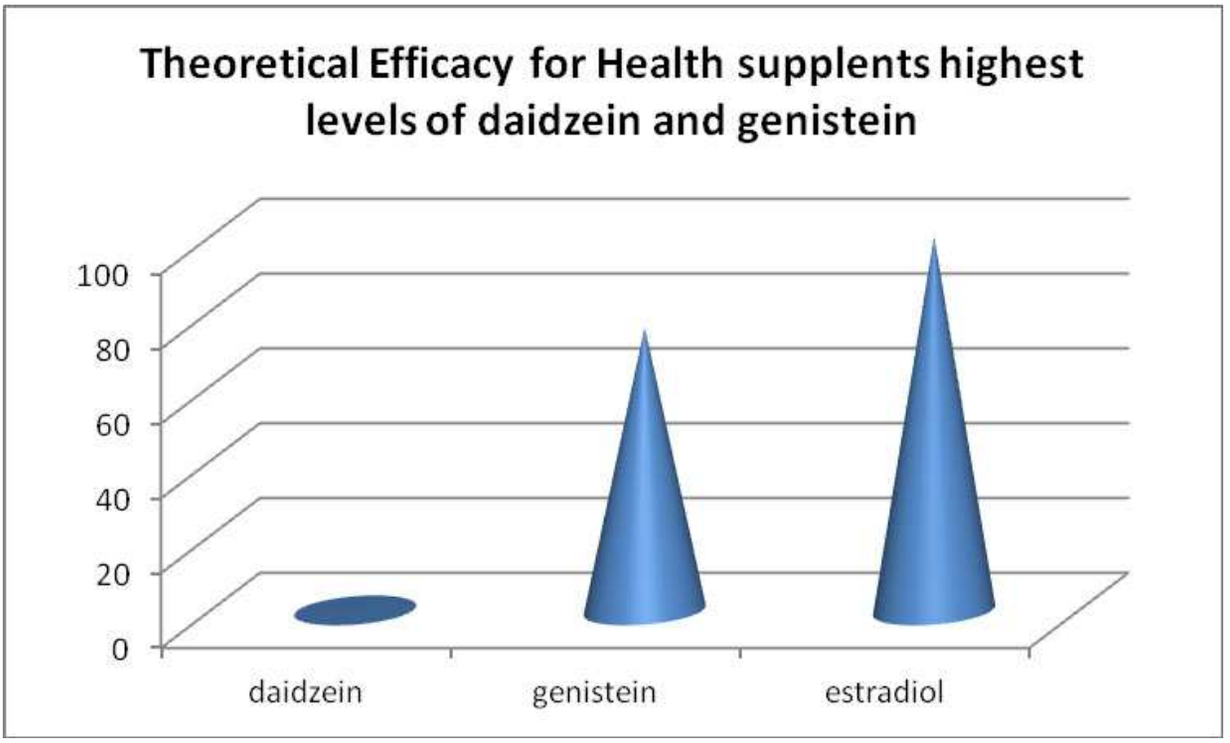

Figure 6. TE values for samples of Group V (only for highest daidzein and genistein content).

4. Conclusions

The above findings emphasize the need for a thorough correct risk assessment to be carried out to evaluate the differences between the various isoflavones, their relative levels in formulations (dietetic supplements, foods soy based, etc) and their safety profile, in order to establish limits for safe therapeutic effects. Various interactions with conventional medicines have already been published and it is important to alert already medicated patients who are also consuming soy or soy based products. The relationship between this intake and cancer and or cardiovascular diseases prevention is unclear moreover the danger of a cancer improvement.

Without such a risk assessment *ad libitum* consumption of these compounds could be hazardous.

Author details

Maria Graça Campos* and Maria Luísa Costa
Observatory of Herb-Drug Interactions & Faculty of Pharmacy, University of Coimbra, Polo III, Azinhaga de Santa Comba, Coimbra, Portugal
Drug Discovery Group, Center of Pharmaceutical Studies, Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Coimbra, Polo III, Azinhaga de Santa Comba, Coimbra, Portugal

Acknowledgement

The authors wish to thank to "Projeto Estratégico - PEst-OE/SAU/UI0177/2011".

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