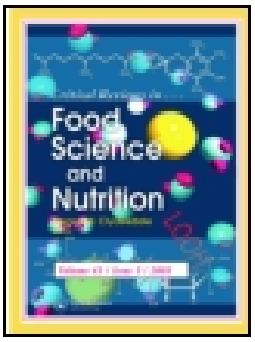


Taylor & Francis
Taylor & Francis Group

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

An Updated Review of Dietary Isoflavones: Nutrition, Processing, Bioavailability and Impacts on Human Health

Khalid Zaheer & M. Humayoun Akhtar

To cite this article: Khalid Zaheer & M. Humayoun Akhtar (2015): An Updated Review of Dietary Isoflavones: Nutrition, Processing, Bioavailability and Impacts on Human Health, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2014.989958

To link to this article: http://dx.doi.org/10.1080/10408398.2014.989958

An Updated Review of Dietary Isoflavones: Nutrition, Processing, Bioavailability and Impacts on Human Health

An Updated Review of Dietary Isoflavones: Nutrition, Processing, Bioavailability and Impacts on Human Health

Khalid Zaheer[*,1] and M. Humayoun Akhtar[2]

[1]Consultant, Toronto, Ontario M3M 2E9, Canada

[2]Guelph Food Research Centre, Agriculture and Agri-Food Canada, Guelph, Ontario N1G 5C9, Canada; E-Mail: humayoun.akhtar@agr.gc.ca

[*]Corresponding author: Dr. Khalid Zaheer, Consultant, Toronto, Ontario M3M 2E9, Canada, E-Mail: kzaheer2000@gmail.com, Phone: 1-416-294-3420: Fax: 1-416-792-6580

ABSTRACT

Isoflavones (genistein, daidzein, and glycitein) are bioactive compounds with mildly estrogenic properties and often referred to as phytoestrogen. These are present in significant quantities (up to 4-5 mg.g^{-1} on dry basis) in legumes mainly soybeans, green beans, mung beans. In grains (raw materials) they are present mostly as glycosides, which are poorly absorbed on consumption. Thus, soybeans are processed into various food products for digestibility, taste and bioavailability of nutrients and bioactives. Main processing steps include steaming, cooking, roasting, microbial fermentation that destroy protease inhibitors and also cleaves the glycoside bond to yield absorbable aglycone in the processed soy products such as miso, natto, soy milk, tofu; and increase shelf lives. Processed soy food products have been an integral part of regular diets in many Asia-Pacific countries for centuries e.g. China, Japan and Korea. However, in the last two decades, there have been concerted efforts to introduce soy products in western diets for their health benefits with some success. Isoflavones were hailed as magical natural component that attribute to prevent some major prevailing health concerns, Consumption of soy products have been linked to reduction in incidence or severity of chronic diseases such as cardiovascular, breast and prostate cancers, menopausal symptoms, bone loss etc. Overall, consuming moderate amounts of traditionally prepared and minimally processed soy foods may offer modest health benefits while minimizing potential for any adverse health effects.

Key Words

Isoflavones, Nutrition, Soybeans, Bioactive compounds, Bioavailability, Health benefits, Chronic diseases

1. Introduction

Soy isoflavones are bioactive compounds of non-steroidal and phenolic nature that are abundantly present in soybeans. The physiological role of these bioactive compounds has received recognition all across the world. Of particular interest in relation to human health these bioactive compounds are known as the phytoestrogens (isoflavones) because of their estrogenic activity [1]. Isoflavones are structurally similar to mammalian estrogens but with mild estrogenic properties. Isoflavones are also considered as a subclass of flavonoids; a large family of compounds synthesized by plants, and thought to have potential antioxidant properties [2]. Antioxidants are substances that protect cells from damage caused by free radicals produced by oxidation during normal metabolism. These free radicals thought to play a role in cancer development [3]. Thus isoflavones have both estrogenic and antioxidant capacity, related to their structural similarity to 17β-estradiol [4]**.** As such there is currently considerable interest in the potential health benefits of isoflavones in functional foods.

Extensive published work and scientific reviews have link isoflavones to bring relief to number of chronic diseases in humans. Possible health benefits include relief of menopausal symptoms [5-7] breast cancer [8-10], prostate cancer [11-13], incidence of cardiovascular disease [14-15], osteoporosis or BMD (bone mineral density) [16-17], obesity and diabetes [18-19], cognitive functions [20-21], and even prevention of virus infections [22]. Isoflavones and their dietary sources have been reported as possible anticarcinogens. The European Prospective Investigation into Cancer and Nutrition (EPIC) is an ongoing multi-centre prospective cohort study designed to investigate the relationship between nutrition and cancer, with the potential for studying other diseases as well

[23]. According to EPIC research findings there was a high variability in the dietary intake of total and phytoestrogen subclasses and their food sources across European regions [24].

In nutshell the intake of isoflavones containing foods have become increasingly recognized worldwide. This is due largely to the apparent health benefits imparted by the traditional Asian diet, which is very high in soy foods, as well as low in saturated fat, and high in dietary fiber. Isoflavone content of soy foods can vary considerably between brands and even between different lots of the same brand. Given the potential health implications of diets rich in soy isoflavones, accurate and consistent labeling of its content is needed. More information on the isoflavone content is available from the USDA nutrient database [25-27]. Over 10,000 scientific papers, reviews have appeared on isoflavones alone in global publications. Considerable global research over the last four decades has identified several benefits of isoflavones in diet. These studies have opened doors for the use of first generation soy-based foods and drinks (soy milk/soy drink, tofu, natto, tempeh etc.), and second generation products (baked goods to which soy-based ingredients have been added). Scientific data continued to be analyzed and reported in books, review articles etc, [1, 28]. Further purified soy isoflavones are now available commercially, and methods of recovering isoflavones have been patented [29]. The purified soy isoflavones may be marketed as pills, concentrates, or extracts.

2. Sources and Occurrence

Isoflavones are biosynthesized in legumes such as soy, red clover, kidney beans, mung bean sprouts, navy beans, Japanese arrowroot (Kudzu) [30], but is abundant in soybeans. They are processed for taste, removal of toxic substances, nutritive value, bioavailability and absorption. Overall, the most significant dietary sources of isoflavone include soybeans, soy flour, soy flakes, isolated soy protein, traditional soy foods (such as tofu and soy milk), and fermented soybean products (such as miso, tempeh), soybean paste, natto and soy sauce [31-32]. Further information and elaboration on soy food products will be given in a separate section later in this review.

Soybean itself is considered as main dietary source of isoflavones. As such some brief details about soybean, its cultivars, worldwide production/cultivation and consumption, nutrients and genome study are mentioned here in this section. Soybeans *Glycine max* (L.), also referred to as soy or soya, and is plants of Asian origin that produce beans used in a variety of food products. There are many kinds of soybean cultivars with different biological composition and economic values. According to the consensus recommendations of the Organization for Economic Cooperation and Development (OECD), soybean nutrients such as amino acids, fatty acids, and isoflavones are important markers in assessing the nutritional quality of soybean varieties [33]. Cultivation of Soybean is being done worldwide and 90% of the world’s soybean production is concentrated in tropical and semi-arid tropical regions which are characterized by high temperatures and low or erratic rainfall. In tropical regions, most of the crops are near their maximum temperature tolerance [34]. The amounts of isoflavones in soybeans vary greatly and can range from 360$\mu g.g^{-1}$ to 2241$\mu g.g^{-1}$ in Eastern Canada [35]. In Southern Ontario the total isoflavone values ranged from a low of 1.4 $mg.\ g^{-1}$ to a high of 4.6 $mg.\ g^{-1}$ on dry weight basis. On the average genistein, daidzein, and glycitein and their derivatives were in ratio of 58:37:5, mostly as malonyl derivatives [36], 21$mg.100\ g^{-1}$ to 134 $mg.100\ g^{-1}$ in Romania [37]; 1176 $\mu g.g^{-1}$ to 3309 $\mu g.g^{-1}$ in the US [38]; and 525 to 986 $mg.kg^{-1}$ in India [39]. Soybeans production for edible oil and protein has seen continuous growth over the years. The top five soybean producers are the US (33%), Brazil (29%), Argentina (19%), China (5%) and India (4%) [40]. Soybean is the major dietary source of isoflavones because of abundant supplies and advanced technologies to process them into a variety of food products. Soybeans contain mostly, around 90% of the total isoflavones as the sugar conjugates of genistein, daidzein, and glycitein along with small amounts of their free form (aglycone) for a total of 12 isoflavones (see details in Chemistry Section). In general, concentrations are in the order genistein> daidzein> and glycitein, which greatly depends on varieties, growing locations, climatic conditions etc. Also, the hot and dry weather yielded low grade soybeans as well as lower total isoflavones contents [41].

Unique features of the soybean genome were characterised through the use of molecular research techniques to determine the variations between wild and cultivated soybean genomes. Genomic variations may be related to the process of domestication and human selection. Wild soybean germplasms exhibited high genomic diversity and hence may be an important source of novel genes/alleles. Accumulation of genomic data will help to refine genetic maps and expedite the identification of functional genes leading to positive impacts on soybean research and breeding programs [42].

It is interesting to note that some American soy varieties have the highest isoflavone contents. American groundnut (*Apios Americana*), an important diet of East Coast Native Americans, contains as high as 8mg. g^{-1} of 7-*O*-glucosylglucoside of genistein [43]. According to research studies American groundnut also contains other novel derivatives which may be converted to genistein by enterobacterial β-glucosidase [44-45]. Likewise clinical trials on the health benefits of pulses show impact on i) total LDL and cholesterol levels, ii) reduce blood pressure, iii) help in weight management, iv) decrease spikes in blood sugar and insulin levels, and v) improve insulin resistance [46]. The presence of common and uncommon isoflavones in pulses may require development of processing technologies to market pulse products for routine dietary consumption for self-health management.

3. Chemistry of Isoflavones

Isoflavones are present in significant quantities in soybeans as glycosides (bound to a sugar molecule) and are called genistin, daidzin, and glycitin. Fermentation or digestion of soybeans or soy products results in the release of the sugar molecule from the isoflavone glycoside, leaving an isoflavone aglycone. Soy isoflavone aglycones are called genistein (5, 7, 4'-trihydroxyisoflavone), daidzein (7, 4'-dihydroxyisoflavone), and glycitein (7, 4'-dihydroxy-6-methoxyisoflavone), sometimes also referred to as isoflavonoids. Chemical structures of major isoflavones are shown in Figure 1.

Overall, soybeans contain three groups of isoflavones in four chemical forms: aglycones, (daidzein, genistein and glycitein); glucosides (daidzin, genistin, and glycitin); acetylglucosides, (acetyldaidzin, acetylgenistin, and acetylglycitin); and malonylglucosides (malonyldaidzin, malonylgenistin and malonylglycitin) for a total 12 isoflavones. The major isoflavones in soybeans are the free and conjugate forms of genistein and daidzein, which make up to 60% and 30% of the total isoflavones, respectively. The glycitein group is a minor component (10 %) in the total of the soy isoflavones. In soybeans the predominant conjugates are the malonyl derivatives [47-48].

Isoflavones are important and physiologically active members of the large flavonoids matrix. Their chemical structures resemble that of an estrogenic compound estradiol (Figure 1). They are biosynthesized following the common phenylpropanoid pathways yielding two major chalcones (naringenin and 4, 2', 4'-trihydroxychalcones)-which in turn with the help of isoflavanoid specific 2-hyroxy isoflavone synthase (IFS) produces daidzein, genistein, and glycetein. Further interaction with UGT (glycosyl-transferase) converts them in daidzin, genistin, and glycitin, respectively. Similarly involvement of malonyl tranferase (MT) yields the malonyl derivatives of diadzein, genistein and glycitein [49]. The main isoflavones with high estrogenic activities are free daidzein, genistein and glycitein (in the text they also referred to as aglycone) and are found in soybeans as sugar derivatives (conjugates). They are also found as methylated derivatives (biochanin, formenotin) in other leguminous plants (alfalfa, red clover, beans) in smaller quantities with considerably low estrogenic activities.

Processing affects the retention and distribution of isoflavone isomers in soy foods. The conversion and loss of isoflavones during processing can affect the nutraceutical values of soybean. Further relevant details about effect of processing and updated information coming up in the proceeding sections.

4. Common Soy Food Products

Soy protein is one of the major sources of vegetable proteins and can possibly act as a good replacement for animal proteins. They are used extensively in many food products to meet the needs of vegan dishes. Soy food products are manufactured with, without fermentation or in combination [1, 28]. Important soy food products are as follows:

a) **Non-fermented** products include soymilk, tofu, yuba, soybean sprouts, okara, roasted soybeans, soy nuts, soy flour, immature soybeans and cooked whole soybean. Processes involved in making some of the important soy products are summarized below:

- **Soy milk/soy drink** is produced from soaked soybean followed by crushing and filtration. The filtrate (water extract) after boiling for a short duration 30 minutes results in soy milk, which is consumed globally at varying rates.
- **Okara** is the residue of crushed soybeans and should contain high amounts of isoflavones.
- **Tofu** is also called soy curd, and is produced by adding a coagulant notably calcium sulphate ($CaSO_4$) to soymilk followed by heating to coagulates and filter. The filter cake (residue) is tofu.
- **Soy cheeses** are made from soy milk, tofu alone or in combination with or without soy protein isolates.

b) **Fermented soy products** include soy paste (Miso, Jiang), Soy sauce, Tempeh, Natto, Soy nuggets, sufu. All fermented products not available globally, but are more specific to a region, some details on their preparation are provided below**:**

- **Miso** is the fermented product of soybeans that have been cooked and inoculated with *Asperigillus orzae* and *Asperigillus soyae,* and further processing as desired. They are consumed mostly in China, Korea, Japan as good substitute for dairy products that cause allergic and intolerance reactions.
- **Tempeh** is used regularly in Indonesian diet and good meat substitutes by vegans. This is produced by fermenting the dehulled boiled soybeans with *Rhizopus oligoporus.* It is eaten with other daily dishes.

- **Fermented tofu**: A variety of fermented tofu is produced with brine made of soy milk and addition of condiments (chilly, as per taste). They are also known as fermented bean curds, fermented cheese, and sufu.
- **Sufu**, a Chinese delicacy is produced by solid-state fermentation of tofu with *Actinomucor elegans* to give phetze, which on salting and maturation yields sufu. The quality and texture of sufu depend greatly on the fermentation temperature. Studies have shown that better quality sufu is produced at 26°C compared with at 32°C.
- **Natto:** This fermented soy product is mainly produced and consumed in Japan. The process involves soaking, steaming, bagging and treating with *Bacillus* Natto and heating the bag at 48°C to 50°C for 16-18 hours. This is consumed with cooked rice.
- **Soy sauce**: It is one of the most commonly used soy-derived products. This is produced from whole bean, soya flakes or soy meal by *Aspergillus* the reactions of *koji* (source of enzymes, bacteria, mold or yeast). The brine is fermented, pressed, filter, and pasteurization.

5. Analytical Methodologies

Due to growing consumption of the dietary isoflavones and reported health benefits to humans, there was a need to develop appropriate analytical methodologies to identify and quantify their content from range of sources. Analytical analysis helps to determine the retention, distribution, and specification of isoflavone isomers in soy foods leading to their consumption by human after processing [50]. A good technique is the one which is reliable, precise, and fast with reproducible results and consume low solvent. The analysis of isoflavones has become more complex, because preparations contain isoflavones from multiple sources. These are biologically active compounds occurring naturally in a variety of plants, with relatively high levels found in soybeans.

Analyses of soybeans and soy food products for isoflavones are multi-step processes. In general, this involves sample collections and proper storage, sub-sampling and addition of internal standard before or after acid/base and enzymatic hydrolysis. This is followed by extraction with appropriate solvent systems, and enrichment (concentration) of the organic extract prior to analysis for detection and quantitation. Extracts are fairly stable at or below -20°C, but degradation sets on with increasing temperature. Separation analysis is performed on high performance liquid chromatography (HPLC) with RP-18 columns. The use of other columns has also been explored for better separation [51]. HPLC instruments are now, everywhere, in food matrix, drug research and development, pharmaceutical manufacturing, quality assurance, diagnostics, toxicology, research and other laboratories to do the needful.

Extensive independent research as well as many in depth reviews have appeared in scientific literatures on the analytical methodologies for the detection and quantification of isoflavones in soy and soy products. Application of these analytical techniques on pharmaceutical formulations is also documented with validation as required by ANVISA [52-57]. Recently a robust automated analytical approach- ultra high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) was used to quantify the soy isoflavones daidzein, genistein and their conjugative metabolites. The application of this method to a pharmacokinetic study in postmenopausal women showed that isoflavones are extensively metabolized in vivo [58]. In short a great abundance of analytical data concerning isoflavones is obtained through the application of latest analytical state of the art research techniques. This led to great success in identification and quantification of the content of isoflavone from range of sources. Further this is backed by procedures, technique development, validation, and retrieval of reproducible research findings published in peer reviewed scientific journals.

6. Processing for Isoflavones

Soybeans are not eaten raw, except occasionally green beans, fried beans as snacks, but processed using heat (high temperature), treatments with enzymes, microbes, molds. The oil is widely used in cooking foods. Processing enhances taste, flavour and more importantly digestibility, bioavailability of nutrients. During the processing including regular cooking, soybeans undergo a variety of reactions to produce compounds that are easily absorbed to exert physiological benefits in controlling some serious health issues- cardiovascular diseases, cancers, diabetes, menopausal symptoms (hot flashes), obesity etc.

In Asian countries such as Japan, China, Korea soybean products have been consumed as part of regular diet for centuries, yet only recently soy products are gaining acceptance in the West. Processing of soybeans leading to the production of edible products involves many steps that may include non-fermentation, fermentation and combination of both techniques depending upon the end product(s) which greatly varies on tastes, benefits etc. Soybeans are cleaned, cracked and dehulled as initial steps in both processes. Soybeans contain approximately 18.5% oil, 38% protein, 7% fiber, 12.5% moisture and 24% others to remove/destroy toxic substances and to soften the tissues for digestion. In general, non-fermented processes soybeans provide i) oil, ii) soy flour, iii) soy protein concentrates, iii) soy isolates, iv) soy fiber (mainly from hulls) and v) bioactives including isoflavones [59].

Soybeans are now processed into a variety of products for human consumption globally. Soy protein isolate is the source of low fat milk, low fat tofu. Soy on heating with water yields high fat soy milk, tofu, and natto. Fermentation of soy produces miso, tempeh, soy sauce - the most commonly used products in Asian countries [47]. Further details including industrial applications as given below:

a) **Crushing**: Moisture content is re- adjusted by heating soybeans between 60° and 90°C for mechanical crushing to prepare soybean cake/soybean chip for solvent extraction with hexane for the removal of oil. Globally about 85% soybeans are processed into vegetable oil and meal (mainly for animal feed) [60]. A small portion of meal is processed further following various techniques for human consumption.

b) **Processing for protein ingredients**: The spent soybean cakes/flakes after removal of the oil are the major source for defatted flour, soybean concentrates and isolates and 40% soybean meal following well established procedures. Total isoflavone contents in soy ingredient were: Soy flour (full fat)> soy flour (textured)> soy flour>soybeans>soy protein concentrate (aqueous washed)>soy protein isolate, all around $100 mg.100g^{-1}$ (Table 1), but little or no isoflavone is detected in oil. Total isoflavones in navy beans, mung beans, chickpeas is $<0.5 mg.100g^{-1}$ of individual produce [26].

c) **Milk/drink**: Soy milk is essentially the water extract of soy. It is the first non-fermented product in which beans are soaked for 18-24 hours; the skin is removed, crushed and filtered to yield raw milk. The raw milk (fresh milk) is then cooked around 100°C for 30 minutes to destroy protease inhibitor, microorganism and add taste by removing beany flavour (produced during wet grinding as a result of lipoxygenase catalyzed reactions), colour and shelf life. The boiled milk is filtered to remove fibrous materials (okara). But for commercial production and marketing of soymilk the raw milk may be heated at higher temperature for longer time as well as may add many more steps including ultra-high temperature heat treatment (UHT) and pasteurization, in-container sterilization [61-62]. In European Union countries “soy milk” is not permitted for sale due to non-compliance with the definition of milk which is “substances secreted from mammary gland” [63], but can be sold as soy drink. Several studies have reported positive relationship between the isoflavone contents in seeds with the content in soy milk cooked at around 90°C [37, 64].

d) **Fermentation products:** Natto, tempeh, miso are the major fermented products. Soy cheeses are produced on further fermentation of tofu (a non-fermented product). Data in Table 1 show that the natto, miso and tempeh, the direct fermentation products have sufficient amounts of isoflavones that range between 42 and 59 mg.100 g^{-1} of edible product. Tempeh in batter when deep-fried for 30 min lost almost 45% of the (205±56 *vs* 113±mg.100 g^{-1}) total isoflavones in raw tempeh [65]. Soy cheeses also contain small amounts (<10mg.100 g^{-1} of edible product) of isoflavones, except American and Monterey varieties that contain isoflavones around 18 mg.100 mg^{-1} [26].

e) **Second generation food products**: Because of high protein contents, presence of other micronutrients and isoflavones, soy protein ingredients are added in baking goods (flour) as replacement for animal fat, dairy products etc. Soy proteins are also good replacement for meat, fish and poultry processed food. Soy derived second generation food products include soy bread, soya cookies, cereal bars, lasagne, soy nugget etc. It is not unexpected that the amount of isoflavones in the edible products was very low and ranged between 2.4

mg.100 g^{-1} to 18.1mg.100 g^{-1} (FW). The highest amounts of isoflavones were in soy kibe and soy sausages, probably due to the large amount of soy protein, are used in the formulation of these products. The low amounts of isoflavones in second generation products may not be considered as a good source for isoflavone requirements to reduce the risk of chronic diseases [66]. Table 1 lists the isoflavone contents in major soy ingredients, first generation processed as well as a few second generation foods (i.e. foods to which soy ingredients were added prior to cooking). Data in Table 1 clearly demonstrate that total isoflavone, daidzein and genistein contents are highest in protein ingredients that have not been subjected to heat treatment or followed fermentation steps. The simplest soy food product is the soy milk- basically the water extract of soybean, also referred to as raw soymilk (unprocessed), has considerably higher total isoflavone than cooked. For example, the total isoflavone content in soymilk skin or film (Foo joke or Yuba) was 196.05 mg.kg^{-1} compared to 44.67 mg.$100g^{-1}$ when cooked [26]. It is interesting that value reported for soymilk varies considerably that raises the concern for the use of the term “soymilk”. This seriously enforces the view that all soy milk is not produced equally and careful vigilance is required on the source (country of origin), process, nutritional and physiological labels.

7. Effects of Processing on Isoflavone Contents

Processing affects the retention and distribution of isoflavone isomers in soy foods. The conversion and loss of isoflavones during processing can affect the nutraceutical values of soybean. The most advanced technical achievements in separation and detection techniques (as mentioned in section 5) are setting standards for study and specification of isoflavones, starting from plant matrix, through food processing, to human intake.

Raw materials containing isoflavones are processed into edible products using physical (soaking, boiling, treatment with acids and bases at ambient and high temperatures), biological (fermentation involving microbes, molds and yeasts) and enzymatic reactions. These actions converts the most abundant malonyl derivatives into acetyl-derivatives and finally into aglycones [67]. Dilution of soy proteins by mixing with other ingredients and heat treatments result in changes of the isoflavone profiles [68]. Different types of processing will have different health/physiological effects that can be achieved when soy products are consumed. Following are the main methods of processing in practice or operation and their effects on the different forms of isoflavones:

a) Thermal Processing and its Effects

Different cultivars present several forms and amount of isoflavones. When the grains were cooked to be soft the malonyl form decreased, and aglycone and glycoside forms increased. It was observed that the heating treatment transformed the malonylglucosides into glucoside isoflavones. After heat treatment at 121ºC for 30 min, nearly all malonyl isoflavones were converted into glucoside [69]. This means isoflavones may not destroy by heat treatment, rather subject to intra-conversions between the different forms. The chemical modification of isoflavones in soy foods (defatted soy flour, toasted soy flour, soymilk and tofu, baking or frying of textured vegetable protein, and baking of soy flour in cookies) have been analyzed during cooking and processing [70-72]. This led to determine the best conditions for extraction of isoflavones from soy foods and the effects of commercial processing procedures and of cooking on isoflavone concentrations and composition. Outcomes of these studies suggested that the chemical form of isoflavones in foods should be taken into consideration when evaluating their availability for absorption from the diet.

Influence of thermal processing such as boiling, regular steaming and pressure steaming were also investigated in yellow and black soybeans. Again, all thermal processing caused significant increases in aglycones and β-glucosides of isoflavones, but caused significant decreases in malonyl glucosides of isoflavones for both kinds of soybeans. The malonyl glucosides decreased dramatically with an increase in β-glucosides and aglycones after thermal processing [73]. According to USDA data [27] total soybean isoflavones contain 37% daidzein, 57% genistein, and 6% glycitein. So the main component of soy isoflavone is genistein which has many physiological actions and benefits [74]. Likewise steaming germinated soybean, which has a high amount of genistein, might be an anticancer functional food through the inhibition of Human DNA Topoisomerase II enzyme activity [75].

b) Fermentation Processing and its Effects

Fermentation process of soy leads to manufacturing of different soy fermented foods, such as tempeh, soy extract, miso and natto. Differences on isoflavones content between non-fermented and fermented soybean products have been extensively studied. Isoflavone glucosides were the major components in soybean and non-fermented products, while isoflavone aglycones were abundant in sufu (a fermented tofu product) and partially in miso of soybean fermented products. Tempeh is a traditional fermented soybean food product from Indonesia. It is normally consumed fried, boiled, steamed or roasted. Soybean is processed into tempeh by fungus mediated fermentation. This way of processing reported an increase of aglycones amount with fermentation time of tempeh, approximately two-fold higher after 24 hours fermentation. Likewise a combined process of fermentation and refrigeration also recorded an increase in aglycone forms [76-78].

Fermentation with microorganisms or natural products containing high β-glucosidase activity converts β-glucosides into corresponding aglycones by breaking the carbohydrate bond [79]. Fermentation increases isoflavone aglycone contents in black soybean pulp [80]. Genistein concentrations in black soybean pulp were recorded higher than controls after fermentation with *Lactobacillus acidophilus* and *Bacillus subtilis*. The conversion of isoflavones from glycosides to aglycones also reported in the fermentation process of whole soybean flour [81].

c) Non-Fermentation Processing and its Effects

Tofu is a popular non-fermented soy food. Processing of tofu involves soaking and heating procedures as well as the addition of protein coagulants (calcium sulfate) to soymilk to coagulate to make tofu. Results of the stability of isoflavone during processing of tofu showed that the concentrations of the three aglycones increased with increasing soaking temperature and time, while a reversed trend was found for the other nine isoflavones. During soaking of soybean malonyl glucosides can be converted to acetyl glucosides, which can further be converted to glycosides or aglycones depending on soaking temperature and time [82]. The increase of aglycones and decrease of glucoside isoflavones during fermentation coincided with the increase of β-glucosidase activity observed in fermented soymilk [83].

8. Soy Infant Formula

Soy infant formula has been in use for over sixty years in the US, and may be classified as the “second generation soy product”. Soy protein ingredients are added as a replacement in infant milk formula to avoid allergic reactions to proteins in pasteurized milk /or other issues. Soy infant formula is also often used as a replacement for mother's milk. Soy formula fed millions of infants worldwide with no observable adverse effects [84]. The patterns of growth, bone health and metabolic, reproductive, endocrine, immune and neurological functions are similar to those observed in children fed with cows' milk-based formulas or Human milk [85]. However use of soy-based infant formula is not recommended if there is indication of other food allergic reactions [86]. Isoflavone contents in marketed soy based infant formula varied significantly. Overall soy infant formula milk support normal growth and may have advantages in promoting bone development [85].

9. **Baked Products**

Very little to almost no isoflavone is found in most marketed baked products with the exception of soya bread that contained isoflavone at 14.67 mg.100 g^{-1} of bread [26.]. The fate and concentration of isoflavones in soy breads made from soy protein isolates and flour obtained from low, intermediate and high level soybean grown in Southern Ontario [87]. The content of isoflavones with the exception of malonyl derivative did not change during the entire process. The malonyl derivatives, on the other hand, were decarboxylated to glycosides and then followed to total deconjugation. Likewise isoflavone aglycone composition evaluated within a soy functional food like soy bread system. Isoflavone malonyl-glucosides (>80%) were converted into acetyl and simple glucoside forms (substrates more favorable for β-glucosidase) in steamed and roasted soy flour/soy milk mixture (SM). Their corresponding breads had isoflavones predominately as aglycones (~75%). Steamed SM bread was more consumer acceptable than roasted [88]. Isoflavones are not found in most cereals except KELLOG's Ready to eat cereal KASHI (17.40 mg.100g^{-1}) and Ready to eat SMART (93.90 mg.100g^{-1}) [26].

10. Irradiation

Like most grains, soy is also irradiated to prevent fungus growth. Soy beans when irradiated between 2.5-10k Gy required less soaking time and 30-60% less time compared to non-treated soy [89]. Irradiation at 0.5-5.0 kGy caused deconjugation and production of aglycone as well as increased anti-oxidation properties [90]. Likewise, the influence of gamma irradiation on isoflavone (genistein, daidzein, and their glycosides genistin and daidzin) contents and hydroxyl radical scavenging effect (HRSE) is also on record [91]. Doses up to up to 10 kGy improve the antioxidant activities of soybean and also nutritional quality with respect to isoflavone content.

11. Storage

Storage of seeds and samples are very critical for the determination of nature of isoflavones and contents. Storage of soybeans between -18°C and 42°C for one month had no effect on the total content of isoflavones, but the profile changed dramatically at 42°C with a significantly decrease in malonyl derivatives with a proportional increase of β-glucosides [92]. Further it has been shown that soybeans and red-clover isoflavone extract profile changes considerably during storage due to mainly hydrolytic reactions. For example, soy beans high in malonyl and acyl derivatives were degraded into glycoside during early days of storage and reached a plateau after extended storage.

Changes also recorded in the compositional components of black soybeans maintained at room temperature for different storage periods. Column chromatography and HPLC-DAD-

ESI/MS spectrometry analysis were performed on hydrolysed extracts of isoflavone and anthocyanin profiles. These components decreased markedly during storage while protein, oil, and fatty acid showed a slight decrease. The scavenging activities of DPPH (diphenylpicrylhydrazyl) and ABTS (2, 2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) radicals during storage also decreased in comparison with those of observed before storage [55].

12. Bioavailability

Isoflavone bioavailability is a measure of the amount of these compounds that becomes available for tissue distribution where they can exert physiological effects. Thus, an understanding of bioavailability is important in assessing the research findings obtained through clinical trials and the possible health benefits of isoflavone, Dietary isoflavone may be metabolized in the intestine to equol, a metabolite, [7-hydroxy-3-(4′-hydroxyphenyl)-chroman] that has greater estrogenic activity than daidzein, and to other metabolites that are less estrogenic. This metabolite has affinity for both estrogen receptors, ERα and ERβ [93-94]. The presence of equol in urine or plasma has been used by researchers to classify subjects with analysis of outcomes in relation to equol-producing ability [95]. More specifically, the proportions of daidzein, genistein and glycitein, will also greatly affect the resulting isoflavone bioavailability and overall physiological effects, due to their different chemical structures and *in vivo* properties.

Number of factors can influence the absorption of food components, including dietary habits, the food matrix, intestinal fermentation and transit time. In bioavailability studies, the soy food used and its isoflavone composition are important determinants of the resulting isoflavone pharmacokinetics and potential physiological effects. The influence of diet is important due to interactions between dietary components. Diet has a strong effect on composition of the gut microbiota, which in turn plays a crucial role in isoflavones bioavailability. Research data from intervention studies in humans, focussing on the factors that affect bioavailability of soy isoflavones, reported an increased concentration of genistein than daidzein in serum. This increased genistein level recorded without the influence of age and gender on the bioavailability of soy isoflavones [96-98]. Further the amount and source of lipid did not affect bio accessibility or uptake and metabolism of isoflavones [99].

Typical fermentation products such as lactic acid or the method of hydrolyzation has no effect on isoflavone metabolism. Isoflavone aglycones were absorbed faster and in greater amounts than isoflavones glucosides. There was no difference recorded in the levels of isoflavones in the blood of volunteers consuming fermented soy milk or soy milk with hydrolyzed isoflavones [100]**.** Further it is worth to point out here that isoflavones are detectable in plasma as soon as 30 minutes after soy intake with an initial peak 1 hour post-meal. This early increase may be due to the presence of a small proportion of aglycones available in the soy isoflavones [101]. Also hydrolysis and initial absorption occur readily in the duodenum and proximal duodenum within the first hour of digestive processing [94]. The majority of the urinary excretion of daidzein and genistein occurs within the first 24 hours after soy ingestion.

To summarise this section it is on record that over the years the substantial evidence supporting the potential for beneficial effects of soy consumption has led to much wider use of traditional soy products even in western countries. This follows the development of new isoflavone and soy-enriched foods and supplements. The role of biotransformation to various conjugates together with the level and duration of isoflavone consumption have been shown to be of importance. Furthermore, the effects of habitual diet on gut microbiota may be one of the most important factors affecting isoflavone bioavailability and thus, modulation of physiological effects. In short, foods are digested, metabolized/ absorbed, distributed (retained) and eliminated after consumption. In general consumed isoflavones follow a number of steps that are greatly influenced by the food matrix i.e. raw, cooked, amount of food, and intestinal microflora and dispersion in the gastric emulsion for metabolism. Isoflavones are then absorbed by the intestinal cell walls for the transportation into blood system (bioavailability) for positive and beneficial effects.

13. Health Benefits and Isoflavones

Over the years epidemiological studies have consistently shown that communities whose diets consist of soy-derived products have lower incidences of chronic diseases such as cardiovascular disease, cancers, menopausal symptoms, diabetes and others [102]. This section reviews the overall health effects of isoflavones by focusing on the important human studies, and discusses the implication of the results from different human trials as under:

A) Positive Benefits

As mentioned before phytoestrogens, as bioactive compounds, has become one of the more important areas of interest in clinical nutrition. Also important the wide range of biological properties of these bioactive compounds that contribute to the many different health-related benefits [47]. Maximal health benefits are most likely to be derived by consuming small amounts of isoflavone-rich foods throughout the day. Isoflavones have characteristics that are consistent with selective estrogen receptor modulators and not estrogens. As such, when consumed at usual dietary intakes, isoflavones are unlikely to have the negative effects associated with estrogens. Research in several areas of healthcare, particularly nutraceutical, health and wellness and clinical nutrition, has shown that consumption of isoflavones may play a role in lowering risk for diseases as well as observed health benefits [1, 24]. Concise and focused details of positive health effects of isoflavones and how these attribute to prevent the incidence or reduction in intensity of some major prevailing health concerns are given below:

a) Isoflavones as Natural Antioxidants

Free radicals are continuously formed in our body as normal by-product of metabolism. Range of research studies has demonstrated that isoflavones have potent antioxidant properties, comparable to that of the well-known antioxidant vitamin E. [2, 3, 103-104]. Antioxidants work by attacking and neutralizing free radicals. The antioxidant powers of isoflavones can reduce the long-term risk of cancer by preventing free radical damage to DNA. Genistein is the most potent antioxidant among the soy isoflavones, followed by daidzein. Genistein seems to increase the production of superoxide dismutase (SOD) which removes the free radicals. Genistein’s ability to act as antioxidant may also explain the antcarcinogen effect of this isoflavones. Genistein is an estrogen receptor (ER)-selective binding phytoestrogen, with a greater affinity to ERβ. Genistein inhibits tyrosine kinases and inhibits DNA topoisomerases I and II, and act as an antioxidant [75].

The isoflavones also demonstrate good antioxidant activity in various systems (both aqueous and lipophilic phases) attributed to a number of antioxidant mechanisms. The inhibition of lipid peroxidation, particularly of low density lipoprotein (LDL) by isoflavones may be an important mechanism by which they positively influence lipid profiles. The conversion of daidzein to equol may be physiologically important as equol has significantly greater antioxidant activity and estrogenic activity (approximately 100-fold higher) on binding to the ER receptor compared with daidzein [105]. Further the consumption of antioxidant/polyphenol rich foods might impart anti-thrombotic and cardiovascular protective effects via their inhibition of platelet hyperactivation or aggregation. Aspirin is commonly used as anti-platelet drugs. Aspirin block the cyclooxygenase (COX)-1 pathway of platelet activation, similar to the action of antioxidants with respect to neutralising hydrogen peroxide (H_2O_2). So the ability of polyphenols rich foods to target additional pathways of platelet activation is possible, Also, dietary isoflavones or polyphenols rich foods may substitute or complements currently used anti-platelet drugs in sedentary, obese, pre-diabetic or diabetic populations who can be resistant or sensitive to pharmacological anti-platelet therapy [106].

b) Lowering of Cholesterol Levels and Cardiovascular Disease

Several clinical trials reported significant decrease in total cholesterol, blood low-density lipoprotein-cholesterol and triglycerides with soy protein intake leading to lower incidences of chronic diseases such as cardiovascular disease (CVD), cholesterol and others. CVD risk factors have been shown a decline among the populations when soy isoflavones added to the diet. This decline in CVD risk factor is recorded both in healthy individuals as well as those on medication. Soy protein directly lowers LDL concentrations. Being low in saturated fat and a good source of essential fatty acids, isoflavones can improve endothelial function and possibly slow the progression of subclinical atherosclerosis [107-109].

Researchers also used biomarkers of soy intake in assessing the relationship between soy consumption and coronary heart disease (CHD). Biomarkers that reflect both intake and metabolism may be more informative than self-reports of dietary intake. Research findings on urinary isoflavonoids and risk of CHD concluded that equol, a bioactive metabolite of soy isoflavone daidzein, may be inversely associated with risk of CHD in women [15]. Over all clinical and epidemiologic data indicate that adding soy foods to the diet can contribute to the health of postmenopausal women by addressing several conditions and diseases associated with the menopausal transition [110].

c) Isoflavones and Epigenetic Changes

De-regulation of gene expression is a hallmark of cancer. Epigenetic changes are mediated by several molecular mechanisms like histone modifications, small non-coding or anti-sense RNA and DNA methylation [111]. The isoflavones have been reported to interact with epigenetic modifications, specifically hypermethylation of tumor suppressor genes. Now the mechanisms are known by which phytoestrogens act on chromatin in breast cancer cell lines, and tend to modify transcription through the demethylation and acetylation of histones in breast cancer cell lines [112]. Latest research findings further enlightened the impact of dietary intake of isoflavones on the epigenetic gene regulation in cancer prevention. These effects have been suggested to contribute in cancer prevention by affecting several key processes such as DNA repair, cell signaling cascades including Wnt-signaling, induction of apoptosis, cell cycle progression [113]. Likewise dietary soy consumption caused deleterious effect on the granulosa cell tumor development (GCT) in human. Genistein modulates estrogen receptor expression in the human granulosa cell tumor-derived COV434 cell line and positively promotes cell growth by suppressing caspase-dependent apoptosis [114].

d) Lowering Risk of Breast Cancer

Considerable research efforts have been made to validate the benefits of soy isoflavones in the prevention and/or treatment of breast cancer. The incidence and mortality rates of breast cancer are high in the Western world compared with countries in Asia, possibly because of selection of dietary intakes. In Asian populations, where soy intake is high, the researchers found an inverse association between soy food intake and breast cancer [115]. Data from over 16000 women, diagnosed with breast cancer recurrence, was presented in the 102nd Annual Meeting of American Association for Cancer Research and recommended that it is beneficial to include soy food as part of healthy diet for women, including those which had breast cancer [116].

e) **Lowering Risk of Prostate Cancer**

Enlargement of the prostate gland and eventually developing into prostate cancer is a rapidly growing disease in men. Different diagnostic testing programs are in operation for random screening and an early detection of prostate cancer or its symptoms. It is estimated that worldwide more than over 1 million new cases of prostate cancer are diagnosed yearly. Prostate cancer affects more North American than Asian men. The difference in the incidence rate has been linked to the presence of microbiota (intestinal bacteria) that converts genistein into equol, which is abundant in Asian population than North Americans and Europeans [117]. This means that prostate cancer has marked geographic variations between countries. Also genetic, epigenetic, and environmental factors co-contribute to the development of the cancer. Mortality from prostate cancer is much higher in the U.S. than in Asian countries, such as Japan and China.

Extensive worldwide research studies investigated the association between dietary factors and prostate cancer, and also potential for soybean and its products to prevent this disease. Soy isoflavone supplementation appeared to slow the rising serum prostate specific antigen (PSA) concentration associated with prostate tumor growth of prostate cancer patients. Multiple meta-analysis of randomized controlled trials were reported on the efficacy of soy and soy isoflavones in men with prostate cancer (PCa) or with a clinically identified risk of PCa, Meta-analyses of these studies including men with identified risk of PCa found a significant reduction in PCa diagnosis after administration of soy isoflavones. However short-term intake of soy isoflavones did not affect serum hormone levels or PSA [118-126].

To determine the mechanisms or pathways as to how consumption of soy foods helps to reduce the risk of prostate cancer, clinical trials were undertaken on clinical pharmacology of isoflavones and its relevance for potential prevention of prostate cancer [127]. Isoflavones are phytoestrogens that have pleiotropic effects in a wide variety of cancer cell lines. Many of these biological effects involve key components of signal transduction pathways within cancer cells, including prostate cancer cells. Epidemiological studies have raised the hypothesis that isoflavones may play an important role in the prevention and modulation of prostate cancer growth. Recently published review article discussed the possible molecular mechanisms behind the reduced risk of prostate cancer (PCa), when soy food added to the diet [119]. Cell-based studies show that soy isoflavones regulate genes that control cell cycle and apoptosis. Food intake rich with soy isoflavones may induce growth arrest and apoptosis of PCa, regulated by estrogen- and androgen-mediated signaling pathways. Other possible mechanisms include antioxidant defense, DNA repair, inhibition of angiogenesis and metastasis, potentiation of radio- and chemotherapeutic agents. Major Phytoestrogens genistein and daidzein have the ability to reverse DNA methylation in cancer cell lines. This action may be mediated through ERβ [120].

Overall, data obtained from clinical studies are much more convincing in regard to the activity of a number of isoflavones, and have led to the development of genistein and phenoxodiol in the clinic as potential treatments for prostate cancer. However, the potential activity of isoflavones in combination with cytotoxics or radiotherapy warrants further investigation. Further evaluation of the role of soy isoflavones in inducing apoptosis and cell cycle control is warranted in the preventive and therapeutic setting. Although these research findings are encouraging, the results of larger randomized controlled trials are needed to determine whether soy isoflavone supplementation can play a targeted role in the prevention or treatment of prostate cancer.

f) **Osteoporosis and Menopausal Symptoms**

Osteoporosis is the thinning of bone tissue and loss of bone mineral density (BMD). About 1 out of 5 American women over the age of 50 suffer from osteoporosis. Menopause is characterised by the loss of estrogen production by the ovaries. The lack of estrogen increases the ability of osteoclasts to absorb bone. Osteoclasts (the cells which produce bone) are not encouraged to produce more bone. The main cause of osteoporosis in ageing women is a decline in estrogen hormone in their body. This decline in menopausal hormone contributes to the risk of osteoporosis. The Hormone Replacement Therapy (HRT) is one of the ways to reduce such a risk [5]. However women are unwilling to start HRT treatment because of increased risk of breast and endometrial cancer. Soy isoflavones, if available, may act as substitute for low or no natural estrogen release to prevent bone loss. As such soy isoflavones have been widely studied for their effects on bone health in the preservation of the bone substance, and fight osteoporosis by improving bone strength in postmenopausal women. Meta-analyses on the effect of soy isoflavones on BMD concluded that six month intake of soy isoflavones was adequate to exert a beneficial effect on it, especially of the lumber spine. These studies also evaluated the effects of soy isoflavones on bone turnover markers and found that these bioactive compounds did significantly reduce the levels of urine deoxypyridinoline (a bone resorption marker) [17]. This is the reason why people in China and Japan experience low incidence of osteoporosis, despite their low consumption of dairy products, whereas in Europe and North America the contrary happens. Recently a research group from China analyzed multiple published international clinical studies on the application of soy isoflavones to prevent osteoporosis, the central cause of hip fractures and other bone fractures, and concluded that soy isoflavones intake increase BMD [128]. Overall, the beneficial effects of soy isoflavones are possibly the results of their chemical similarity to human estrogen, which is known to increase BMD in menopausal women. Also there are molecular mechanisms behind the action of genistein (soy isoflavone) in reducing the risk of bone loss, when soy food added to the diet. Genistein is an estrogen receptor (ER)-selective binding phytoestrogen, with a greater affinity to ERβ. Genistein enhances osteoblastic differentiation and maturation by activation of estrogen receptor (ER), p38MAPK-Runx2, and NO/cGMP pathways. It also inhibits osteoclast formation and bone resorption through inducing osteoclastogenic inhibitor osteoprotegerin (OPG) and blocking NF-κB signaling [129].

g) **Relief in Menopausal Symptoms**

Most women experience hot flashes or night sweats during their menopause. This result in impaired concentration, disturbs sleep and other physical problems like joint and muscle pain. To reduce hot flashes hormone treatment often recommended but can have side effect. As such isoflavones intake may play a role in controlling hot flashes by replacing hormone treatment [130].

This may possibly be due to the structural similarity of isoflavones and human estrogen. Recently reported clinical and epidemiologic data ("meta-analysis"-which is the largest and most comprehensive conducted to date) indicate that adding soy foods to the diet can contribute to the health of postmenopausal women by reducing the frequency and severity of hot flushes [6, 110, 131]. Genistein, a predominant soybean isoflavone, supplement can alleviate menopausal hot flashes in postmenopausal women. Based on these latest research findings it can be concluded that the use of soy isoflavones (phyto-oestrogens) can lead to a significant reduction in some of the disorders linked with the menopause, especially hot flushes by making up the decline of endogenous estrogen hormone.

Regarding the role of soy isoflavone and their metabolites including equol, clinical studies compared outcomes among women whose intestinal bacteria have the ability to convert daidzein to equol (equol producers) with those that lack that ability (equol non-producers). This comparison may help to determine if equol producers derive greater benefits from soy supplementation. Also consensus appearing that soy isoflavone and its metabolite S-equol supplements provides relief from menopausal discomforts like hot flash frequency as well as muscle and joint pain [132-134].

h) **Obesity and Diabetes**

It is for the most part accepted that obesity is caused by an imbalance between energy intake and energy consumed. The increased body weight contributes to the development of metabolic syndrome a pre-cursor to diabetes, an insulin resistant phenomenon. There have been many speculations that soy isoflavones could play important role in body weight (obesity) management issues. These speculations are based on the knowledge that isoflavones are mildly estrogenic and that data from animal models for obesity showed reductions in body fat accumulation and improvement in insulin resistance, a hall mark for obesity [18-19]. Currently, there is no direct evidence of managing diabetes from soy isoflavones in diets, although efforts and laboratory trials are continuing. Likewise, there are only a few human studies, with limited information on loss in body weight [135]. Recent evidence has indicated that dietary polyphenols may modulate mitochondrial function, substrate metabolism and energy expenditure in humans. Effects of short-term supplementation of two combinations of polyphenols increases energy expenditure and alters substrate metabolism in overweight subjects. Positive effect of soy isoflavones may possibly be due to their higher lipolytic potential [136]. These findings further suggested that long-term supplementation of these dosages may improve metabolic health and body weight regulation.

i) **Cognitive Functions**

Cognitive activities generally refer to reception, learning, memory and expression. These factors are greatly affected by aging processes. As the world aging population is increasing, there is growing concern that incidences of Alzheimer/dementia (memory loss and learning) will also increase accordingly along with health care cost. Studies have shown that estrogen replacement therapy (ERT) can possibly increase verbal memory, and may prevent Alzheimer disease in postmenopausal women. Isoflavones being structurally similar to 17β-estradiol have been shown to bind estrogen receptors and may positively influence learning and memory expression in women [20-21].

A range of randomized double-blind, crossover, placebo-controlled studies involving healthy postmenopausal women with variable age groups receiving isoflavone tablets showed an increase in cognitive functions like working and visual memory [137-140]. This area of research has also shown some inconsistent results. This may, possibly, be due to substandard or poor experimental designs that did not include other factors such as identities, purities, source of isoflavones as well large number of participants. This topic has been thoroughly reviewed, analyzed and recommendations made for future research [141].

B) Negative Effects and Inconclusive Results

Alongside side hope and positivity backed by strong research based evidence, results of some studies on soy isoflavones are inadequate, inconsistent or statistically not significant in supporting some of the suggested health benefits of consuming soy protein or isoflavones, except for a modest effects [142-143]. There are also real concerns that excessive amounts of isoflavones in serum may promote other hormone-related problems. Although there are not many documented cases but a few incidences of feminizing effects-reduced libido and erectile dysfunction, reduced sperm concentration without morphological changes are reported [144-146]. However, meta- analysis on the clinical evidences concluded that soy food or isoflavones did not affect semen and sperm [147].

Having said that more rigorous studies are required to assess dose-response relationships while consuming soy food and supplementation. Possible fertility issues among males and the unknown long-term health effects of consuming highly processed modern soy foods needs cautious approach. Overall, consuming moderate amounts of traditionally prepared and minimally processed soy foods may offer modest health benefits while minimizing potential for adverse health effects [148].

14. Future Research Directions

Given the recent upsurge in soy products in the human food market, it may be important to indicate, in addition to the amount of isoflavones, the type of isoflavones in these products. Processing can, possibly, affects the retention and distribution of isoflavone isomers in soy foods. The conversion and loss of isoflavones during processing may affect the nutraceutical values of soybean. Different types of processing will have different health/physiological effects that can be achieved when soy products are consumed. New processing technologies may be needed to meet the growing demands of soy products with or without isoflavones, especially increased genistein as meat replacement and/or for managing health. Although soy isoflavones were hailed as magical natural component that provides health benefits to human, but some clinical trials have raised doubts on the validity of such claims. There are urgent needs to address these inconsistencies. One of the major issues appears to be ill conceived and/or not well-thought out experimental designs that lacked information on the nature, quality and bioavailability of isoflavones. This may require establishing well defined international guidelines for investigations on health benefits for soy protein and isoflavones. In addition there is a stronger need to keep an eye on the reported side effects of over use of soy drink, beverage and meatless products that contain soy ingredients as animal protein substitutes. Also, possible fertility issues among male humans, and the unknown long-term health effects of consuming highly processed modern soy foods needs cautious approach. And finally, greater standardization and documentation of clinical trial data of soy isoflavones are needed to further substantiate health benefit claims.

15. References

1. Preedy, V. R. (2013). Isoflavones: chemistry, analysis, function and effects. **In**: Preedy, V. R., Eds., Royal Society of Chemistry (RSC Publishing), Cambridge, UK

2. Patel, R. P., Boersma, B. J., Crawford, J. H., Hogg, N., Kirk, M., Kalyanaraman, B., Parks, D. A., Barnes, S., and Darley-Usmar, V. (2001). Antioxidant mechanisms of isoflavones in lipid systems: paradoxical effects of peroxyl radical scavenging. *Free Radic. Biol. Med.*, **31**:1570-1581.

3. National Cancer Institute (NCI). (2004). Antioxidants and cancer prevention: Fact sheet. Retrieved November 18, 2008, from *http://www.nci.nih.gov/cancertopics/factsheet/antioxidantsprevention*.

4. Tham, D., Gardner, C., and Haskell, W. (1998). Potential health benefits of dietary phytoestrogens: a review of the clinical, epidemiological, and mechanistic evidence. *J. Clin. Endocrinol. Metabol.*, **83**: 2223-2235**.**

5. Messina, M. (1998). Soy foods: An Alternative to Hormone Replacement Therapy. *Vegetarian Nutrition and Health Letter*, **1(5)**.

6. Taku, K., Melby, M. K., Kronenberg, F., Kurzer, M. S., and Messina, M. (2012). Extracted or synthesized soybean isoflavones reduce menopausal hot flash frequency and severity: systematic review and meta-analysis of randomized controlled trials. *Menopause*, **19**: 776-790.

7. Clarkson, T. B. (2000). Soy phytoestrogens: what will be their role in postmenopausal hormone replacement therapy? *Menopause*, **7**: 71-75.

8. Loibl, S., Lintermans, A., Dieudonné, A., and Neven, P. (2011). Management of menopausal symptoms in breast cancer patients. *Maturitas*, **68**: 148-154.

9. Magee, P. J., Mcglynn, H., and Rowland, I. R. (2004). Differential effects of isoflavones and lignans on invasiveness of MDA-MB-231 breast cancer cells *in vitro*. *Cancer Lett.*, **208**: 35-41.

10. Patisaul, H. B., and Jefferson, W. (2010). The pros and cons of phytoestrogens. *Front. Neuroendocrinol*, **31**: 400-419.

11. Ganry, O. (2005). Phytoestrogens and prostate cancer risk. *Prev. Med.*, **41**:1-6.

12. Nagata, Y., Sonoda, T., Mori, M., Miyanaga, N., Okumura, K., Goto, K., Naito, S., Fujimoto, K., Hirao, Y., Takahashi, A., Tsukamoto, T., and Akaza, H. (2007). Dietary isoflavones may protect against prostate cancer in Japanese men. *J. Nutr.*, **137**: 1974-1979.

13. Zuniga, K. E., Clinton, S. K., and Erdman, Jr, J. W. (2013). The interactions of dietary tomato powder and soy germ on prostate carcinogenesis in the TRAMP model. *Cancer Prev. Res.*, **6**: 548-557.

14. Merz-Demlow, B. E., Duncan, A. M., Wangen, K. E., Xu, X., Carr, T. P., Phipps, W. R., and Kurzer, M. S. (2000). Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women. *Am. J. Clin. Nutr.*, **71**: 1462-1469.

15. Zhang, X., Gao, Y. T., Yang, G., Li, H., Cai, Q., Xiang, Y. B., Ji, B. T., Franke, A. A., Zheng, W., and Shu, X. O. (2012). Urinary isoflavonoids and risk of coronary heart disease. *Int. J. Epidemiol.*, **41**:1367-1375.

16. Ma, D. F., Qin, L. Q, Wang, P. Y., and Katoh, R. (2008). Soy isoflavone intake inhibits bone resorption and stimulates bone formation in menopausal women: meta-analysis of randomized controlled trials. *Eur. J. Clin. Nutr.*, **62**:155-161.

17. Wei, P., Liu, M., Chen, Y., and Chen, D. C. (2012). Systematic review of soy isoflavone supplements on osteoporosis in women. *Asian Pac. J. Trop. Med.*, **5**: 243-248.

18. Velasquez, M. T., and Bhathena, S. J. (2007). Role of dietary soy protein in obesity. *Int. J. Med. Sci*., **4**: 72-82.

19. Zimmermann, C., Cederroth, C. R., Bourgoin, L., Foti, M., and Nef. S. (2012). Prevention of diabetes in *db/db* mice by dietary soy is independent of isoflavone levels. *Endocrinol.*, **153**: 5200-5211.

20. Henderson, V. W., Paganinin-Hill, A., Miller, B. L., Elble, R. J., Reyes, P. F., Shoupe, D., McCleary, C. A., Klein, R. A., Hake, A. M., and Farlow, M. R. (2000). Estrogen for Alzheimer disease in women, randomized, double-blind placebo-controlled trial. *Neurolog*y, **54**: **2**95-301.

21. Neese, S. L., Bandara, S. B., Doerge, D. R., Helferich, W. G., Korol, D. L., and Schantz, S. L. (2012). Effects of multiple daily genistein treatments on delayed alternation and differential reinforcement of low rates of responding task in middle-aged rats. *Neurotoxicol. Teratol*., **34**:187-195.

22. Andres, A., Donovan, S. M., and Kuhlenschmidt, M, S. (2009). Soy isoflavones and virus infections. *J. Nutr. Biochem.*, **20**: 563-569.

23. Riboli, E., Hunt, K. J., Slimani, N., Ferrari, P., Norat, T., Fahey, M., Charrondière, U. R., Hémon, B., Casagrande, C., Vignat, J., Overvad, K., Tjønneland, A., Clavel-Chapelon, F., Thiébaut, A., Wahrendorf, J., Boeing, H., Trichopoulos, D., Trichopoulou, A., Vineis, P., Palli, D., Bueno-De-Mesquita, H. B., Peeters, P. H., Lund, E., Engeset, D., González, C. A., Barricarte, A., Berglund, G., Hallmans, G., Day, N. E., Key, T. J., Kaaks, R., and Saracci, R. (2002). European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr*., **5**:1113-1124.

24. Zamora-Ros, R., Knaze, V., Luján-Barroso, L., Kuhnle, G. G., Mulligan, A. A., Touillaud, M., Slimani, N., Romieu, I,., Powell, N., Tumino, R., Peeters, P. H., de Magistris, M. S., Ricceri, F., Sonestedt, E., Drake, I,., Hjartåker, A., Skie, G., Mouw, T., Wark, P. A., Romaguera, D., Bueno-de-Mesquita, H. B., Ros, M., Molina, E., Sieri, S., Quirós, J. R., Huerta, J. M., Tjønneland, A., Halkjær, J., Masala, G., Teucher, B., Kaas, R., Travis, R. C., Dilis, V., Benetou, V., Trichopoulou, A., Amiano, P., Ardanaz, E., Boeing, H., Förster, J., Clavel-Chapelon, F., Fagherazzi, G., Perquier, F., Johansson, G., Johansson, I,., Cassidy, A., Overvad, K., and González, C. A. (2012). Dietary intakes and food sources of phytoestrogens in the European Prospective Investigation into Cancer and Nutrition (EPIC) 24-hour dietary recall cohort. *Eur. J. Clin. Nutr*., **66**: 932–941.

25. USDA. (2002). Iowa State University Isoflavones Database. United States Department of Agriculture [Web page]. March 15, 2002.Available at: http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav/isoflav.html.

26. USDA. (2008). USDA National Nutrient Database *http://www.ars.usda.gov/nutrientdata*. Accessed August 20, 2013. Also available at *www.isoflavones.info/isoflavones-content.php* (accessed August 20, 2013).

27. United States Department of Agriculture (USDA). (2012). World Agricultural Supply and Demand Estimates: WASDE- 504 (ISSN: 1554-9089). *www.usda.gov/oce/commodity/wasde/latest.pdf*

28. Thompson, M. J. (2010). Isoflavones: Biosynthesis, Occurrence and Health Effects. Nova Publishers, New York, USA.

29. Waggle, D., and Bryan, B. (2000). Recovery of Isoflavones from Soy Molasses. United States Patent 6,083,552.

30. Mazur, W. M., Duke, J. A., Wahala, K., Rasku, S., and Adlercreutz, H. (1998). Isoflavones and lignans in legumes: nutritional and health aspects in human. *J. Nutr. Biochem.*, **9:**193-200.

31. Ho, H., Chen, R., Leung, L., Chan, F., Huang, Y., and Chen, Z. (2002). Difference in flavonoid and isoflavone profile between soybean and soy leaf. *Biomed. Pharmacother*, **56***:* 289-295.

32. Reinli, K., and Block, G. (1996). Phytoestrogen content of foods--a compendium of literature values. *Nutr Cancer*, **26:**123-148.

33. Jiao, Z., Si, X., Zhang, Z. M., Li, G. K., and Cai, Z. W. (2012). Compositional study of different soybean (Glycine max L.) varieties by 1H NMR spectroscopy, chromatographic and spectrometric techniques. *Food Chem.*, **135**: 285-291.

34. Thuzar, M., Puteh, A. B., Abdullah, N. A. P., Lassim, M. B. M., and Jusoff, K. (2010). The Effects of Temperature Stress on the Quality and Yield of Soya Bean [(Glycine max L.) Merrill.]. *J. Agric. Sci.*, **2:** 172-179.

35. Seguin, P., Zheng, W., Smith, D. L., and Deng, W. (2004). Isoflavone content of soybean cultivars grown in eastern Canada, *J. Sci. Food Agric.*, **84**: 1327-1332.

36. Akhtar. M. H., Reid, C., and Bryan, M. (2002). Isoflavone contents in fifty soybean varieties grown in Canada. *5th AAFC Food Network meeting, Kentville, NS*. June 18-20, Abs. No. 36.

37. Sertovic, E., Mujic, I., Jokic, S., Alibabic, V., and Saric, Z. (2012). Effect of soybean cultivars and the content of isoflavone on soymilk, *Romanian Biotechnol. Lett.*, **17**: 7151-7159.

38. Wang, H. J., and Murphy, P. A. (1994). Isoflavone composition of American and Japanese Soybeans in Iowa- Effects of variety, crop year, and location. *J. Agric. Food Chem.*, **42**: 1674-1677.

39. Devi, M. K., Gondi, M., Sakthivelu, G., Girdhar, P., Rajasekaran, T., and Ravishankar, G. A. (2009). Functional attributes of soybean seeds and products with reference to isoflavone content and antioxidant activity. *Food Chem.*, **114**: 771-776.

40. World Statistics: *http://soybeans.com/2012/page_30.htm* accessed July 22, 2013.

41. Lozovaya, V. V. , Lygin, A. V. , Ulanov, A. V. , Nelson, R. L. , Dayde, J. , and Widholm, J. M. (2005). Effect of temperature and soil moisture status during seed development on soybean seed isoflavone concentration and composition. *Crop Sci*., **45**: 1934-1940.

42. Chan, C., Qi, X., Li, M. W., Wong, F. L., and Lam, H. M. (2012). Recent Developments of Genomic Research in Soybean. *J. Genet. Genomics*, **39**: 317-324.

43. Barnes, S., Wang, C. C., Kirk, M., Smith-Johnson, M., Coward, L., Barnes, N. C., Vance, G., and Boersma, B. (2002). HPLC-mass spectrometry of isoflavonoids in soy and the American groundnut, *Apios americana*. *Adv. Exp. Med. Biol*., **505**: 77-88.

44. Ichige, M., Fukuda, E., Miida, S., Hattan, J. I., Misawa, N., Saito, S., Fujimaki, T., Imoto, M., and Shindo, K. (2013). Novel Isoflavone Glucosides in Groundnut (*Apios americana* Medik) and Their Antiandrogenic Activities. *J. Agric. Food Chem*., **61**:2183-2187.

45. Nara, K., Nihei, K., Ogasawarara, Y., Koga, H., and Yoji Kato, Y. (2011). Novel isoflavone diglycoside in groundnut (*Apios americana* Medik). *Food Chem*., **124**: 703-710.

46. Pulse Canada (2013). The health benefits of pulses- Clinical trial findings. *http://www.pulsecanada.com/uploads/81/3c/813*. (accessed September 22, 2013)

47. Barnes, S. (2010). The Biochemistry, chemistry and physiology of the isoflavones in soybeans and their food products. *Lymphat. Res. Biol.*, **8**: 89-98.

48. Collison, M. W. (2008). Determination of Total Soy Isoflavones in Dietary Supplements, Supplement Ingredients, and Soy Foods by High-Performance Liquid Chromatography with Ultraviolet Detection: Collaborative Study. *J. AOAC Int.*, **91**: 489-500.

49. Dhaobhadel, S. (2011). Regulation of Isoflavonoid Biosynthesis in Soybean Seeds. **In**: Biochemistry, Chemistry and Physiology. pp. 244-258. Prof. Tzi-Bun Ng., Eds., InTech, Europe.

50. Mortensen, A., Kulling, S. E., Schwartz. H., Rowland, I., Ruefer, C. E., Rimbach, G., Cassidy, A., Magee, P., Millar, J., Hall, W. L., Kramer Birkved, F., Sorensen, I. K., and Sontag, G. (2009). Analytical and compositional aspects of isoflavones in food and their biological effects. *Mol. Nutr. Food Res*., **53**: S266-309.

51. Rostagno, M. A., Villares, A., Guillamon, E., García-Lafuente, A., and Martínez, J. A. (2009). Sample preparation for the analysis of isoflavones from soybeans and soy foods. *J. Chromatogr A*., **1216**: 2-29.

52. Saracino, M. A., Mercolini, L., Musenga, A., Bugamelli, F., and Raggi, M. A. (2008). Comparison of analytical methods for the quality control of new formulation containing soy extract and melatonin. *J. Sep. Sci*., **31**: 1851-1859.

53. Deshmukh, K. A., and Amin, P. D. (2012). Stability Indicating RP-HPLC Method for Analysis of Soy Isoflavones in Pharmaceutical Formulations. *Analyt. Chem. Lett.*, **2**: 327-336.

54. Auwerter, L. C. C., Wanczinski, A. E., and Chiandotti, R. S. (2012). Development of an analytical method to quantify total isoflavones in phytotherapic capsules using high-performance liquid chromatography. *Rev. bras. Farmacogn.*, **22**.

55. Lee, J. H., and Cho, K. M. (2012). Changes occurring in compositional components of black soybeans maintained at room temperature for different storage periods. *Food Chem.*, **131**: 161-169.

56. Oomah, B. D., and Hosseinian, F. S. (2002). Phytoestrogens. **In:** Methods of Analysis for Functional Foods and Nutraceuticals. pp. 1-63. Hurst, W. J., Eds., CRC Press, Florida.

57. ANVISA. (2003). Guia para validação de métodos analíticos e bioanalíticos, Resolução no. 899 de 29 de maio de 2003. *Agência Nacional de Vigilância Sanitária, Ministério da Saúde*.

58. Soukup, S. T., Al-Maharik, N., Botting, N., Kulling, S. E. (2014). Quantification of soy isoflavones and their conjugative metabolites in plasma and urine: an automated and validated UHPLC-MS/MS method for use in large –scale studies. *Anal. Bioanal. Chem.*, **406**:6007-6020.

59. National Soybean Research Laboratory (NSRL). *http://www.nsrl.uiuc.edu/aboutsoy/images/processing_diagram.gif* (accessed July 23, 2013).

60. Soyatech: *http://soyatech.com/soy_facts.htm*, accessed July 22, 2013.

61. Eisen, B., Unger, Y., and Shimoni, E. (2003). Stability of isoflavones in soymilk stored at elevated and ambient temperatures. *J. Agric. Food Chem.*, **51:** 2212-2215.

62. Kwok, K-C., and Niranjan, K. (1995). Review: Effect of thermal processing on soy milk. *Int. J. Food Sci. Tech.*, **30**: 263-295.

63. EEC Regulations. (1987). No. 1898/87 dated July 02, 1987 on the protection of designations used in marketing of milk and milk products (accessed August 02, 2013) *http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32006R1628:en:NOT*

64. Hiroshi, E., Masahiro, O., Katsuo, T., Shinji, S., and Kentaro, K. (2003). Effect of variety of soybean seed and processing of soy milk on the isoflavone content of tofu. *Food Preserv. Sci*., **29**:165-172.

65. Haron, H., Ismail, A., Azlan, A., Shahar, L. S., and Peng, L. S. (2009). Daidzein and genestein contents in tempeh and selected soy products. *Food Chem.*, **115**: 1350-1356.

66. Alezandro, M. R., Granato, D., Lajolo, F. M., and Genovese, M. I. (2011). Nutritional aspects of second generation soy foods. *J. Agric. Food. Chem.* **59**: 5490-5497.

67. Coward, L., Smith, M., Kirk, M., and Barnes, S. (1998). Chemical modification of isoflavones in soy foods during cooking and processing. *Am. J. Clin. Nutr.,* **68**: 1486S-1491S.

68. Baiano, A. (2010). Effects of Processing on Isoflavone Content and Profile in Foodstuffs: A Review. **In:** Isoflavones: Biosynthesis, occurrence and health effects. pp. 111-135. Melanie J Thompson Eds., Nova Publishers, USA.

69. Aguiar, C. L. (2010). Effects of the Processing Techniques on Isoflavone Profiles: A review. **In:** Isoflavones: Biosynthesis, occurrence and health effects. pp. 225-237. Melanie J Thompson Eds., Nova Publishers, USA.

70. Jackson, C. J. C., Dini, J. P., Lavandier, C., Rupasinghe, H. P. V., Faulkner, H., Poysa, V., Buzzell, D., and DeGrandis, S. (2002). Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. *Process Biochem.,* **37**: 1117-1123.

71. Uzzan, M., Nechrebeki, J., and Labuza, T. P. (2007). Thermal and storage stability of nutraceuticals in a milk beverage dietary supplement. *J, Food Sci.,* **72**: E109-114.

72. Pananun, T., Montalbo-Lomboy, M., Noomhorm, A., Grewell, D., and Lamsal, B. (2012). High-power ultrasonication-assisted extraction of soybean isoflavones and effect of toasting. *Food Sci. Tech.,* **47**: 1999-2007.

73. Xu, B., and Chang, S. K. (2008). Total phenolics, phenolic acids, isoflavones, and anthocyanins and antioxidant properties of yellow and black soybeans as affected by thermal processing. *J. Agric. Food Chem.,* **56**: 7165-7175.

74. Davis, T. A., Mungunsukh, O., Zins, S., Day, R. M., and Landauer, M. R. (2008). Genistein induces radioprotection by hematopoietic stem cell quiescence. *Int. J. Radiat. Biol.,* **84:** 713-726.

75. Kuriyama, I., Yoshida, H., Mizushina, Y., and Takahashi, Y. (2013). Inhibitory Effect of Isoflavones from Processed Soybeans on Human DNA Topoisomerase II Activity. *J. Plant Biochem. Physiol.*, **1**: 106.

76. Chen, T. R., and Wei, Q. K. (2008). Analysis of bioactive aglycone isoflavones in soybean and soybean products. *Nutr. Food Sci.*, **38**: 540-547.

77. Astuti, M., and Dalais, F. S. (2000). Tempeh, a nutritious and health food from Indonesia. *Asia Pacific J.* Clin*.* *Nutr.*, **9**: 322-325.

78. Ferreira, M. P., Oliveira, M. C. N., Mandarino, J. M. G., Silva, J. B., Ida, E. I., and Carrão-Panizzi, M. C. (2011). Changes in the isoflavone profile and in the chemical composition of tempeh during processing and refrigeration. *Pesquisa Agropecuária Brasileira*, **46**: 1555-1561.

79. Yang, S. O., Chang, P. S., and Lee, J. H. (2006). Isoflavone distribution and beta-glucosidase activity in Cheonggukjang, a traditional Korean whole soybean-fermented food. *Food Sci. Biotech*., **15:** 96-101.

80. Hong, G. E., Mandal, P. K., Lim, K. W., and Lee-H, C. (2012). Fermentation increases isoflavone aglycone contents in black soybean pulp. *Asian J. Animal Vet. Advances*, **6**: 502-511.

81. Silva, L. H., Celeghini, R. M. S., and Chang, Y. K. (2011). Effect of the fermentation of whole soybean flour on the conversion of isoflavones from glycosides to aglycones. *Food Chem.*, **128**: 640-644.

82. Simonne, A. H., Smith, M., Weaver, D. B., and Wei, C. I. (2000). Retention and changes of soy isoflavones and carotenoids in immature soybean seeds (Edamame) during processing. *J. Agric. Food Chem.*, **48**: 6061–6069.

83. Chien, H. L., Huang, H. Y., and Chou, C. C. (2006). Transformation of isoflavone phytoestrogens during the fermentation of soymilk with lactic acid bacteria and bifidobacteria. *Food Microbiol*., **23**: 772-778.

84. Badger, T. M., Gilchrist, J. M., Pivik, R. T., Andres, A., Shankar, K., Chen, J., and Ronis, M. J. (2009). “The health implications of soy infant formula.” *Am J Clin. Nutr.*, **89**:1668S–1672S.

85. Vandenplas, Y., Castrellon, P. G., Rivas, R.. Gutiérrez, C. J., Garcia, L. D., Jimenez, J. E., Anzo, A., Hegar B., and Alarcon, P. (2014). Safety of soya-based infant formulas in children. *Br. J. Nutr*., **111**: 1340-1360.

86. Bhatia, J., Greer, F., and the Committee on Nutrition, American Academy of Pediatrics. (2009). “Soy Protein-based Formulas: Recommendations for Use in Infant Feeding.” *Pediatrics*, **101**: 1062-1068.

87. Shao, S., Duncan, A. M., Yanga, R., Marcone, M. F., Rajcan, I., and Tsao, R. (2009). Tracking isoflavones: From soybean to soy flour, soy protein isolates to functional soy bread. *J. Functional Foods,* **1**: 119-127.

88. Ahn-Jarvis, J. H., Riedl, K. M., Schwartz, S. J., and Vodovotz, Y. (2013). Design and selection of soy breads used for evaluating isoflavone bioavailability in clinical trials. *J. Agric. Food Chem.*, **61**: 3111-3120.

89. Pednekar, M., Das, A. K., Rajalakshmi, V., and Sharma, A. (2010). Radiation processing and functional properties of soybean (Glycine max). *Radiat. Phys. Chem.*, **79**: 490-494.

90. Dixit, A. K., Bhatnagar, D., Kumar, V., Rani, A., Manjaya, J. G., and Bhatnagar, D. (2010). Gamma irradiation induced enhancement in isoflavones, total phenol, anthocyanin and antioxidant properties of varying seed coat colored soybean. *J. Agric. Food Chem.*, **58**: 4298-4302.

91. Popović, B. M., Stajner, D., Mandić, A., Canadanović-Brunet, J., and Kevrešan, S. (2013). Enhancement of antioxidant and isoflavones concentration in gamma irradiated soybean. *Scientific World Journal*. Nov 5; 2013:383574. doi: 10.1155/2013/383574.

92. Pinto, M. S., Lajolo, F. M., and Genovese, M. I. (2005). Effect of storage temperature and water activity on the content of profile of isoflavones, antioxidant activity and in vitro protein digestibility of soy protein isolates and defatted soy flours. *J. Agric. Food Chem.*, **53**: 6340-6346.

93. Rowland, I., Faughnan, M., Hoey, L., Wähälä, K., Williamson, G., and Cassidy A. (2003). Bioavailability of phyto-oestrogens. *Br. J. Nutr.*, **89**: S45-58.

94. Setchell, K. D., Brown, N. M., and Lydeking-Olsen, E. (2002). The clinical importance of the metabolite equol-A clue to the effectiveness of soy and its isoflavones. *J. Nutr.*, **132**: 3577-3584.

95. Karr, S. C., Lampe, J. W., Hutchins, A. M., and Slavin, J. L. (1997). Urinary isoflavonoid excretion in humans is dose dependent at low to moderate levels of soy-protein consumption. *Am. J. Clin. Nutr.*, **66**: 46-51.

96. Cassidy, A., Brown, J. E., Hawdon, A., Faughnan, M. S., King, L. J., Millward, J., Zimmer-Nechemias, L., Wolfe, B., and Setchell, K. D. (2006). Factors affecting the bioavailability of soy isoflavones in humans after ingestion of physiologically relevant levels from different soy foods. *J. Nutr.*, **136**: 45-51.

97. Nielsen, I. L., and Williamson, G. (2007). Review of the factors affecting bioavailability of soy isoflavones in humans. *Nutr. Cancer*, **57**: 1-10.

98. Rowland, I., Wiseman, H., Sanders, T., Adlercreutz, H., and Bowey, E. (1999). Metabolism of oestrogens and phytoestrogens: role of the gut microflora. *Biochem. Soc. Trans.*, **27**: 304-308.

99. Simmons, A. L., Chitchumroonchokchai, C., Vodovotz, Y., and Failla, M. L. (2012). Isoflavone retention during processing, bioaccessibility, and transport by Caco-2 cells: effects of source and amount of fat in a soy soft pretzel. *J. Agric. Food Chem.*, **60**: 12196-12203.

100. Kano, M., Takayanagi, T., Harada, K., Sawada, S., and Ishikawa, F. (2006). Bioavailability of isoflavones after ingestion of soy beverages in healthy adults. *J. Nutr.*, **136**: 2291-2296.

101. King, R. A., and Bursill, D. B. (1998). Plasma and urinary kinetics of the isoflavones daidzein and genistein after a single soy meal in humans. *Am. J. Clin. Nutr.*, **67**: 867-872.

102. Kozłowska, A., and Szostak-Wegierek, D. (2014). Flavonoids-food sources and health benefits. *Rocz. Panstw. Zakl. Hig*., **65**: 79-85.

103. Wei, H., Bowen, R., Cai, Q., Barnes, S., and Wang, Y. (1995). Antioxidant and antipromotional effects of the soybean isoflavone genistein. *Proc. Soc. Exp. Biol. Med.*, **208**:124-130.

104. Djuric, Z., Chen, G., Doerge, D. R., Heilbrun, L. K., and Kucuk, O. (2001). Effect of soy isoflavone supplementation on markers of oxidative stress in men and women. *Cancer Lett.*, **172**: 1-6.

105. Zheng, G., and Zhu, S. (1999). Antioxidant effects of soybean isoflavones. **In:** Antioxidants in Human Health. pp. 123-130. Basu, T. K., Temple, N. J., and Garg, M. L., Eds., CAB Publishing, Oxon, UK.

106. Santhakumar, A. B., Bulmer, A. C., and Singh, I. (2014). A review of the mechanisms and effectiveness of dietary polyphenols in reducing oxidative stress and thrombotic risk. *J. Hum. Nutr. Diet*., **27**: 1–21.

107. Anderson, J. W., Johnstone, B. M., and Cook-Newell, M. E. (1995). Meta-analysis of the effects of soy protein intake on serum lipids. *N. Engl. J. Med.,* **333**: 276-282.

108. Curtis, P. J., Sampson, M., Potter, J., Dhatariya, K., Kroon, P. A., and Cassidy, A. (2012). Chronic ingestion of flavan-3-ols and isoflavones improves insulin sensitivity and lipoprotein status and attenuates estimated 10-Year CVD risk in medicated postmenopausal women with type 2 diabetes: A 1-year, double-blind, randomized, controlled trial. *Diabetes Care,* **35**: 226-232.

109. González Cañete, N., and Durán Agüero, S. (2014). Soy isoflavones and evidences on cardiovascular protection. *Nutr. Hosp*., **29**:1271-1282.

110. Messina, M. (2014). Soy Foods, isoflavones, and the health of postmenopausal women. *Am. J. Clin. Nutr*., **100**: 423S-430S.

111. Dagdemir, A., Durif, J., Ngollo, M., Bignon, Y. J., and Bernard-Gallon, D. (2013a). Breast Cancer: Mechanisms Involved in Action of Phytoestrogens and Epigenetic Changes. *In Vivo,* **27**: 1-9.

112. Dagdemir, A., Durif, J., Ngollo, M., Bignon, Y. J., and Bernard- Gallon, D. (2013b). Histone lysine trimethylation or acetylation can be modulated by phytoestrogen, estrogen or anti-HDAC in breast cancer cell lines. *Epigenomics,* **5**: 51-63.

113. Pudenz, M., Roth, K., and Gerhauser, C. (2014). Impact of soy isoflavones on the epigenome in cancer prevention. *Nutrients,* **6**: 4218-4272.

114. Mansouri-Attia, N., James, R., Ligon, A., Li, X., and Pangas, S. A. (2014). Soy promotes juvenile granulosa cell tumor development in mice and in the human granulosa cell tumor-derived COV 434 cell line. *Biol Reprod.*, **91**:100. doi: 10.1095/biolreprod.114.120899. Epub 2014 Aug 27.

115. Nagata, C., Mizoue, T., Tanaka, K., Tsuji, I., Tamakoshi, A., Matsuo, K., Wakai, K., Inoue, M., Tsugane, S., and Sasazuki, S. (2014). Soy intake and breast cancer risks: an evaluation based on s systematic review of epidemiologic evidence among the Japanese population. *Jpn. J. Clin. Oncol.*, **44**: 282-295.

116. AACR (2011). American Association for Cancer Research's 102nd Annual Meeting (held Saturday, April 2 - Wednesday, April 6, 2011) at the Orange County Convention Center in Orlando, Florida.

117. Akaza, H. (2012). Prostate cancer chemoprevention by soy isoflavones: role of intestinal bacteria as "second genome". *Cancer Sci.*, **103**: 969-975.

118. Miyanaga, N., Akaza, H., Hinotsu, S., Fujioka, T., Naito, S., Namiki, M., Takahashi, S., Hirao, Y., Horie, S., Tsukamoto, T., Mori, M., and Tsuji, H. (2012). Prostate cancer chemoprevention study: an investigative randomized control study using purified isoflavones in men with rising prostate-specific antigen. *Cancer Sci.*, **103**:125-130.

119. Mahmoud, A. M., Yang, W., and Bosland, M. C. (2014). Soy isoflavones and prostate cancer: a review of molecular mechanisms. *J. Steroid Biochem. Mol. Biol.*, **140**:116-132.

120. Adjakly, M., Ngollo, M., Lebert, A., Dagdemir, A., Penault-Llorca, F., Boiteux, J. P., Bignon, Y. J., Guy, L., and Bernard-Gallon, D. (2014). Comparative effects of soy phytoestrogens and 17β-estradiol on DNA methylation of a panel of 24 genes in prostate cancer cell lines. *Nutr. Cancer*, **66**: 474-482.

121. Hamilton-Reeves, J. M., Banerjee, S., Banerjee, S. K., Holzbeierlein, J. M., Thrasher, J. B., Kambhampati, S., Keighley, J., and Van Veldhuizen, P. (2013). Short-Term Soy Isoflavone Intervention in Patients with Localized Prostate Cancer: A Randomized, Double-Blind, Placebo-Controlled Trial. *PLoS One*, **8**: e68331. doi: 10.1371/journal.pone.0068331. Print 2013.

122. Dalais, F. S., Meliala, A., Wattanapenpaiboon, N., Frydenberg, M., Suter, D. A., Thomson, W. K., and Wahlqvist, M. L. (2004). Effects of a diet rich in phytoestrogens on prostate-specific antigen and sex hormones in men diagnosed with prostate cancer. *Urol.*, **64**: 510-515.

123. Pendleton, J. M., Tan, W. W., Anai, S., Chang, M., Hou, W., Shiverick, K. T., and Rosser, C. J. (2008). Phase II trial of isoflavone in prostate-specific antigen recurrent prostate cancer after previous local therapy. *BMC Cancer*, **8**: 132.

124. Messina, M., Kucuk, O., and Lampe, J. W. (2006). An overview of the health effects of isoflavones with an emphasis on prostate cancer risk and prostate-specific antigen levels. *J. AOAC Int.*, **89**: 1121-1134.

125. Yan, L., and Spitznagel, E. L. (2009). Soy consumption and prostate cancer risk in men: a revisit of a meta-analysis. *Am. J. Clin. Nutr.*, **89**: 1155-1163.

126. van Die, M. D., Bone, K. M., Williams, S. G., Pirotta, M. V. (2014). Soy and soy isoflavones in prostate cancer: a systematic review and meta-analysis of randomized controlled trials. *BJU Int.*, **113**: E119-130.

127. De Souza, P. L., Russell, P. J., Kearsley, J. H., and Howes, L. G. (2010). Clinical pharmacology of isoflavones and its relevance for potential prevention of prostate cancer. *Nutr. Rev.*, **68**: 542-55.

128. Taku, K., Melby, M. K., Nishi, N., Omori, T., and Kurzer, M. S. (2011). Soy isoflavones for osteoporosis: an evidence-based approach. *Maturitas*, **70**: 333-8.

129. Ming, L. G., Chen, K. M., and Xian, C. J. (2013). Functions and action mechanisms of flavonoids genistein and icariin in regulating bone remodeling. *J. Cell Physiol.*, **228**: 513-521.

130. Li, L., Lv, Y., Xu L., and Zheng, Q. (2014). Quantitative efficacy of soy isoflavones on menopausal hot flashes. *Br. J. Clin. Pharmacol.*, 2014 Oct 15. doi: 10.1111/bcp.12533. [Epub ahead of print]

131. Chen, M. N., Lin, C. C., and Liu, C. (2014). Efficacy of phytoestrogens for menopausal symptoms: a meta-analysis and systematic review. *Climacteric*, 1-21. [Epub ahead of print]

132. Clarkson, T. B., Utian, W. H., Allmen, T. I., Aso, T., Barnes, S., Basaria, S. S., and Brinton, B. D. (2011). The role of soy isoflavones in menopausal health: report of The North American Menopause Society/Wulf H. Utian Translational Science Symposium in Chicago, IL (October 2010). *Menopause,* **18**: 732-753.

133. Jenks, B. H., Iwashita, S., Nakagawa, Y., Ragland, K., Lee, J., Carson, W. H., Ueno, T., and Uchiyama, S. (2012). A Pilot Study on the effects of S-Equol compared to soy isoflavones on menopausal hot flash frequency. *J. Women's Health (Larchmt),* **21**: 674-692.

134. NAMS (The North American Menopause Society). (2011).The role of soy isoflavones in menopausal health: Report of The North American Menopause Society/Wulf H. UtianTranslational Science Symposium in Chicago, IL (October 2010). *Menopause*, **18**: 732-753.

135. Orgaard, A., and Jensen, L. (2008). The effects of soy isoflavones on obesity. *Exp. Biol. Med.,* **233**: 1066-1080.

136. Most, J., Goossens, G. H., Jocken, J. W., and Blaak, E. E. (2013). Short-term supplementation with specific combination of dietary polyphenols increases energy expenditure and alters substrate metabolism in overweight subjects. *Int. J. Obes (Lond).*, doi: 10.1038/ijo.2013.231. [Epub ahead of print]

137. Greendale, G. A., Huang, M. H., Leung, K., Crawford, S., Gold, E. B., Wight, R., Waetjen, E., and Karlamangla, A. S. (2012). Dietary phytoestrogen intakes and cognitive

function during the menopause transition: Results from the SWAN Phytoestrogen Study. *Menopause*, **19**: 894–903.

138. Casini, M. L., Marelli, G., and Papaleo, E., Ferrari, A., D'Ambrosio, F., and Unfer, V. (2006). Psychological assessment of the effects of treatment with phytoestrogens on postmenopausal women: A randomized double-blind, crossover, placebo-controlled study. *Fertil. Steril.*, **85**: 972-978.

139. Santos- Galduroz, R. F., Galduroz, J. C., Facco, R. L., Hachul, H., and Tufik, S. (2010). Effects of isoflavones on the learning and memory of women in menopause: A double-blind placebo-controlled study. *Braz. J. Med. Biol. Res.*, **43**: 1123-1126.

140. Henderson, V. W., St-John, J. A., Hodis, H. N., Kono, N., McCleary, C. A., Franke, A. A., and Mack, W. J. (2012). Long-term soy isoflavone supplementation and cognition in women- a randomized, controlled trial. *Neurology*, **78**:1841-1848.

141. Wrenn, C. C. (2013). Dietary isoflavones and learning and memory. **In:** Isoflavones: Chemistry, Analysis, Function and Effects. pp. 451-464. Preedy, V. R., Eds., Royal Society of Chemistry (RSC publishing), UK

142. Alekel, D. L., Van Loan, M. D., Koehler, K. J., Hanson, L. N., Stewart, J. W., Hanson, K. B., Kurzer, M. S., and Peterson, C. T. (2010). The soy isoflavones for reducing bone loss (SIRBL) study: a 3-y randomized controlled trial in postmenopausal women. *Am. J. Clin. Nutr.*, **91**: 218-230.

143. Thai, T. Y., Tsai, K. S., Tu, S. T., Wu, J. S., Chang, C. I., Chen, C. L., Shaw, N. S., Peng, H. Y., Wang, S. Y., and Wu, C. H. (2012). The effect of soy isoflavones on bone mineral density in post-menopausal Taiwanese women with bone loss: a 2 year randomized double blind placebo-controlled study. *Osteoporos Int.*, **23**: 1571-1580.

144. Siepmann, T., Roofeh, J., Kiefer, F. W., and Edelson, D. G. (2011). Hypogonadism and erectile dysfunction associated with soy product consumption. *Nutrition*, **27**: 859-862.

145. Yin, D., Zhu, Y., Liu, L., Xu, H., Huang, J., and Li, Y. (2014). Potential detrimental effect of soy isoflavones on testis sertoli cells. *Zhong Nan Da Xue Xue Bao Yi Xue Ban.*, **39**: 598-604.

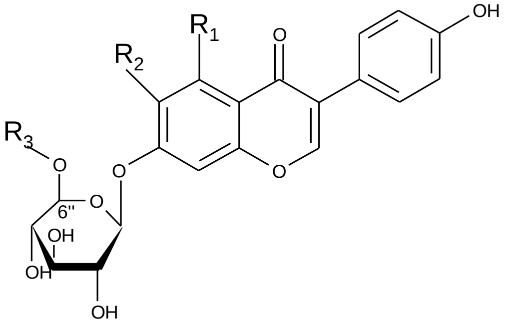
146. Cederroth, C. R., Zimmermann, C., and Nef, S. (2012). Soy, phytoestrogens and their impact on reproductive health. *Mol. Cell. Endocrinol.*, **355**:192-200.

147. Hamilton-Reeves, J. M., Vazquez, G., Duval, S. J., Phipps, W. R., Kurzer, M. S., and Messina, M. J. (2010). Clinical studies show no effects of soy protein or isoflavones on reproductive hormones in men: results of a meta-analysis. *Fertil. Steril.*, **94**: 997-1007.

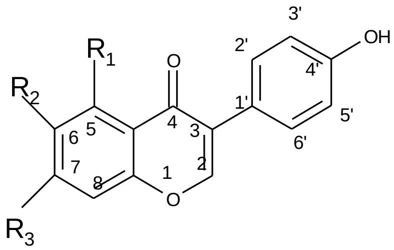
148. D'Adamo, C. R., and Sahin, A. (2014). Soy foods and supplementation: a review of commonly perceived health benefits and risks. *Altern. Ther. Health Med.*, **20**: 39-51.

Table 1: Isoflavone contents of food (mg per 100 g) [26]

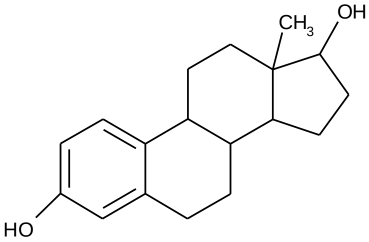
Items	Total Isoflavones	Daidzein	Genestein
Soybeans	128.34	46.46	73.76
Ingredients:			
Okara	13.51	5.39	6.48
Soybean chips (paste)	54.16	26.71	27.45
Soy flour (full-fat)	171.89	96.83	71.19
Soy flour (textured)	148	59.62	78.62
Soy flour (defatted)	131.19	57.47	71.21
Soy protein (water)	102.07	43.04	59.59
Soy protein (alcohol ext)	12.47	6.63	5.33
Soy protein isolate	97.43	33.59	59.62
Food: non-fermented			
Soy milk	9.65	4.45	6.06
Soy drink	7.01	2.41	4.6
Tofu (firm)	22.70	8.00	12.75
Tofu (fried)	48.53	17.83	28.00
Tofu (soft)	29.24	8.59	20.65
Food: fermented			
Natto	58.93	21.85	29.04
Tempeh	43.52	17.58	24.85
Miso	42.55	16.13	24.56
Cheese (American)	17.95	5.75	8.75
Cheese, Monterey	18.70	7.80	8.80
Yogurt			
Food: 2nd generation products			
Vegetable burger	9.30	2.95	5.28
Soy hot dog	15.00	3.40	8.20
Bacon- meatless	12.10	2.8	6.9
Sausage, meatless	14.34	4.46	9.23



R_1=H	R_2=H	R_3=H	Daidzin
R_1=H	R_2=H	R_3=COOH$_3$	Acetyldaidzin
R_1=H	R_2=H	R_3=CO-CH$_2$-COOH	Malonyldaidzin
R_1=OH	R_2=H	R_3=H	Genistin
R_1=OH	R_2=H	R_3=COOH$_3$	Acetylgenistin
R_1=OH	R_2=H	R_3=CO-CH$_2$-COOH	Malonylgenistin
R_1=H	R_2=OCH$_3$	R_3=H	Glycitin
R_1=H	R_2=OCH$_3$	R_3=COOH$_3$	Acetylglycitin
R_1=H	R_2=OCH$_3$	R_3=CO-CH$_2$-COOH	Malonylglycitin



R_1=H	R_2=H	R_3=OH	Daidzein (DAZ)
R_1=H	R_1=OH	R_3=OH	Genestein (GEN)
R_1=H	R_2=OCH$_3$	R_3=OH	Glycitein (GLY)



Estradiol

Figure 1: Chemical structures of isflavones and 17β-estradiol