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Chapter 24

Food, Nutrition and Health

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Additional information is available at the end of the chapter

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1. Introduction

Botanically, soybean belongs to the order *Rosaceae*, family *Leguminosae* or *Papillonaceae* or *Fabaceae*, subfamily *Papilionoidae*, the genus *Glycine* and the cultivar Glycine max. It is an annual plant that measures up to 1.5 m tall, with pubescent leaves and pods; the stems are erect and rigid. In its primary and secondary roots, are located a variable number of nodes. One of the characteristics of the root system development is its sensitivity to variations in the supply and distribution of inorganic nutrients in the soil. The root system has a main root which can reach a meter deep, with an average being between 40 and 50 centimeters [1].

The soybean is a traditional oriental food, a leguminous plant native to eastern Asia, especially in China. Soybean is cultivated worldwide, the United States is the country that grows more than 50% of the world production of this important food, which has been utilized in the diet of humans around the world, due to its high content of essential amino acids (Table 1) and calcium. It is consumed as cooked beans, soy sauce, soymilk and tofu (soybean curd). Also, a vegetable oil is obtained from soybeans, rich in polyunsaturated fatty acids [2].

Soybean is an annual plant, whose seeds are the edible organ. Soybean grains are rich in protein, and also a good source of various phytochemicals such as isoflavones and lignans, molecules with antioxidant and antiplatelet activities, among other effects; also may help fight and prevent various diseases, so constitute a useful source of food. For these reasons, these compounds have been intensively studied at basic and clinical level [3].

Soybean consumption benefits, especially in several chronic diseases, have been related to its important protein content, high levels of essential fatty acids, vitamins and minerals. Consequently, the present chapter aimed at the comprehensive characterization of the anti-

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oxidant and antiplatelet activities of bioactive compounds, of soybean and its derivatives, and the extent to which soybean is a health-promoting food.

Amino acid	g/16 g Nitrogen
Isoleucine	4.54
Leucine	7.78
Lysine	6.38
Methionine	1.26
Cysteine	1.33
Phenylalanine	4.94
Tyrosine	3.14
Threonine	3.86
Tryptophan	1.28
Valine	4.80
Arginine	7.23
Histidine	2.53
Alanine	4.26
Aspartic acid	11.70
Glutamic acid	18.70
Glycine	4.18
Proline	5.49
Serine	5.12

Table 1. Amino acid composition of soybeans seeds. Source: Adapted by authors from FAO (1970) and FAO/WHO (1973).

2. Soybean: foods and bioactive compounds

Soybeans are consumed as cooked beans, which previously should be boiled for at least three hours. With these grains are prepared meals, salads and soups, which in turn, are a source of preparation of other foods. From soybean grains also is possible to obtain soy sauce, which is used especially in oriental foods, such as sushi. The soy sauce is usually made by fermenting soya grains with cracked roasted wheat, which are arranged in blocks and immersed in a cold salt water, the process takes about a year in pots mud, sometimes dried mushrooms are added as mushrooms. In Japan, it is illegal to produce or import artificial soy sauce and therefore all Japanese soy sauces are made by the traditional way [4].

Another food derived from soy is tofu, which is a widely used food in the East as well as vegetarian meals around the world. Required for preparing tofu soybeans are water and a coagulant. Initially, you get the coagulated soymilk, then is pressed and separated the liquid portion from the solid. Tofu has a firm texture similar to cheese milk; the color is cream and

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served in buckets. Also, from soybeans is possible to obtain vegetable oil, which is characterized by high polyunsaturated fatty acids [5].

Soy is a source not only of proteins, vitamins and minerals, but also of many bioactive compounds, such as isoflavones, protease inhibitors, saponins, and phytates. The great importance of these compounds is based on their biochemical activity, which results in health promotion and disease prevention, by their antioxidant, and antiplatelet activities.

Antioxidant Activity

Soybeans contain a variety of bioactive phytochemicals such as phenolic acids, flavonoids, isoflavones, saponins, phytosterols and sphingolipids; being the phenolic compounds with the highest antioxidant capacity. The key benefits of soy are related to their excellent protein content, its high content of essential fatty acids, numerous vitamins and minerals, their isoflavones and their higher fiber content.

Polyphenolic compounds are a class of secondary metabolites biosynthesized by the vegetal kingdom [6] and involve a wide range of substances that possess one or more aromatic rings with at least one hydroxyl group. Among them, can be mentioned flavonoids, isoflavones, anthraquinones, anthocyanins, xanthones, phenols, hydroxycinnamic acids, lignin and others.

All of them act as scavengers or stabilizers of free radicals, and can produce chelation of metals, those having carboxyl groups at its end. Works have also been reported that its antioxidant action can be attributed to the inhibition of prooxidants enzymes as lipoxygenase [7].

A study by Xu (2008) in 30 samples of soybean from different regions of North Dakota, Minnesota (USA), found that some cultivars of black soybean had a higher antioxidant capacity measured as ORAC, FRAP and DPPH than yellow soybeans and that the phenolic acid content, isoflavones and antiochians was different, suggesting that some selected cultivars can be used as producers of high quality soy, because it provides a high content of phenolic phytochemicals and antioxidant properties [8].

Isoflavones and equol. Of all plants, soybean contains the highest amount of different isoflavones, a variety of phytoestrogens that have a structure similar to estrogen (figure 1). The interest in soybean isoflavones has gained importance since the 90's to today, there are a lot of evidence that these phytoestrogens possess a powerful and wide range of biological activities. Isoflavones are not a steroid structure, however, has a phenolic ring than is capable of binding to the estrogen receptor (ER) and according to Makela (1995) can act as either an agonist or an antagonist [9].

The discovery of high concentrations of isoflavones in urine of adults who consume soy protein, in addition to the evidence supporting its biological action, elevate the soybean to the category of functional food. The FDA in 1999 gave approval to give foods containing 6.25 g of soy protein the seal of protector of cardiovascular health, increasing significantly the sales of foods fortified with soy and isoflavone constituent.

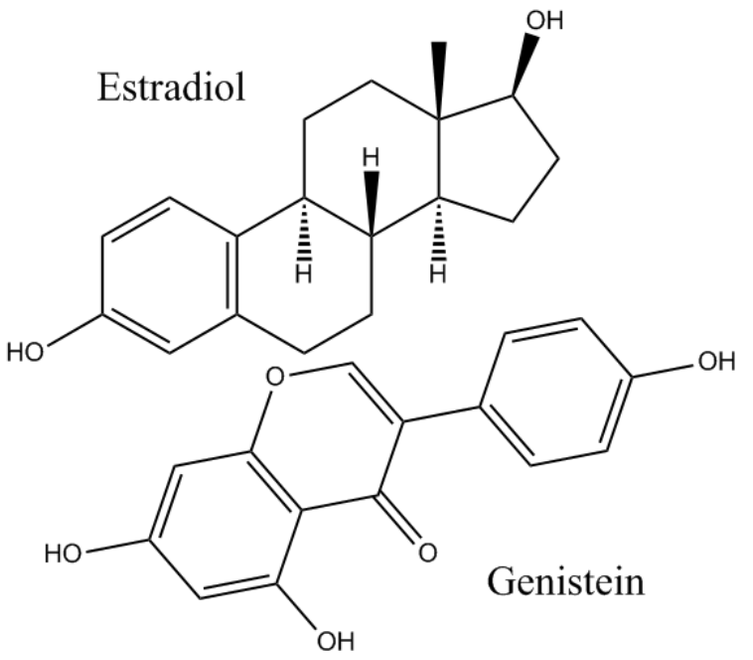


Figure 1. Comparison between the structure of the derivative of Isoflavone (genistein) and estrogen (estradiol), which shows the similarities between the two molecules.

On the other hand, traditional foods in the East, such as extracts and broth of rice and soybeans fermented with microorganisms for 21 days, were reported as antioxidants by Yen (2003) [10] and Yang (2000) [11]. The authors attributed the antioxidant power at the content of polyphenol and the presence of reductons that only occur during the fermentation process. The antioxidant supplements or foods containing antioxidants may be used to reduce the oxidative damage related to age and diseases such as artherosclerosis, diabetes, cancer, cirrhosis, among other [12].

For 10 to 15 years, has been a strong interest in the use of products of botanical origin for the protection, whitening and skin aging. According Baunmann (2009) the mechanism of action of botanical products has been known given the use of advanced technologies applied to research, this is how there are several reports showing that soy components play an important role in the extracellular matrix of the dermis [13].

Moreover, it is accepted that isoflavones act through different mechanisms such as modulation of cell growth and proliferation, extracellular matrix synthesis, inhibition of inflammation and oxidative stress. The isoflavones reduced renal injury by decreasing the concentration of lipoproteins in plasma and acting as an antioxidant reducing the lipid peroxidation [14].

The Equol, whose structure corresponds to 7-hydroxy-3-(4'-hydroxyphenyl)-chroman, is a nonesteroidal estrogen, which was discovered in the early 80's in the urine of adults who consumed soy foods [15]. It has been shown to be a metabolite of daidzein, one of the major isoflavones present in foods containing soy, which is formed after hydrolysis of the isofla-

vone glycoside [16] at intestinal level and subsequent bacterial biotransformation in the colon [17], leaving an intermediary called dihydro-equol [18-20].

Equol does not originate in plants, but is the product of degradation of the isoflavone glycoside in the intestine [21], situation that was confirmed in infants of 4 months who were fed with formulations containing soy [22, 23]. All mammals can biotransform isoflavone glycoside permanently, except the man who for reasons still unexplained, only 20-35% of adults produces equol after eating foods made from soybeans or that have been enriched with pure isoflavones [17, 24, 25].

Several studies have suggested that those mammals who are equol producers show a greater response to isoflavone-enriched diets, leading to the conclusion that equol is a more potent isoflavone than genistein and is the only one that has a chiral carbon at position 3 of the furan ring, making two enantiomeric forms, S and R that differ significantly in their conformational structure [26].

Gopaul (2012) investigated the effect of equol on gene expression of proteins in the skin, using a cellular model of human dermis and found that equol significantly increased gene expression of collagen, elastin (ELN), and tissue factor inhibitor of metalloproteinases and decreased metalloproteinases (MMPs), causing positive changes in the skin's antioxidant and anti-aging genes. The same occurred in cultured human fibroblasts (hMFC), in which equol significantly increased type I collagen (COL1A1), while 5a-dihydrotestosterone (5a-DHT) significantly decreased cell viability. These findings suggest that equol has great potential for topical applications to the skin, for the treatment and prevention of aging of the skin by increasing the extracellular matrix components [27].

Has been found that Equol have affinity for the estrogen receptor beta, which is abundant in keratinocytes of the epidermis and dermal fibroblasts [28-30]. On the other hand, equol is a selective androgen modulator and has the ability to bind to 5a-dihydrotestosterone (5a-DHT) and inhibit its potent action on the skin [31]. In this sense, we can quote the opposite effect that have androgens and estrogens, the former producing an injury to the skin by increasing MMPs, while the latter have a positive effect on the aging of the skin by increasing collagen, elastin and decreasing MMPs [13, 32-35].

In turn, Muñoz (2009) studied the inhibitory effect of soy isoflavones and the metabolite equol derived from daidzein, an agonist that has biological effects attributed to an antagonism of the thromboxane A2 receptor (TXA2R), which helps explain the beneficial effects of dietary isoflavones in the prevention of thrombotic events [36].

Recent works by Ronis (2012) studied the effect of mice fed with extracts of soy protein or isoflavones finding that can reduced the metabolic syndrome in rats via activation of peroxisome proliferation activated receptor (PPAR), liver X receptor (LXR) and decreased signaling protein binding to the sterol regulatory element binding proteins (SREBP) [37].

Anthocyanins. The anthocyanins are known to have antioxidant effects and play an important role in preventing various degenerative diseases. Structurally, this are a suitable chemical structure to act as antioxidants, since they can donate hydrogens or electrons to free

radicals or catch and move them in its aromatic structure. There are about 300 anthocyanins in nature, with different glycosidic substitutions in the basic structure of the ion 2-phenyl-benzopirilio or flavilio [38].

Paik (2012) examined the effect of anthocyanin extracts from the cover of black soybean in an animal model of retinal degeneration (RD), the leading cause of death of the photoreceptor cells that lead to blindness and noted that extracts of anthocyanins may protect retinal neurons from damage induced by degenerative agents such as N-methyl-N-nitrosourea (MNU) at a dose of (50mg/kg), which acts as a methylating agent that causes DNA damage to the photoreceptors [39].

In general, bioactive compounds from soybean are many, but still exists wide variety of information of the beneficial effects and also adverse effects of isoflavones and anthocyanins, so likewise it is necessary to study more thoroughly this compound and its relation to chronic diseases through scientific studies with larger number of patients and longer study periods, in order to clarify all the diffuse concepts, labile or poorly sustained, so as to give the isoflavones, a clear place in the diet therapy.

Antiplatelet Activity

There has been much recent interest in the cardiovascular benefits of dietary soybean on potential anti-thrombogenic and anti-atherogenic effects [40]. Extracts containing isoflavones and soy saponins inhibit the platelet aggregation *ex vivo* induced by ADP and collagen in diabetic rats [41]. Moreover, black soybean extracts inhibited platelet aggregation induced by collagen *in vitro* and *ex vivo*, and attenuates the release of serotonin and P-selectin expression [42].

The effects of soybean products on platelet aggregation were initially described for genistein [43]. In these reports, genistein was able to inhibit platelet activation induced by collagen and thromboxane A2 analog (TXA2), but not by thrombin. Genistein (10 mg/kg) in mouse significantly prolonged the thrombotic occlusion time and significantly inhibited *ex vivo* and *in vitro* (30 μM) platelet aggregation induced by collagen [44]. Genistein is a well-known inhibitor of protein tyrosine kinases, however, on platelet functions *in vitro* genistein inhibits activation of phospholipase C in stimulated platelets, apparently independent of its effects on tyrosine kinases. These results suggest that dietary supplementation of soy may prevent the progression of thrombosis and atherosclerosis [45]. Daidzein, another soy flavonoid that lacks tyrosine kinase inhibitory activity also inhibited the response to collagen and TXA2, suggesting that these flavonoids inhibit platelet aggregation by competition for the thromboxane A2 receptor (TXA2R) rather than through tyrosine kinase inhibition. Genistein and daidzein have effect on platelets, macrophages and endothelial cells: inhibited collagen-induced platelet aggregation in a dose-dependent manner and in macrophage cell line activated with interferon γ, plus lipopolysaccharide inhibit tumoral necrosis factor α (TNF-α) secretion, dose-dependently. Both isoflavones also dose-dependently decreased monocyte chemoattractant protein-1 secretion induced by TNF-α in human umbilical vein endothelial cells [40].

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Equol is more active than soy isoflavone itself to compete for binding to TXA2R in human platelets (in the range of micromoles / L), so that inhibits the platelet aggregation and secretion induced by U46619 [36]. Under equilibrium conditions, the following order of the relative affinity in inhibiting [(3)H]-SQ29585 binding was: equol > genistein > daidzein > glycitein > genistin > daidzin > glycitin [36]. Guerrero and colleagues suggested that this competitive binding was due to structural features of these flavonoids such as the presence of a double bond in C2-C3 and a keto group in C4 [36].

From a extraction of soy sauce, two kinds of components with anti-platelet activity were isolated and structurally identified: 1-methyl-1,2,3,4-tetrahydro-β-carboline (MTBC) and 1-methyl-β-carboline (MBC). MTBC shows IC_{50} ranging from 2.3 to 65.8 μg/mL for aggregation response induced by epinephrine, platelet-activating factor (PAF), collagen, ADP and thrombin [46]. Membrane fluidity regulates the platelet function and various membrane-fluidizing agents are known to inhibit platelet aggregation [47]. Certain β-carbolines influence the fluidity of model membranes. The alteration of membrane fluidity may be involved in the antiplatelet effects of MTBC and MBC [48].

Soybean protein inhibits platelet aggregation induced by thrombin, collagen and ADP, and prolongs the clotting time [49]. Also was observed that most fractions obtained of soy protein hydrolysates (gel filtration chromatography, reverse-phase HPLC and cation exchange HPLC) inhibited rat platelet aggregation induced by ADP, which suggests that most peptides have some degree of antiplatelet effect. From the inhibitory fractions, two new peptides were identified, SSGE and DEE, and at concentrations of 480 and 460 μM, respectively, inhibited in 50% platelet aggregation [50].

The diet may be the most important factor influencing the risk of cardiovascular disease. Soybean derivatives can be denominated as functional ingredients as they contain bioactive compounds that inhibit platelet aggregation, which gives a preventive effect on thrombus formation [51].

3. Functional Food

Soybean is a very rich source of essential nutrients and one of the most versatile foodstuffs. It possesses good quality protein and highly digestible (92–100%) and contains all the essential amino acids. Soybean-protein products also contain a high concentration of isoflavones (1 g/kg) [52]. Therefore, consumption of soy-based foods has been associated with multiple health benefits [53, 54]. Among a variety of soybeans, black soybean is known to display diverse biological activities superior to those of yellow and green soybeans, such as antioxidant, anti-inflammatory and anticancer activities [42].

Soy food intake has been shown to have beneficial effects on cardiovascular disease risk factors. Data directly linking soy food intake to clinical outcomes of cardiovascular disease, evidence that soy food consumption may reduce the risk of coronary heart disease in women and may be protective against the development of subarachnoid hemorrhage [54, 55]. Based

on that 1% reduction in cholesterol values is associated with an approximate 2-3% reduction in the risk of coronary heart disease, it can be assumed that a daily intake of 20-50 grams of isolated soy protein could result in a 20- 30% reduction in heart disease risk [56, 57].

Several components associated with soy protein have been implicated in lowering cholesterol: trypsin inhibitors, phytic acid, saponins, isoflavins, fiber and proteins [58]. Apparently, there is a synergy among the components of intact soy protein, which provides the maximum hypocholesterolemic benefit. A variety of clinical trials have demonstrated that consuming 25 to 50 g/daily of soy protein is both safe and effective in reducing LDL cholesterol by ≈4% to 8% [58]. Therefore, maturation of SREBP and induction of SRE-regulated genes produce an increase in surface LDL receptor expression that increases the clearance of plasma cholesterol, thus decreasing plasma cholesterol levels [59]. However, other results present direct evidence for the existence of LDL receptor and plasma lipoprotein-independent pathways by which dietary soy protein isolate inhibits atherosclerosis [60, 61].

The addition of soy protein in diet or replacing animal protein in the diet with soy, lowers blood cholesterol. Moreover, defatted soy flour is a widely used in these applications as a partial replacement for nonfat dry milk [62-64]. Soy protein can increase protein content and its used in compounded foods (breads, crackers, doughnuts, and cakes) for their functional properties, including water and fat absorption, emulsification, aeration (whipping), heat setting, and for increasing total protein content and improving the essential amino acids profile [65].

The low breast cancer mortality rates in Asian countries and the putative anti-estrogenic effects of isoflavones have fueled speculation that soyfood intake reduces breast cancer risk [66]. Soy sauce promotes digestion, because the consumption of a cup of clear soup containing soy sauce enhances gastric juice secretion in humans. The feeding of a diet containing 10% soy sauce to male mice for 13 months also reduces the frequency and multiplicity of spontaneous liver tumors [67]. Over the past decades, enormous research efforts have been made to identify bioactive components in soy [68]. The Health effects of soy dietary are variable depending on individuals' metabolism and in particular to their ability to convert daidzein to equol that seems to be restricted to approximately 1/3 of the population. Equol production has been indeed linked to a decreased on arterial stiffness and antiatherosclerotic effects via nitric oxide production [69]. Despite being a biotransformation product of daidzein, the equol at low dosage can prevent skeletal muscle cell damage induced by H_2O_2 [70] and possesses anticancer activity via apoptosis induction in mammary gland tumors of rats [71].

Hydroponic cultivation improved the nutritional quality of soybean seeds with regard to fats and dietary fiber. This suggest that specific cultivars should be selected to obtain the desired nutritional features of the soybean raw material [72]. Irrigation enhanced the isoflavone content of both early- and late-planted soybeans as much as 2.5-fold. Accumulation of individual isoflavones, daidzein and genistein, are also elevated by irrigation [53].

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4. Digestion and Absorption

A number of factors including the amount consumed, chemical speciation, interactions with other ingredients, physiological state (e.g., gender, ethnicity, age, health status) and intestinal microflora influence the absorption of dietary isoflavonoids by the gastrointestinal tract [73]. Additionally, the absorption and disposition of isoflavones (daidzein and genistein) appears to be independent of age, menopausal status and probiotic or prebiotic consumption [74, 75].

After absorption, isoflavones are reconjugated predominantly to glucuronic acid and to a lesser extent to sulphuric acid. Only a small portion of the free aglycone has been detected in blood, demonstrating that the rate of conjugation is high [76]. The isoflavone aglycones are absorbed faster and in greater amounts than glucosides in humans, dependent on the initial concentrations. Thus, products rich on aglycone can be more effective in the prevention of chronic diseases [77]. Concentrations of isoflavones and their gut microflora metabolites in the plasma, urine, and feces are significantly higher in the subjects who consume a high-soy diet than in those who consume a low-soy diet [78].

Bioavailability of isoflavone glycosides (daidzein and genistein) as pure compounds or in a soyfood matrix (soymilk) requires initial hydrolysis of the sugar moiety by intestinal β-glucosidases for the uptake to the peripheral circulation [16]. Twenty-four hours after dosing, both plasma and urine isoflavone concentrations were nearly null [79]. The genistein compound is absorbed from the lumen partly unhydrolyzed and transported directly (by an unknown transporter or diffusion) to the vascular side [80]. Conjugates of daidzein are more bioavailable than those of genistein. Thus, after oral administration of soy extract in rats providing 74 micromol of genistein and 77 micromol of daidzein / kg (as Conjugates), were found that plasma concentration of daidzein was maximal at 2 h and it was almost double that of genistein. Since about 50% of the genistein dose is excreted as 4-ethyl phenol (the main end product from genistein) [81]. The end product of the biotransformation of the phytoestrogen daidzein, is the equol, that is not produced in all healthy adults in response to dietary challenge with soy or daidzein [21]. However, plasma genistein concentrations are consistently higher than daidzein when equal amounts of the two isoflavones are administered, and this is accounted for by the more extensive distribution of daidzein (236 L) compared with genistein (161 L). In addition to the conjugated state, the chemical structures of isoflavones play a major role in its pharmacokinetics with marked qualitative and quantitative differences depending on the type of supplement ingested [82-84].

5. Health

Functional food may act as an adjunctive therapy/alternative treatment of different pathologies, and scientific studies are appearing more frequently demonstrating that this hypothesis is, indeed, a reality. Soybean containing isoflavone and protein is considered a functional food item [85].

Epidemiological studies suggest that soybean consumption is associated, at least in part, with lower incidences of a number of chronic diseases. The lower rates of several chronic diseases in Asia, including type 2 diabetes, certain types of cancer and cardiovascular diseases, between others, have been partly attributed to consumption of large quantities of soy foods (figure 2) [86].

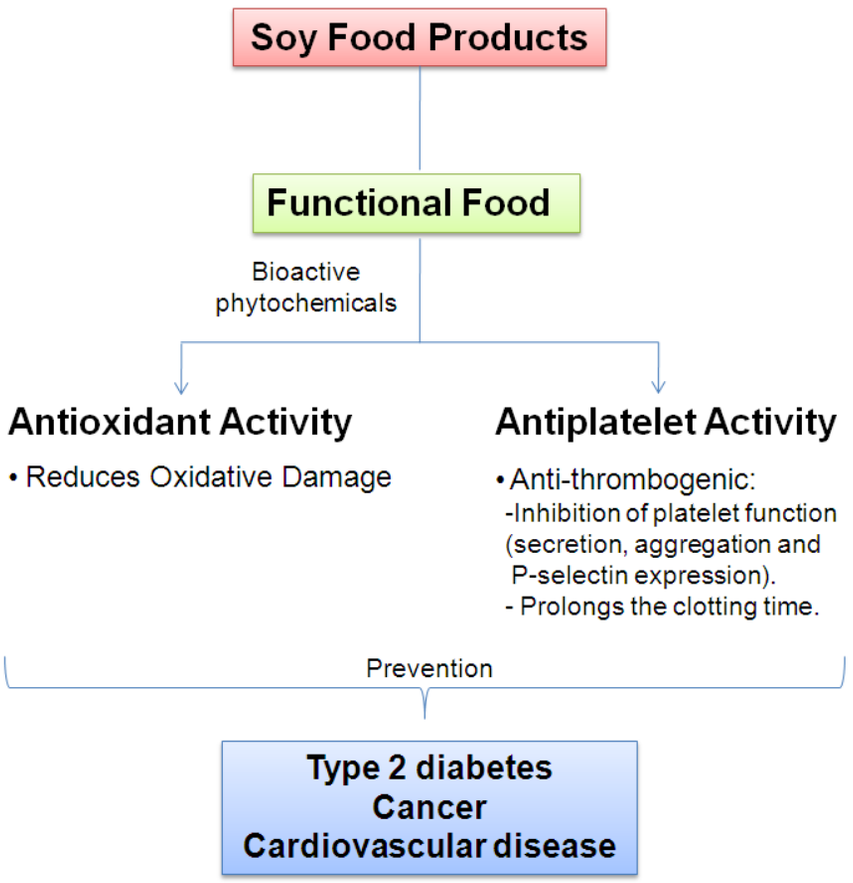


Figure 2. Biological activities from soy food products and its effect on health.

a) Soybean and type 2 diabetes. Type 2 diabetes mellitus is a multifactorial metabolic disorder disease, which results from defects in both insulin secretion and insulin action. Insulin stimulates uptake, utilization and storage of glucose in cells throughout the body by inducing multiple signaling pathways in the tissues that express the transmembrane insulin receptor [87], especially in skeletal muscle that accounts for 75% of whole-body insulin-stimulated glucose uptake. The reduced responsiveness of cells to insulin is due to defective intracellular signaling processes [88, 89]. Millions of people have been diagnosed with type 2 diabetes, and many more are unaware they are at high risk [90]. Obesity is the major risk factor for diabetes and accounts for ≈70% of the variance in the prevalence of this common disease [91]. Dry beans and soyfoods offer benefits in the prevention of diabetes and in the clinical management of established diabetes, soybeans, in particular, have a low glycemic index.

They are rich in phytates, soluble fiber, and tannins, all of which correlate inversely with carbohydrate digestion and glycemic response [62, 92].

Soybean and its natural bioactive products have been studied as an antidiabetic potential. Studies have been conducted to examine the therapeutic effect of different bioactive compounds such as aglycin, phenolic-rich extract and glyceollins, all derivates of soybean. Soybean peptides have been widely used as a natural health food and supplement. It should be good for preventing obesity and diabetes because long-term feeding of soy peptide induced weight loss in obese mice [93]. In healthy and diabetic animal models, soybean peptides decreased blood glucose by increasing insulin sensitivity and improving glucose tolerance [89, 94].

Aglycin, a natural bioactive peptide isolated from soybean, structurally, it has a high stability with six cysteines embedded in three disulfide bonds. It is also resistant to digestion by trypsin, pepsin, Glu-C and bovine rumen fluid and has an antidiabetic potential [95, 96], at this respect, Lu J (2012), studied the effect of aglycin administration on the glucose homeostasis. For this, the diabetes was induced in BALB/c mice fed with a high-fat diet and a single intraperitoneal injection of streptozotocin. With onset of diabetes, the mice were administered daily with aglycin (50 mg/kg/d) for 4 weeks, blood glucose was monitored once a week [89]. The administration of aglycin restored insulin-signaling transduction by maintaining the insulin receptor (IR) and the insulin receptor substrate 1 (IRS1) expression at both the mRNA and protein levels, as well as elevating the expression of p-IR, p-IRS1, p-Akt and membrane GLUT4 protein. The results hence demonstrate that oral administration of aglycin can potentially attenuate or prevent hyperglycemia by increasing insulin receptor signaling pathway in the skeletal muscle of streptozotocin/high-fat-diet-induced diabetic mice [89].

Complex polysaccharides are hydrolyzed by α-amylase to oligosaccharides that are further hydrolyzed to liberate glucose by intestinal α-glucosidase before being absorbed into the intestinal epithelium and entering blood circulation. Therefore, α-amylase and α-glucosidase inhibitors may help to reduce postprandial hyperglycemia by inhibiting the enzymatic hydrolysis of carbohydrates, and hence may delay the absorption of glucose [97]. Therefore, effort has been directed in finding a natural and safer α-amylase and α-glucosidase inhibitors. Phytochemicals such as phenolics with strong antioxidant properties has been reported to be good inhibitors of these enzymes [98]. The phenolic compounds of soybean have also been studied, Ademiluyi (2011) [99] assessed the inhibitory activities of different phenolic-rich extracts from soybean on key-enzyme linked to type 2 diabetes (α-amylase and α-glucosidase) [99]. Their results shown that the different phenolic-rich extracts used inhibited α-amylase, α-glucosidase activities in a dose dependent pattern and the free phenolic extract had higher α-glucosidase inhibitory activity when compared to that of α-amylase; this property confer an advantage on soybean phenolic-rich extracts over commercial antidiabetic drugs with little or no side effect [99].

In turn, the flavonoid family of phytochemicals, particularly those derived from soy, has received attention regarding their estrogenic activity as well as their effects on human health and disease. In addition to these flavonoids other phytochemicals, including phytostilbene,

enterolactone, and lignans, possess endocrine activity; the health benefits of soy-based foods may, therefore, be dependent upon the amounts of the various hormonally active phytochemicals within these foods, especial attention have recieved the isoflavonoid phytoalexin compounds, glyceollins, in soy plants grown under stressed conditions [100]. A glyceollin-containing fermented soybeans was assessed by Park S (2012), where diabetic mice, induced by intraperitoneal injections of streptozotocin (20 mg/kg bw), were administered a high fat diet with no soybeans (control), 10% unfermented soybeans and 10% fermented soybeans containing glyceollins (FSG), respectively, for 8 weeks; among the diabetic mice, FSG-treated mice exhibited the lowest peak for blood glucose levels with an elevation of serum insulin levels during the first part of oral glucose tolerance testing. FSG also made blood glucose levels drop quickly after the peak and it decreased blood glucose levels more than the control during insulin tolerance testing [101]. The enhancement of glucose homeostasis was comparable to the effect induced by rosiglitazone, a commercial peroxisome proliferator-activated receptor-γ agonist, but it did not match the level of glucose homeostasis in the non-diabetic mice [101].

In vitro studies suggest that isoflavones have antidiabetic properties such as the inhibition of the intestinal brush border uptake of glucose, α-glucosidase inhibitor actions, and tyrosine kinase inhibitory properties [102]. Animal studies have indicated that soy protein or isoflavones improve glycemic control, lower insulin requirement, and increase insulin sensitivity [96, 103]. Several observational studies have also suggested that soy intake was associated with improved glycemic control or lowered risk of diabetes [104, 105].

Nevertheless, data from human clinical trials that evaluated the possible beneficial effects of isoflavone-rich soy products on glycemic control and insulin sensitivity have generated mixed findings. Some studies showed that soy products and foods significantly improve glycemic control [106-108], whereas others observed no significant effect [109-111]. This inconsistency could be due to a number of possible reasons. A wide variety of soy products, such as traditional soy foods, isolated soy protein, soy extracts, or purified isoflavones, and a variety of controls have been used [112]. Varied amounts and compositions of protein and isoflavones in soy products and the menopausal status of participants, study duration, baseline health status of participants, intervention adherence, and degree to which dietary intake is controlled may have contributed to variations in studies [113].

b) Soybean and cancer. For a long time has been described the effect of soy on human health, this legume present since ancient times in the Oriental diet is now present all over the world. The eastern population has low rates of various cancers, among these; breast cancer is undoubtedly a classic example.

Liener and Seto in 1955, described an *in vivo* effect in rats inoculated with Walker tumor. Daily injection of a soy extract at 25 mg / kg in these animals results in a decrease in tumor size and weight at the end of the period. This effect was observed only in animals that were treated from the time of inoculation of the tumor and not in those treated after tumor establishment [114]. From Liener investigations have been reported countless works that characterize the effect of soy on tumor growth.

Since 1996, it has been reported the use of extracts rich in soy isoflavones in human clinical studies. Has recently been published the results of a clinical study of soy isoflavones used in women at high risk of breast cancer, the results at 6 months of intervention is that soy isoflavones decrease epithelial proliferation in women with risk, modulating the expression of a large number of genes involved in carcinogenesis [115]. One of the markers associated with risk of breast cancer is the IGF-1 (Insulin-like growth factor 1), this growth factor is involved in various processes that stand between growth and cell development. The increase in plasma concentration is associated with up to seven times greater risk of developing this malignancy. However, high intake of soy in American women produces a slight increase in the concentration of IGF-1, these data are extremely complex to interpret. Eating a diet high in soy and algae produce a decrease of up to 40% of the levels of IGF-1 [116].

There is a wide range of cancers in which have been found some degree of association with soy consumption. Recently, a meta-analysis has shown that high-soy intake determined a low risk of lung cancer [117]. In this context, researchers have demonstrated *in vivo*, in nude mice (immune compromised) that were intravenously injected human tumor cell line A549, these cells generate tumor nodules in the lungs of these animals. By analyzing histologically the tissues of mice that received a treatment of 1 mg / day for 30 days orally soybeans, it was determined that the tumor cells were more sensitive to radiation therapy in addition to reducing vascular damage, inflammation and fibrosis caused by radiation on healthy tissue [118].

The relationship between breast cancer and the presence of estrogen receptor in these tumor cells is a fact and is related primarily to the aggressiveness of the tumor and its size. The non-steroidal phytoestrogens chemical compounds present in plants and especially in soy and structurally resemble human estrogen, may play a central role in the effect on the risk of breast cancer in postmenopausal women. This effect, albeit has been reported, is still not entirely clear, while some studies are very conclusive as that conducted by Zaineddin et al in 2012, recently, over 3000 cases and over 5000 controls in a case-control study in German women, the results did show a relationship between the consumption of phytoestrogens including soy and reduced the relative risk of breast cancer in postmenopausal women [119].

Genistein a predominant isoflavonoid in soybeans has long shown a beneficial effect on the prevention and treatment of some cancers. Has been studied this soy isoflavone and the mechanisms that may be associated with its antitumor effect. Among the mechanisms described is the inhibition of nuclear factor NFkappa B a key molecule in tumor cell survival, in 2011 a group of researchers reported that genistein inhibited the proliferation and induced apoptosis in the BGC-823 cell line, a cell line of human gastric cancer, in a dose and time dependent. The mechanism by which this isoflavonoid is capable of producing this effect is by decreasing cyclooxygenase 2 (COX-2), through inhibition of the transcriptional activation of NFkB [120]. Recently another research group reported that genistein is capable of reducing the growth of the cell line of breast cancer MDA-MB-231 inhibit NFkB transcriptional activity through a mechanism dependent signaling pathway Notch-1 [121], These researchers found that genistein negatively regulates the expression of cyclin B1, Bcl-2 and Bcl-xl. Genistein has proven to be a potentiating agent of other compounds with anticarcinogenic effect, a recent example is the reported effect on the cell line A549 lung cancer, where

genistein potentiates the apoptotic effect of Trichostatin A, the mechanism involved in this effect would be enhancing the positive regulation of the expression of mRNA encoding the tumor necrosis factor receptor 1(TNFR-1), which play a role of death receptor and therefore may at least partly explain this phenomenon [122]. One line of research to elucidate the mechanisms associated with the anti tumor effect of genistein is the ability of this molecule to inhibit the progression of tumor stem cells, in this context, Zhang et al 2012 have found that genistein has an effect until now not reported to prevent carcinogenesis in a model of early colon cancer using as markers of this process the signaling pathway WNT / beta-catenin, some of the genes that are under the control of this pathway are: cyclin D1 and c-myc, being genistein and an extract of soy protein capable of inhibiting various genes involved in the WNT pathway including the mentioned Wnt5a, sFRP1, sFRP2 and Sfrp5 [123]. It has also been shown that genistein could also exert its effect by regulating the immune system, is how Iranian researchers showed that genistein is capable of protecting carcinogenesis in a mouse model of cervical cancer by an immune modulatory mechanism [124].

In summary, it appears that soy consumption is a protective agent in carcinogenesis of various cancers. The mechanisms involved in this process are still a mystery although there are efforts to discover them. One of the molecules characterized in this protective effect is genistein, a soy isoflavone, to which today have described multiple benefits and is now the therapeutic target for the generation of other molecules with structural similarity to her and that enhance its effects *in vivo*.

c) Soybean and cardiovascular disease. Another major concern in today's world is cardiovascular disease, in which the nutritional properties of soybean proteins are well known. Within the past 25 years, numerous studies have reported inverse associations between soy protein intake and plasma cholesterol concentrations; this association is particularly evident in hyper-cholesterolemic men and women [125-127].

Several studies comparing isoflavone-rich soy diets with isoflavone-free soy diets have been performed in experimental animals and humans. Soy consumption could reduce the cardiovascular disease risk factors through its beneficial components, including complex carbohydrates, unsaturated fatty acids, vegetable protein, soluble fiber, oligosaccharides, vitamins, minerals, inositol-derived substances such as lipintol and pinitol, and phytoestrogens, particularly the isoflavones genistein, diadzein, and glycitein [128, 129].

Different studies have been carried out to assess the influence of soy-protein on serum concentrations of total cholesterol, LDL cholesterol, triacylglycerol, and apoB-100. Beneficial results have also been seen among subjects with different types of diseases [130, 131]. Beneficial effects of soy consumption on blood lipids were the most consistently reported findings by Azadbakht (2007) [108]. In turn, Anderson (1995), showed significant reductions in total cholesterol (9%), LDL cholesterol (13%), and triacylglycerol's (11%) with the consumption, on average, of 47 g soy-protein/daily [125].

For its part, Jenkins (2002) studied the effects of high- and low-isoflavone soy-protein foods on both lipid and nonlipid risk factors for coronary artery disease. They found that compared with the control diet, however, both soy diets resulted in significantly lower total cho-

lesterol, estimated CAD risk, and ratios of total to HDL cholesterol, LDL to HDL cholesterol, and apolipoprotein B to A-I [132]. No significant sex differences were observed, except for systolic blood pressure, which in men was significantly lower after the soy diets than after the control diet. On the basis of blood lipid and blood pressure changes, the calculated CAD risk was significantly lower with the soy diets, by 10.1 ± 2.7% [132].

Other group reported that monkeys fed isoflavone-rich soy-protein-isolate diets had significantly better serum lipid values (lower total cholesterol and higher HDL-cholesterol concentrations) than monkeys fed isoflavone-poor soy-protein-isolate diets. Whereas the administration of the antiestrogen tamoxifen is accompanied by an increase in serum triacylglycerol concentrations, soy-protein administration is associated with a decrease in serum triacylglycerol concentrations [133].

Other cardiac benefits of soy intake, independent of cholesterol reduction, have been identified and investigated. Clarkson (1994) [134] used monkeys with experimental atherosclerosis as a model to examine the effects of estrogen administration on vascular dilatation *in vivo*. Using a similar animal model they also showed that an isoflavone-rich soy-protein-isolate diet has a favorable effect on dilatation of coronary arteries similar to that of estrogen administration [62, 135].

The amount of soy protein that should be recommended for use to achieve "therapeutic effects" is unknown. Also, further research is required to determine the safety of isoflavones in pharmaceutical doses. Animal studies suggest that small amounts of isoflavones have favorable effects on lipoprotein oxidation and cholesterol reduction. Much more work is required to determine the minimum amount needed to have a specific beneficial health effect.

6. Conclusion

Epidemiological studies suggest that soybean consumption is associated, at least in part, with lower incidences of a number of chronic diseases, including type 2 diabetes, certain types of cancer and cardiovascular diseases, between others, mainly due antioxidant and antiplatelet activities.

Soybean derivatives can be denominated as functional ingredients as they contain bioactive compounds that inhibit platelet aggregation, which gives a preventive effect on thrombus formation. Also soybeans contain a variety of bioactive phytochemicals such as phenolic acids, flavonoids, isoflavones, saponins, phytosterols and sphingolipids; being the phenolic compounds with the highest antioxidant capacity. The key benefits of soy are related to their excellent protein content, its high content of essential fatty acids, numerous vitamins and minerals, their isoflavones and their higher fiber content. The experimental evidences related soy protein more than soy isoflavones as responsible by effects observed. At the present is not possible to discard another component present in soy as responsible by the effects.

Functional food may act as an adjunctive therapy/alternative treatment of different pathologies, and scientific studies are appearing more frequently demonstrating that this hypothesis is, indeed, a reality.

These evidences were considered by FDA, that published claims that recommended soy protein extract as an alternative to reduce blood cholesterol concentrations and prevent cancer, diabetes and increase protection cardiovascular.

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References

[1] Forde, B., & Lorenzo, H. (2001). The nutritional control of root development. *Plant and Soil*, 232, 51-68.

[2] Liu, X., Jin, H., Wang, G., & Herbert, S. (2008). Soybean yield physiology and development of high-yielding practices in Northeast China. *Field Crops Research*, 105, 157-171.

[3] Setchell, K. D. (1998). Phytoestrogens the biochemistry, physiology, and implications for human health of soy isoflavones. *Am J Clin Nutr*, 68, 1333S -1346S .

[4] Heaney, R. P., Weaver, C. M., & Fitzsimmons, M. L. (1991). Soybean phytate content: effect on calcium absorption. *Am J Clin Nutr*, 53, 745-747.

[5] Liu, Z. S., & Chang, S. K. (2004). Effect of soy milk characteristics and cooking conditions on coagulant requirements for making filled tofu. *J Agric Food Chem*, 52, 3405-3411.

[6] Wood, J. E., Senthilmohan, S. T., & Peskin, A. V. (2002). Antioxidant activity of procyanidin-containing plant extracts at different pHs. *Food Chemistry*, 77, 155-161.

[7] Decker, E. A. (1995). The role of phenolics, conjugated linoleic acid, carnosine, and pyrroloquinoline quinone as nonessential dietary antioxidants. *Nutr Rev*, 53, 49-58.

[8] Xu, B., & Chang, S. K. (2008). Characterization of phenolic substances and antioxidant properties of food soybeans grown in the North Dakota-Minnesota region. *J Agric Food Chem*, 56, 9102-9113.

[9] Makela, S., Poutanen, M., Lehtimaki, J., Kostian, M. L., et al. (1995). Estrogen-specific 17 beta-hydroxysteroid oxidoreductase type 1 (E.C. 1.1.1.62) as a possible target for the action of phytoestrogens. *Proc Soc Exp Biol Med*, 208, 51-59.

[10] Yen, G. C., Chang, Y. C., & Su, S. W. (2003). Antioxidant activity and active compounds of rice koji fermented with Aspergillus candidus. *Food Chemistry*, 83, 49-54.

[11] Yang, J. H., Mau, J. L., Ko, P. T., & Huang, L. C. (2000). Antioxidant properties of fermented soybean broth. *Food Chemistry*, 71, 249-254.

[12] Siddhuraju, P., Mohan, P. S., & Becker, K. (2002). Studies on the antioxidant activity of Indian Laburnum (Cassia fistula L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chemistry*, 79, 61-67.

[13] Baunmann, L.S. (2009). Cosmetic Dermatology: Principles and Practice, Revised 2nd ed. *McGraw-Hill, Columbus, Ohio*.

[14] Ranich, T., Bhathena, S. J., & Velasquez, M. T. (2001). Protective effects of dietary phytoestrogens in chronic renal disease. *J Ren Nutr*, 11, 183-193.

[15] Axelson, M., Kirk, D. N., Farrant, R. D., Cooley, G., et al. (1982). The identification of the weak oestrogen equol [7-hydroxy-3-(4'-hydroxyphenyl)chroman] in human urine. *Biochem J*, 201, 353-357.

[16] Setchell, K. D., Brown, N. M., Zimmer-Nechemias, L., Brashear, W. T., et al. (2002). Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. *Am J Clin Nutr*, 76, 447-453.

[17] Setchell, K. D., Borriello, S. P., Hulme, P., Kirk, D. N., & Axelson, M. (1984). Nonsteroidal estrogens of dietary origin: possible roles in hormone-dependent disease. *Am J Clin Nutr*, 40, 569-578.

[18] Kelly, G. E., Nelson, C., Waring, M. A., Joannou, G. E., & Reeder, A. Y. (1993). Metabolites of dietary (soya) isoflavones in human urine. *Clin Chim Acta*, 223, 9-22.

[19] Heinonen, S., Wahala, K., & Adlercreutz, H. (1999). Identification of isoflavone metabolites dihydrodaidzein, dihydrogenistein, 6'-OH-O-dma, and cis-4-OH-equol in

human urine by gas chromatography-mass spectroscopy using authentic reference compounds. *Anal Biochem*, 274, 211-219.

[20] Atkinson, C., Berman, S., Humbert, O., & Lampe, J. W. (2004). In vitro incubation of human feces with daidzein and antibiotics suggests interindividual differences in the bacteria responsible for equol production. *J Nutr*, 134, 596-599.

[21] Setchell, K. D., Brown, N. M., & 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.

[22] Setchell, K. D., Zimmer-Nechemias, L., Cai, J., & Heubi, J. E. (1998). Isoflavone content of infant formulas and the metabolic fate of these phytoestrogens in early life. *Am J Clin Nutr*, 68, 1453S -1461S .

[23] Setchell, K. D., Zimmer-Nechemias, L., Cai, J., & Heubi, J. E. (1997). Exposure of infants to phyto-oestrogens from soy-based infant formula. *Lancet*, 350, 23-27.

[24] Lampe, J. W., Karr, S. C., Hutchins, A. M., & Slavin, J. L. (1998). Urinary equol excretion with a soy challenge: influence of habitual diet. *Proc Soc Exp Biol Med*, 217, 335-339.

[25] Rowland, I. R., Wiseman, H., Sanders, T. A., Adlercreutz, H., & Bowey, E. A. (2000). Interindividual variation in metabolism of soy isoflavones and lignans: influence of habitual diet on equol production by the gut microflora. *Nutr Cancer*, 36, 27-32.

[26] Setchell, K. D, Clerici, C, Lephart, E. D., Cole, S. J., et al. (2005). S-equol, a potent ligand for estrogen receptor beta, is the exclusive enantiomeric form of the soy isoflavone metabolite produced by human intestinal bacterial flora. *Am J Clin Nutr*, 81, 1072-1079.

[27] Gopaul, R., Knaggs, H. E., & Lephart, E. D. (2012). Biochemical investigation and gene analysis of equol: a plant and soy-derived isoflavonoid with antiaging and antioxidant properties with potential human skin applications. *Biofactors*, 38, 44-52.

[28] Kuiper, G. G., Lemmen, J. G., Carlsson, B., Corton, J. C., et al. (1998). Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology*, 139, 4252-4263.

[29] Kuiper, G. G., Carlsson, B., Grandien, K., Enmark, E., et al. (1997). Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology*, 138, 863-870.

[30] Thornton, M. J., Taylor, A. H., Mulligan, K., Al-Azzawi, F., et al. (2003). The distribution of estrogen receptor beta is distinct to that of estrogen receptor alpha and the androgen receptor in human skin and the pilosebaceous unit. J. *Investig Dermatol Symp Proc*, 8, 100-103.

[31] Lund, T. D., Munson, D. J., Haldy, M. E., Setchell, K. D., et al. (2004). Equol is a novel anti-androgen that inhibits prostate growth and hormone feedback. *Biol Reprod*, 70, 1188-1195.

[32] Makrantonaki, E., & Zouboulis, C. C. (2009). Androgens and ageing of the skin. *Curr Opin Endocrinol Diabetes Obes*, 16, 240-245.

[33] Nitsch, S. M., Wittmann, F., Angele, P., Wichmann, M. W., et al. (2004). Physiological levels of 5 alpha-dihydrotestosterone depress wound immune function and impair wound healing following trauma-hemorrhage. *Arch Surg*, 139, 157-163.

[34] Stevenson, S., & Thornton, J. (2007). Effect of estrogens on skin aging and the potential role of SERMs. *Clin Interv Aging*, 2, 283-297.

[35] Verdier-Sevrain, S., Bonte, F., & Gilchrest, B. (2006). Biology of estrogens in skin: implications for skin aging. *Exp Dermatol*, 15, 83-94.

[36] Munoz, Y., Garrido, A., & Valladares, L. (2009). Equol is more active than soy isoflavone itself to compete for binding to thromboxane A(2) receptor in human platelets. *Thromb Res*, 123, 740-744.

[37] Ronis, M. J., Chen, Y., Badeaux, J., & Badger, T. M. (2009). Dietary soy protein isolate attenuates metabolic syndrome in rats via effects on PPAR, LXR, and SREBP signaling. *J Nutr*, 139, 1431-1438.

[38] Harborne, J. B, & Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry*, 55, 481-504.

[39] Paik, S. S., Jeong, E., Jung, S. W., Ha, T. J., et al. (2012). Anthocyanins from the seed coat of black soybean reduce retinal degeneration induced by N-methyl-N-nitrosourea. *Exp Eye Res*, 97, 55-62.

[40] Gottstein, N., Ewins, B. A., Eccleston, C., Hubbard, G. P., et al. (2003). Effect of genistein and daidzein on platelet aggregation and monocyte and endothelial function. *Br J Nutr*, 89, 607-616.

[41] Yin, X. Z., Quan, J. S., Takemichi, K., & Makoto, T. (2004). Anti-atherosclerotic effect of soybean isofalvones and soyasaponins in diabetic rats. *Zhonghua Yu Fang Yi Xue Za Zhi*, 38, 26-28.

[42] Kim, K., Lim, K. M., Kim, C. W., Shin, H. J., et al. (2011). Black soybean extract can attenuate thrombosis through inhibition of collagen-induced platelet activation. *J Extract Black*, 22, 964-970.

[43] Mc Nicol, A. (1993). The effects of genistein on platelet function are due to thromboxane receptor antagonism rather than inhibition of tyrosine kinase. *ProstaglandinsLeukot Essent Fatty Acids*, 48, 379-384.

[44] Kondo, K., Suzuki, Y., Ikeda, Y., & Umemura, K. (2002). Genistein, an isoflavone included in soy, inhibits thrombotic vessel occlusion in the mouse femoral artery and in vitro platelet aggregation. *Eur J Pharmacol*, 455, 53-57.

[45] Atsushi, O. (2004). Anti-platelets Effects of Genistein, an Isoflavonoid from Soybean. *Soy Protein Research*, 7, 145-148.

[46] Tsuchiya, H., Sato, M., & Watanabe, I. (1999). Antiplatelet activity of soy sauce as functional seasoning. *J Agric Food Chem*, 47, 4167-4174.

[47] Kitagawa, S., Orinaka, M., & Hirata, H. (1993). Depth-dependent change in membrane fluidity by phenolic compounds in bovine platelets and its relationship with their effects on aggregation and adenylate cyclase activity. *Biochim Biophys Acta*, 1179, 277-282.

[48] Peura, P., Mackenzie, P., Koivusaari, U., & Lang, M. (1982). Increased fluidity of a model membrane caused by tetrahydro-beta-carbolines. *Mol Pharmacol*, 22, 721-724.

[49] Silva, M., Santana, L., Silva-Lucca, R., Lima, A., et al. (2011). Immobilized Cratylia mollis lectin: An affinity matrix to purify a soybean (Glycine max) seed protein with in vitro platelet antiaggregation and anticoagulant activities. *Process Process*, 46, 74-80.

[50] Lee, K., & Kim, S. (2005). SSGE and DEE, new peptides isolated from a soy protein hydrolysate that inhibit platelet aggregation. *Food Chemistry*, 90, 389-393.

[51] Tsuchiya, H., Sato, M., & Watanabe, I. (1999). Antiplatelet activity of soy sauce as functional seasoning. *J Agric Food Chem*, 47, 4167-4174.

[52] Setchell, K. D., & Cassidy, A. (1999). Dietary isoflavones: biological effects and relevance to human health. *J Nutr*, 129, 758S -767S .

[53] Bennett, J. O., Yu, O., Heatherly, L. G., & Krishnan, H. B. (2004). Accumulation of genistein and daidzein, soybean isoflavones implicated in promoting human health, is significantly elevated by irrigation. *J Agric Food Chem*, 52, 7574-7579.

[54] Okamoto, K., & Horisawa, R. (2006). Soy products and risk of an aneurysmal rupture subarachnoid hemorrhage in Japan. *Eur J Cardiovasc Prev Rehabil*, 13, 284-287.

[55] Zhang, X., Shu, X. O., Gao, Y. T., Yang, G., et al. (2003). Soy food consumption is associated with lower risk of coronary heart disease in Chinese women. *J Nutr*, 133, 2874-2878.

[56] Potter, S. M., Bakhit, R. M., Essex-Sorlie, D. L., Weingartner, K. E., et al. (1993). Depression of plasma cholesterol in men by consumption of baked products containing soy protein. *Am J Clin Nutr*, 58, 501-506.

[57] Bakhit, R. M., Klein, B. P., Essex-Sorlie, D., Ham, J. O., et al. (1994). Intake of 25 g of soybean protein with or without soybean fiber alters plasma lipids in men with elevated cholesterol concentrations. *J Nutr*, 124, 213-222.

[58] Erdman, J. W., Jr. (2000). AHA Science Advisory: Soy protein and cardiovascular disease: A statement for healthcare professionals from the Nutrition Committee of the AHA. *Circulation*, 102, 2555-2559.

[59] Mullen, E., Brown, R. M., Osborne, T. F., & Shay, N. F. (2004). Soy isoflavones affect sterol regulatory element binding proteins (SREBPs) and SREBP-regulated genes in HepG2 cells. *J Nutr*, 134, 2942-2947.

[60] Adams, M. R., Golden, D. L., Anthony, M. S., Register, T. C., & Williams, J. K. (2002). The inhibitory effect of soy protein isolate on atherosclerosis in mice does not require the presence of LDL receptors or alteration of plasma lipoproteins. *J Nutr*, 132, 43-49.

[61] Adams, M. R., Register, T. C., Golden, D. L., Anthony, M. S., et al. (2002). The atheroprotective effect of dietary soy isoflavones in apolipoprotein E-/- mice requires the presence of estrogen receptor-alpha. *Arterioscler Thromb Vasc Biol*, 22, 1859-1864.

[62] Anderson, J. W., Smith, B. M., & Washnock, C. S. (1999). Cardiovascular and renal benefits of dry bean and soybean intake. *Am J Clin Nutr*, 70, 464S -474S .

[63] Iritani, N., Sugimoto, T., Fukuda, H., Komiya, M., & Ikeda, H. (1997). Dietary soybean protein increases insulin receptor gene expression in Wistar fatty rats when dietary polyunsaturated fatty acid level is low. *J Nutr*, 127, 1077-1083.

[64] Zhan, S., & Ho, S. C. (2005). Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. *Am J Clin Nutr*, 81, 397-408.

[65] Lusas, E. W., & Riaz, M. N. (1995). Soy protein products: processing and use. J Nutr ., 125, 573S-580S.

[66] Messina, M. J. (1999). Legumes and soybeans: overview of their nutritional profiles and health effects. *Am J Clin Nutr*, 70, 439S -450S .

[67] Kataoka, S. (2005). Functional effects of Japanese style fermented soy sauce (shoyu) and its components. *J Biosci Bioeng*, 100, 227-234.

[68] Kang, J., Badger, T. M., Ronis, M. J., & Wu, X. Non-isoflavone phytochemicals in soy and their health effects. *J Agric Food Chem*, 58, 8119-8133.

[69] Gil-Izquierdo, A., Penalvo, J. L., Gil, J. I., Medina, S., et al. Soy isoflavones and cardiovascular disease epidemiological, clinical and-omics perspectives. *Curr Pharm Biotechnol*, 13, 624-631.

[70] Wei, X., Wu, J., Ni, Y., Lu, L., & Zhao, R. (2011). Antioxidant effect of a phytoestrogen equol on cultured muscle cells of embryonic broilers. *In Vitro Cell Dev Biol Anim*, 47, 735-741.

[71] Choi, E., & Kim, G. (2011). Anticancer mechanism of equol in dimethylbenz(a)anthracene-treated animals. 7 12 . *Int J Oncol*, 39, 747-754.

[72] Palermo, M., Paradiso, R., De Pascale, S., & Fogliano, V. Hydroponic cultivation improves the nutritional quality of soybean and its products. *J Agric Food Chem*, 60, 250-255.

[73] Hendrich, S., & Fisher, K. (2001). What do we need to know about active ingredients in dietary supplements? Summary of workshop discussion. *J Nutr*, 131, 1387S -8S .

[74] Setchell, K. D., Brown, N. M., Desai, P. B., Zimmer-Nechimias, L., et al. (2003). Bioavailability, disposition, and dose-response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes. *J Nutr*, 133, 1027-1035.

[75] Larkin, T. A., Astheimer, L. B., & Price, W. E. (2009). Dietary combination of soy with a probiotic or prebiotic food significantly reduces total and LDL cholesterol in mildly hypercholesterolaemic subjects. *Eur J Clin Nutr*, 63, 238-245.

[76] Rowland, I., Faughnan, M., Hoey, L., Wahala, K., et al. (2003). Bioavailability of phyto-oestrogens. *Br J Nutr, Suppl 1, S*, 89, 45-58.

[77] Izumi, T., Piskula, M. K., Osawa, S., Obata, A., et al. (2000). Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *J Nutr*, 130, 1695-1699.

[78] Wiseman, H., Casey, K., Bowey, E. A., Duffy, R., et al. (2004). Influence of 10 wk of soy consumption on plasma concentrations and excretion of isoflavonoids and on gut microflora metabolism in healthy adults. *Am J Clin Nutr*, 80, 692-699.

[79] Xu, X., Wang, H. J., Murphy, P. A., Cook, L., & Hendrich, S. (1994). Daidzein is a more bioavailable soymilk isoflavone than is genistein in adult women. *J Nutr*, 124, 825-832.

[80] Andlauer, W., Kolb, J., & Furst, P. (2000). Isoflavones from tofu are absorbed and metabolized in the isolated rat small intestine. *J Nutr*, 130, 3021-3027.

[81] King, R. A. (1998). Daidzein conjugates are more bioavailable than genistein conjugates in rats. *Am J Clin Nutr*, 68, 1496S -1499S .

[82] Setchell, K. D., Brown, N. M., Desai, P., Zimmer-Nechemias, L., et al. (2001). Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. *J Nutr*, 131, 1362S -75S .

[83] Piazza, C., Privitera, M. G., Melilli, B., Incognito, T., et al. (2007). Influence of inulin on plasma isoflavone concentrations in healthy postmenopausal women. *Am J Clin Nutr*, 86, 775-780.

[84] Benvenuti, C., & Setnikar, I. (2011). Effect of Lactobacillus sporogenes on oral isoflavones bioavailability: single dose pharmacokinetic study in menopausal women. *Arzneimittelforschung*, 61, 605-609.

[85] Miguez, A. C., Francisco, J. C., Barberato, S. H., Simeoni, R., et al. (2012). The functional effect of soybean extract and isolated isoflavone on myocardial infarction and ventricular dysfunction: The soybean extract on myocardial infarction. *J Nutr Biochem*.

[86] Palomo, I., Guzmán, L., Leiva, E., Mujica, V., et al. (2011). Soybean and Health. *Hany El-Shemy: InTech*.

[87] Ruiz-Alcaraz, A. J., Liu, H. K., Cuthbertson, D. J., Mc Manus, E. J., et al. (2005). A novel regulation of IRS1 (insulin receptor substrate-1) expression following short term insulin administration. *Biochem J*, 392, 345-352.

[88] Bjornholm, M., & Zierath, J. R. (2005). Insulin signal transduction in human skeletal muscle: identifying the defects in Type II diabetes. *Biochem Soc Trans*, 33, 354-357.

[89] Lu, J., Zeng, Y., Hou, W., Zhang, S., et al. (2012). The soybean peptide aglycin regulates glucose homeostasis in type 2 diabetic mice via IR/IRS1 pathway. *J Nutr Biochem*.

[90] Mizokami-Stout, K., Cree-Green, M., & Nadeau, K. J. (2012). Insulin resistance in type 2 diabetic youth. *Curr Opin Endocrinol Diabetes Obes*, 19, 255-262.

[91] Everhart, J. E., Pettitt, D. J., Bennett, P. H., & Knowler, W. C. (1992). Duration of obesity increases the incidence of NIDDM. *Diabetes*, 41, 235-240.

[92] Liener, I. E. (1994). Implications of antinutritional components in soybean foods. *Crit Rev Food Sci Nutr*, 34, 31-67.

[93] Ishihara, K., Oyaizu, S., Fukuchi, Y., Mizunoya, W., et al. (2003). A soybean peptide isolate diet promotes postprandial carbohydrate oxidation and energy expenditure in type II diabetic mice. *J Nutr*, 133, 752-757.

[94] Davis, J., Higginbotham, A., O'Connor, T., Moustaid-Moussa, N., et al. (2007). Soy protein and isoflavones influence adiposity and development of metabolic syndrome in the obese male ZDF rat. *Ann Nutr Metab*, 51, 42-52.

[95] Dun, X. P., Wang, J. H., Chen, L., Lu, J., et al. (2007). Activity of the plant peptide aglycin in mammalian systems. *FEBS J*, 274, 751-759.

[96] Lu, M. P., Wang, R., Song, X., Chibbar, R., et al. (2008). Dietary soy isoflavones increase insulin secretion and prevent the development of diabetic cataracts in streptozotocin-induced diabetic rats. *Nutr Res*, 28, 464-471.

[97] Saito, N., Sakai, H., Suzuki, S., Sekihara, H., & Yajima, Y. (1998). Effect of an alpha-glucosidase inhibitor (voglibose), in combination with sulphonylureas, on glycaemic control in type 2 diabetes patients. *J Int Med Res*, 26, 219-232.

[98] Kwon, Y. I., Vattem, D. A., & Shetty, K. (2006). Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. *Asia Pac J Clin Nutr*, 15, 107-118.

[99] Ademiluyi, A. O., & Oboh, G. (2011). Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (alpha-amylase and alpha-glucosidase) and hypertension (angiotensin I converting enzyme) in vitro. *Exp Toxicol Pathol*.

[100] Burow, M. E., Boue, S. M., Collins-Burow, B. M., Melnik, L. I., et al. (2001). Phytochemical glyceollins, isolated from soy, mediate antihormonal effects through estrogen receptor alpha and beta. *J Clin Endocrinol Metab*, 86, 1750-1758.

[101] Park, S., Kim, da. S., Kim, J. H., Kim, J. S., & Kim, H. J. (2012). Glyceollin-containing fermented soybeans improve glucose homeostasis in diabetic mice. *Nutrition*, 28, 204-211.

[102] Vedavanam, K., Srijayanta, S., O'Reilly, J., Raman, A., & Wiseman, H. (1999). Antioxidant action and potential antidiabetic properties of an isoflavonoid-containing soybean phytochemical extract (SPE). *Phytother Res*, 13, 601-608.

[103] Ascencio, C., Torres, N., Isoard-Acosta, F., Gomez-Perez, F. J., et al. (2004). Soy protein affects serum insulin and hepatic SREBP-1 mRNA and reduces fatty liver in rats. *J Nutr*, 134, 522-529.

[104] Villegas, R., Gao, Y. T., Yang, G., Li, H. L., et al. (2008). Legume and soy food intake and the incidence of type 2 diabetes in the Shanghai Women's Health Study. *Am J Clin Nutr*, 87, 162-167.

[105] Nanri, A., Mizoue, T., Takahashi, Y., Kirii, K., et al. (2010). Soy product and isoflavone intakes are associated with a lower risk of type 2 diabetes in overweight Japanese women. *J Nutr*, 140, 580-586.

[106] Aubertin-Leheudre, M., Lord, C., Khalil, A., & Dionne, I. J. (2008). Isoflavones and clinical cardiovascular risk factors in obese postmenopausal women: a randomized double-blind placebo-controlled trial. *J Womens Health (Larchmt)*, 17, 1363-1369.

[107] Azadbakht, L., Atabak, S., & Esmaillzadeh, A. (2008). Soy protein intake, cardiorenal indices, and C-reactive protein in type 2 diabetes with nephropathy a longitudinal randomized clinical trial. *Diabetes Care*, 31, 648-654.

[108] Azadbakht, L., Kimiagar, M., Mehrabi, Y., Esmaillzadeh, A., et al. (2007). Soy inclusion in the diet improves features of the metabolic syndrome: a randomized crossover study in postmenopausal women. *Am J Clin Nutr*, 85, 735-741.

[109] Blakesmith, S. J., Lyons-Wall, P. M., George, C., Joannou, G. E., et al. (2003). Effects of supplementation with purified red clover (Trifolium pratense) isoflavones on plasma lipids and insulin resistance in healthy premenopausal women. *Br J Nutr*, 89, 467-474.

[110] Gonzalez, S., Jayagopal, V., Kilpatrick, E. S., Chapman, T., & Atkin, S. L. (2007). Effects of isoflavone dietary supplementation on cardiovascular risk factors in type 2 diabetes. *Diabetes Care*, 30, 1871-1873.

[111] Liao, F. H., Shieh, M. J., Yang, S. C., Lin, S. H., & Chien, Y. W. (2007). Effectiveness of a soy-based compared with a traditional low-calorie diet on weight loss and lipid levels in overweight adults. *Nutrition*, 23, 551-556.

[112] Erdman, J. W., Jr , , Badger, T. M., Lampe, J. W., Setchell, K. D., & Messina, M. (2004). Not all soy products are created equal: caution needed in interpretation of research results. *J Nutr*, 134, 1229S -1233S .

[113] Liu, Z. M., Chen, Y. M., & Ho, S. C. (2011). Effects of soy intake on glycemic control: a meta-analysis of randomized controlled trials. *Am J Clin Nutr*, 93, 1092-1101.

[114] Liener, I. E., & Seto, T. A. (1955). Nonspecific effect of soybean hemagglutinin on tumor growth. *Cancer research*, 15, 407-409.

[115] Khan, S. A., Chatterton, R. T., Michel, N., Bryk, M., et al. (2012). Soy isoflavone supplementation for breast cancer risk reduction: a randomized phase II trial. *Cancer Prev Res (Phila)*, 5, 309-319.

[116] Teas, J., Irhimeh, M. R., Druker, S., Hurley, T. G., et al. (2011). Serum IGF-1 concentrations change with soy and seaweed supplements in healthy postmenopausal American women. *Nutr Cancer*, 63, 743-748.

[117] Yang, W. S., Va, P., Wong, M. Y., Zhang, H. L., & Xiang, Y. B. (2011). Soy intake is associated with lower lung cancer risk: results from a meta-analysis of epidemiologic studies. *Am J Clin Nutr*, 94, 1575-1583.

[118] Hillman, G. G., Singh-Gupta, V., Runyan, L., Yunker, C. K., et al. (2011). Soy isoflavones radiosensitize lung cancer while mitigating normal tissue injury. *Radiotherapy and oncology journal of the European Society for Therapeutic Radiology and Oncology*, 101, 329-336.

[119] Zaineddin, A. K., Buck, K., Vrieling, A., Heinz, J., et al. (2012). The association between dietary lignans, phytoestrogen-rich foods, and fiber intake and postmenopausal breast cancer risk: a german case-control study. *Nutr Cancer*, 64, 652-665.

[120] Li, Y. S., Wu, L. P., Li, K. H., Liu, Y. P., et al. (2011). Involvement of nuclear factor kappaB (NF-kappaB) in the downregulation of cyclooxygenase-2 (COX-2) by genistein in gastric cancer cells. *The Journal of international medical research*, 39, 2141-2150.

[121] Pan, H., Zhou, W., He, W., Liu, X., et al. (2012). Genistein inhibits MDA-MB-231 triple-negative breast cancer cell growth by inhibiting NF-kappaB activity via the Notch-1 pathway. *International journal of molecular medicine*, 30, 337-343.

[122] Wu, T. C., Yang, Y. C., Huang, P. R., Wen, Y. D., & Yeh, S. L. (2012). Genistein enhances the effect of trichostatin A on inhibition of A549 cell growth by increasing expression of TNF receptor-1. *Toxicol Appl Pharmacol*, 262, 247-254.

[123] Zhang, Y., Li, Q., Zhou, D., & Chen, H. (2012). Genistein, a soya isoflavone, prevents azoxymethane-induced up-regulation of WNT/beta-caten in signalling and reduces colon pre-neoplasia in rats. *Br J Nutr*, 1-10.

[124] Ghaemi, A., Soleimanjahi, H., Razeghi, S., Gorji, A., et al. (2012). Genistein induces a protective immunomodulatory effect in a mouse model of cervical cancer. *Iranian journal of immunology : IJI*, 9, 119-127.

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

[126] Carroll, K. K. (1991). Review of clinical studies on cholesterol-lowering response to soy protein. *J Am Diet Assoc*, 91, 820-827.

[127] Wangen, K. E., Duncan, A. M., Xu, X., & Kurzer, M. S. (2001). Soy isoflavones improve plasma lipids in normocholesterolemic and mildly hypercholesterolemic postmenopausal women. *Am J Clin Nutr*, 73, 225-231.

[128] Kim, J. I., Kim, J. C., Kang, M. J., Lee, M. S., et al. (2005). Effects of pinitol isolated from soybeans on glycaemic control and cardiovascular risk factors in Korean patients with type II diabetes mellitus: a randomized controlled study. *Eur J Clin Nutr*, 59, 456-458.

[129] Crisafulli, A., Altavilla, D., Marini, H., Bitto, A., et al. (2005). Effects of the phytoestrogen genistein on cardiovascular risk factors in postmenopausal women. *Menopause*, 12, 186-192.

[130] Hoie, L. H., Graubaum, H. J., Harde, A., Gruenwald, J., & Wernecke, K. D. (2005). Lipid-lowering effect of 2 dosages of a soy protein supplement in hypercholesterolemia. *Adv Ther*, 22, 175-186.

[131] Hermansen, K., Hansen, B., Jacobsen, R., Clausen, P., et al. (2005). Effects of soy supplementation on blood lipids and arterial function in hypercholesterolaemic subjects. *Eur J Clin Nutr*, 59, 843-850.

[132] Jenkins, D. J., Kendall, C. W., Jackson, C. J., Connelly, P. W., et al. (2002). Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. *Am J Clin Nutr*, 76, 365-372.

[133] Anthony, M. S., Clarkson, T. B., Hughes, C. L. Jr, Morgan, T. M., & Burke, G. L. (1996). Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. *J Nutr*, 126, 43-50.

[134] Clarkson, T. B., Anthony, M. S., & Klein, K. P. (1994). Effects of estrogen treatment on arterial wall structure and function. *Drugs*, 2, 42-51.

[135] Williams, J. K., & Clarkson, T. B. (1998). Dietary soy isoflavones inhibit in-vivo constrictor responses of coronary arteries to collagen-induced platelet activation. *Coron Artery Dis*, 9, 759-764.