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Biologically Active Molecules from Soybeans

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1. Introduction

Soybeans (Glycine max [L.] Merr.) contain an impressive array of biologically active components. People have been eating soybeans for almost 5,000 years. Unlike most plant foods, soybeans are high in protein. Researchers are interested in both the nutritional value and the potential health benefits of soybeans. Research of the health effects of soyfoods and soybean constituents has been received significant attention to support the health improvements or health risks observed clinically or in vitro experiments. This research includes a wide range of areas, such as cancer, coronary heart disease (cardiovascular disease), osteoporosis, cognitive function (memory related), menopausal symptoms, renal function, and many others. This chapter provides up-to-date coverage on biologically active and related organic molecules isolated from soy and soy products. Their biological activities are briefly summarized. Molecules discussed in this chapter are as follows: isoflavones, phytic acid, soy lipids, soy phytoalexins, soyasaponins, lectins, hemagglutinin, soy toxins, and vitamins.

2. Health benefit of soy or soy products

Soy products have been considered as great source of protein for many decades. Japanese, in particular, eat a soy-based diet. Japanese monks eat soy products as their main protein source. They are known to live longer and have lower rates of chronic diseases. Because soybeans contain practically no starch, soybeans are an important part of a diabetic diet. Soybeans are 1) rich in protein (vide supra), calcium, and vitamins, and 2) high in monosaturated fatty acids. In addition, soybeans contain several biologically interesting phytochemicals as minor components, which scientists are interested in understanding their biological functions.

Many studies have shown that American and European males have a ten-fold increase in the risk of prostate cancer development as compared with East Asian countries. The observed difference prevalence of prostate cancers is considered due, in part, to difference in soy consumption. Some studies indicated that isoflavones found in soybeans contribute to the risk reduction of prostate cancer. Although there is a lack of unequivocal evidences that consuming soy as an adult may reduce the risk of breast cancer, several researches have reported that consuming soy products as a teenager may help reduce breast cancer risk as an adult. Some medical research has determined that ingredients of soybeans may help reduce the risk of colon cancer and heart diseases. The dramatic increase in soy products is
due largely to the fact that the US FDA approved soy products as an official cholesterol-lowering food, along with other heart and health benefits due to the evidence that soy product intake is correlated with significant decreases in serum cholesterol, low-density lipoprotein (LDL, bad cholesterol) and triglycerides. Although a significant health benefit has been observed in people with a high soy intake (vide supra), some scientists have argued that reported health benefits are poorly supported by the available experimental evidences. It seems to be difficult to support soy product benefit by in vitro studies using individually isolated phytochemicals (a wide variety of compounds produced by plants) or crude products prepared from soy. It is important to note that all phytochemicals isolated from soybeans show an array of weak biological activities, thus, normal consumption of foods that contain these phytochemicals should not provide sufficient amounts to elicit a visible physiological response in humans in short-time clinical researches.

3. Chemical composition of soybeans

Remarkably, seeds of soy contain very high levels of protein, carbohydrate conjugates, fatty acids (soybean oil), amino acids, and inorganic materials (minerals). Among these soybean components, protein and fatty acid content account for about 40% and 20%, respectively. The remaining components consist of carbohydrate conjugates, inorganic constituents, and the minor components of biologically interesting small molecules (molecules highlighted below). Thus, soybeans constitute important nutritional components. Soybeans are considered to be a good substitute of protein (essential amino acids), amongst other major vegetables, for animal products. This chapter reviews secondary metabolites isolated from soy and soy products that show interesting biological activities.

3.1 Isoflavones

Isoflavones (a subgroup of flavonoids) are known to be highly potent antioxidants (Fig.1). As stated above, the consumption of soy products has many health benefits, including protection against breast cancer, prostate cancer, menopausal symptoms, heart disease and osteoporosis. Many of the health benefits of soy are derived from its isoflavones. Isoflavones are produced via a branch of the general phenylpropanoid pathway biosynthesis (begins from phenylalanine) that produces flavonoid compounds in legumes and stored as glucosyl- and malonyl-glucose conjugates (Graham, 1991). The major isoflavones in soybean are genistein, daidzein, and glycitein, comprising about 50%, 40%, and 10% of total isoflavone profiles, respectively. The chemical structure of isoflavones is similar to that of the primary female sex hormone, estrogen. Because of this similarity in structure, they can interfere with the action of estrogen. Thus, isoflavones are often called “phytoestrogens”. The common biological roles of phytoestrogens are to protect plants from stress and to act as part of a plant’s defence mechanism. Some scientists postulate that phytoestrogen may have evolved to protect the plants by interfering with the reproductive ability of grazing animals. The estrogen effects of isoflavones are much less effective than estrogen; its effectiveness represents around 1/1000 of estrogen. Isoflavones can also reduce the effect of the estrogen on cells and skin layers when the hormone levels are high, reducing the risk of estrogen linked cancers. There are two other classes (lignans and coumestans) of phytoestrogens that have estrogen-like actions in the human body. Isoflavones have been reported to show estrogenic, antifungal, anti-tumor and anti-mutagenic properties (Rishi, 2002; Dorge and Sheehan, 2002; Coward et al., 1993; Miyazawa, 1999). Isoflavones remain the subject of many scientific studies, as illustrated by the more than 18,000 scientific publications.
3.1.1 Genistein

Genistein is found in a number of plants including soybeans, lupin, fava beans, kudzu, psoralea, and coffee. Genistein is the most discussed phytoestrogenic substance, because it is very well represented in soybeans. Genistein influences several targets in living cells. Due to its structural similarity to estrogen (i.e. 17β-estradiol, Fig. 2), genistein can bind to estrogen receptors. Genistein shows much higher affinity toward estrogen receptor β (ERβ) than toward estrogen receptor α (ERα).

Recent studies have shown that ERβ is lost in majority of breast tumors and thus ESR2 gene, encoding ERβ, is suggested to be a possible tumor suppressor gene. Similarly, ERβ overexpression in ovarian cancer cells is suggested to exert antitumoral effects. ERβ is highly expressed in prostate cancer cells, and is the predominant estrogen receptor in the colonic epithelium. Thus, effects of estrogen in these cells or tissues are mediated by ERβ. Therefore, non-steroidal ERβ-antagonist has potential to be a clinically useful drug.
In the 1960’s, many researches regarding the physiological effects of genistein were limited to its estrogenic activity. Genistein have also been shown to possess antifungal activities (Weidernbörner, et al. 1989), antiangiogenic effects (blocking formation of new blood vessels), and may block the uncontrolled cell growth associated with cancer, most likely by inhibiting the activity of substances in the body that regulate cell division and cell growth factors. Various studies have found moderate doses of genistein to have inhibitory effects on cancers of the prostate, cervical, brain, breast, and colon. Additionally it has been shown that genistein makes some cells more sensitive to radio-therapy. Genistein has shown a protein tyrosine kinase inhibitory activity. Tyrosine kinases are implicated in almost all cell growth and proliferation signal cascades. Genistatin’s inhibition of DNA topoisomerase II also plays an important role in the cytotoxic activity of genistein.

3.1.2 Daidzein
Daidzein is also present in a number of plants. Soy foods typically contain more genistein than daidzein. Structurally, daidzein lacks the 5-hydroxy group of genistein (Fig. 1). Genistein and daidzein can transfer across the human placenta at environmentally relevant levels and their influence to early puberty in children is unknown. In vitro and in vivo studies have shown that daidzein stimulates the growth of estrogen-sensitive breast cancer cells. Some epidemiological evidence indicates that soy intake may be more protective when the exposure occurs prior to puberty. More research needs to be conducted on the association between breast cancer risk and daidzein specifically before conclusions can be drawn. Daidzein is metabolized in the colon by bacteria to equol and another isoflavones. Daidzein is available as a dietary supplement.

3.1.3 Glycitein
Glycitein is unique in that it is an isoflavone found in soy with a methoxy group. Methylated isoflavones have been shown to be more bioavailable and biologically stable than non-methylated isoflavones. Glycitein accounts for 5-10% of the total isoflavones in soy food products. Glycitein shows a weak estrogenic activity, comparable to that of the other soy isoflavones.

3.1.4 Formononetin
Formononetin is an O-methylated isoflavone. It is found in the family Fabaceae and Ranunculaceae (i.e. clovers, soybeans, and cohosh). Formononetin is known to be converted in the rumen (in sheep and cow) into a potent phytoestrogen, equol. Although, O-demethylase, catalysing O-demethylation, has been attributed to metabolism by gut microflora, incubation of formononetin with human liver microsomes resulted in 4’-O-demethylation to yield daidzein (Tolleson, et al. 2002) (Scheme 1).

3.1.5 Biochanin A
Biochanin A is an O-methylated isoflavone. Biochanin A can be found in red clover, soy, alfalfa sprouts, peanuts, chickpea and other legumes. Biochanin A-containing supplements are derived from red clover and, in addition to biochanin A, usually contain genistein, daidzein and formononetin. In red clover, biochanin A exists as its glycoside (Fig. 3). However, the glycoside undergoes hydrolysis during extraction to form the aglycone (non-sugar component). Biochanin A has weak estrogenic activities as measured in in vivo and in vitro assays. In comparison with other isoflavones, biochanin A is expected to have possible
anti-osteoporotic activity. Structurally, biochanin A would be expected to be able to scavenge reactive oxygen species and inhibit lipid peroxidation. *In vitro* and *in vivo* studies using rodents indicated that biochanin A has anticarcinogenic activity.

Scheme 1. Biotransformation of formononetin, daidzein, and equol from ononin.

### 3.1.6 Equol
Equol has the 3S configuration and is produced by bacterial flora in the intestines as a metabolite of daidzein (Scheme 1). However, only about 30-50% of people have intestinal bacteria that produce equol. Equol is a non-steroidal estrogen that acts as an anti-androgen by blocking the hormone dihydrotestosterone. Equol has the ability to bind to ERβ. This may make equol advantageous in estrogen-related cancers, including breast cancer. Equol is unique because it not only has the ability to bind to ERβ, but also acts as an antagonist to androgen actions. Unlike anti-androgen drugs, equol does not bind to androgen receptors, but it binds directly to dihydrotestosterone. This mode of action has prompted studies to determine if men who are equol-producers may have an advantage against prostate cancer.

### 3.1.7 Isoflavone glycosides
Isoflavones generally exist as aglycones (Fig. 1) and their glycoside forms. Isoflavone glycosides isolated from soybeans are β-glucosidated at C7-position of isoflavone core structure. Soybeans are known to contain daidzein, glycitin, 6’-acetylgenistin, 6’-acetyldaizin (Waltz, 1931; Naim, et al. 1973; Ohta, et al. 1979). Later, malonylglycosides (6’-O-malonyldaidzin, 6’-O-malonylgenistin, 6’-O-malonylglycitin) and succinylglycosides (6’-O-succinylldaidzin, 6’-O-succinylgenistin, 6’-O-succinylglycitin) are found in soybeans or soy products (Wang et al., 1994; Toda, 1999) (Fig. 3). Careful analyses of isoflavones in soybeans revealed that the malonylglycosides are the predominant isoflavones in soybeans. Mass balance of isoflavone glycosides vary depending on manufacturing process of soy products. As describe above, biological effects of aglycones of isoflavone glycosides found in soybeans have been of great interest in food science, food technology, nutrition and dietary supplements, and disease prevention or treatment. Due to the fact that isoflavones in soybeans are conjugated almost exclusively to sugars, thus, understanding of the mechanism of intestinal absorption of isoflavones in humans is an important subject. Evidence from intestinal perfusion and *in vitro* cell culture studies indicates that isoflavone glycosides are poorly absorbed, yet isoflavones are bioavailable and appear in high
concentrations in plasma, irrespective of whether they are ingested as aglycones or glycoside conjugates. Therefore, it was suggested that hydrolysis of the sugar moiety is an essential prerequisite for bioavailability of soy isoflavones (Setchell et al. 2002).

![Fig. 3. Structures of soy isoflavone glycosides](image)

### 3.1.8 Controversial effect of soy isoflavones

Recently, a lot of articles regarding the negative aspects of soy have been published. However, several controversy reports about the adverse effects are always not clear. This may be due to the lack of understanding of metabolism and bioavailability of isoflavones in soy products. Some studies concluded that the bioavailability and pharmacokinetics of isoflavones are significantly influenced by type of soy products. Examples of adverse effects of isoflavones are that genistein 1) increased the rate of proliferation of estrogen-dependent breast cancer *in vitro* when not co-treated with an estrogen antagonist, 2) decreased efficiency of tamoxifen and letrozole, drugs commonly used in breast cancer therapy, and 3) inhibited immune response towards cancer cells due in part to the reduction of thyroid function. In some analyses of current concerns regarding the estrogen-like effects of isoflavones in the breast cancers on the clinical trial data and recent evidence regarding estrogen therapy use in postmenopausal women, Messinia and Wood concluded that there is little clinical evidence to suggest that isoflavones will increase breast cancer risk in healthy women or worsen the prognosis of breast cancer patients. They also pointed out that the clinical trials often involved small numbers of subjects, and there is no evidence that
isoflavone intake increases breast tissue density in pre- or postmenopausal women or increases breast cell proliferation in postmenopausal women with or without a history of breast cancer. The Israeli health ministry has recommended only moderate consumption of soy products because of reported adverse effects of isoflavones (vide supra). On the other hand, the British Dietary Association concluded that evidence suggesting isoflavones reduce the symptoms of menopause is inconsistent. Although more clinical research should be performed to definitively alleviate above concerns, the existing data should provide some degree of assurance that isoflavone exposure at levels consistent with a large amount of soy product intake does not result in adverse effects on breast tissue (Messina et al, 2008).

3.2 Phytic acid
Phytic acid [hexakisphosphate (IP6)] or phytate is present in the brans and hulls of most grains, beans, nuts, and seeds. Rich sources of phytic acid are wheat bran and flaxseed. Phytic acid is inositol hexaphosphate, and thus it is highly charged, which provided chelative (or binding) properties. Phytic acid binds to minerals and metals. Phytate is not digestible to humans or nonruminant animals. The chelated forms of phytic acid with Zn, Ca, and Mg make them impermeable molecules through cell membranes. Phytic acid blocks the body's uptake of essential minerals such as magnesium, calcium, iron and especially zinc. On the other hand, phytic acid is known to be an antioxidant as well as helpful in eradication of heavy metals and other toxic cation species from the body.

![Phytic acid](image_url)

3.3 Soybean lipids
Lipids are broadly defined as hydrophobic or amphiphilic molecules. Lipids include fatty acids, sterols, lipid-soluble vitamins (vitamins A, D, E and K), glycerolipids, phospholipids, glycolipids, and sphingolipids. Soybeans contain 82% of triacylglycerol, 13% of phospholipids, about 1% of sterols, and 4% of unsaturated and saturated fatty acids in a total lipid extracted with chloroform-methanol (2/1). Phospholipid composition in a soybean lipid extract is phosphatidylcholine (42%), phosphatidylethanolamine (30%), phosphatidylinositol and phosphatidylserine (20%), lysophosphatidylcholine (1%), sphingomyelin (0.6%), phosphatidic acid and others, respectively (Takagi et al. 1985).

Lipids remain an important research subject because of associations between consumption of lipids and the incidence of some chronic conditions including coronary artery disease, diabetes, cancer and obesity. Dietary lipids (or fats) serve multiple purposes. The importance of antioxidant ability of unsaturated fatty acids including β-carotene in the prevention of cardiovascular disease as well as many cancers is being increasingly recognised. Although saturated fatty acids are generally considered cholesterolemic, it is now evident that the effect of some fatty acids on blood lipids and lipoproteins suggest that the major dietary fats containing in some food products (i.e. soybeans or palm oils) do not
raise plasma total fatty acids and LDL cholesterol levels. In recent times, adverse health concerns from the consumption of trans fatty acids arising from hydrogenation of oils and fats have been the subject of much discussion and controversy.

Fig. 5. Structures of representative lipids isolated from soybeans

3.3.1 Fatty acids and soybean oil
Soybean oil is rich in polyunsaturated fatty acids, including the two essential fatty acids, linoleic and linolenic, that are not produced in the human body. Linoleic and linolenic acids aid the body’s absorption of vital nutrients and are required for human health. In many applications, the higher saturate oils have been replaced with partially hydrogenated vegetable oils. Partially hydrogenated oils make the oil more stable and more resistant to air oxidation. Saturated fatty acids are more difficult to digest than unsaturated fatty acids and are seldom used for food product industry applications. Nature makes most mono- and polyunsaturated fatty acids in the cis form. However, during the partial hydrogenation process, the cis geometry of unsaturated fatty acids is partially isomerized to the trans form. Numerous research and epidemiological studies have been conducted to determine the impact of trans-fatty acids on cholesterol levels and coronary heart disease. The study by Troisi, et al. suggested a correlation between increased consumption of trans fatty acids and an increase in LDL (bad) cholesterol, which increases lipoprotein level and is
an independent risk factor for the development of coronary heart disease, and decrease in HDL (good) cholesterol. This could represent an increased risk of heart attacks by trans-fatty acid intake.

Fig. 6. Structures of plant sterols from soybeans

### 3.3.2 Soy sterol

Soybeans contain plant sterols, β-sitosterol, β-sitostanol, campesterol, campestanol, brassicasterol, stigmasterol, and Δ5-avenasterol, and cholesterol (Fig. 6). Plant sterols are natural dietary components, and known to have serum cholesterol-lowering properties. The lowering of serum cholesterol by plant sterols is believed to be the result of an inhibition of cholesterol absorption in small intestine. Several studies suggested that unsaturated or saturated plant sterols showed different effects on cholesterol absorption and sterol excretion (Normén et al. 2007).

### 3.3.3 Glycerolipids

Glycerolipids are composed of mono-, di- and tri-substituted glycerols. In these compounds, the three hydroxy groups of glycerol are esterified (triacylglycerols) or one of hydroxy group forms the ether linkage. Subclasses of glycerolipids are represented by glycosyl glycerols and glycerophospholipids. Glycerophospholipids are subdivided into distinct classes which are characterized by the presence of one or more sugar or phosphate residues (i.e. phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, and phosphatidyl serine).

Soybeans contain a wide variety of triacylglycerols, however LC-MS analyses revealed that fatty acids incorporated in soy triacylglycerols are stearic acid, palmitic acid, oleic acid, linoleic acid, and linolenic acid (Neff et al. 1995). Fatty acids in soybeans are considered to be stored as triacylglycerides.

Lecithin can easily be extracted from soybeans (or egg yolk) and soy lecithin is an additive found in many everyday foods (Fig. 5). It has low solubility in water. Due to its amphipathic characteristic, lecithin phospholipids can form either liposomes, bilayer sheets, micelles, or lamellar structures in aqueous solution. In cooking, it is sometimes used as an emulsifier and to prevent sticking (for example, in non-stick cooking spray) and or as a stabilizer in various food applications. Lecithin has been a popular supplement because it’s high choline (N,N,N-trimethyllethanol) content. Choline is an essential nutrient that has benefit for heart health and brain development, as choline deficiency plays a role in liver disease,
atherosclerosis, and possibly neurological disorders. It is particularly important for pregnant women to get enough choline, since low choline intake may raise the rate of neural tube defects in infants, and may affect their child’s memory.

Phosphatidylinositol (PI) is classified as a glycerophospholipid that contains a glycerol backbone, two non-polar fatty acid tails, a phosphate group substituted with an inositol (myo-D-inositol in animals) polar head group. The most common fatty acids of PIs are stearic acid in the SN$_1$ position and arachidonic acid in the SN$_2$ position. Phosphatidylinositols play important roles in lipid signaling, cell signaling and membrane trafficking. The inositol ring can be phosphorylated by a variety of kinases.

3.3.4 Cerebroside

Sphingolipids are structural components of eukaryotic cell membranes. A large number of recent reports have indicated that sphingolipid are involved in a number of important regulatory processes in cell development. Cerebrosides (monoglycosylceramide) is the common name for a group of glycosphingolipids.

Soya-cerebroside (Fig. 5) is a glucosylceramide isolated from soybeans, exhibited a Ca$^{2+}$-binding activity. The basic structure of soya-cerebroside II including the absolute stereochemistries of (2R)-hydroxy fatty acids are identical to one of the neural glucosylceramide. However, the main long-chain base (sphingosine moiety) is C$_{18}$-4,8-diunsaturated (E/Z). Biological functions of the cerebrosides in soybeans have not been thoroughly studied. Recently, a soya-cerebroside was reported to exhibit moderate tyrosinase inhibitory activity, and applied for making skin-care cosmetics for removal of (black) freckles.

3.3.5 Sphingomyelin

Sphingomyelin is a type of sphingolipid found in animal cell membranes, especially in the membranous myelin sheath that surrounds some nerve cell axons. It consists of phosphorylcholine and ceramide (Fig.5). In humans, sphingomyelin represents ~85% of all sphingolipids. On the other hand, only 0.6% of sphingomyelin was found in a total phospholipid isolated from soybeans (Takagi, et al. 1985). The accumulation of sphingomyelin (i.e. Niemann-Pick Disease) in brain causes irreversible neurological damage. Sphingomyelin in food products is not bioavailable, and thus the accumulation of sphingomyelin in human body is not considered possible by sphingomyelin containing food intake.

3.3.6 Vitamin K

Vitamin K is a lipid-soluble essential vitamin that is stable to air but susceptible to air under sunlight. The “K” is derived from the German word “koagulation”. Natural forms of vitamin K, vitamin K$_1$ (phylloquinone) and vitamin K$_2$ (menaquinone), exist in the human liver and other tissues at very low concentrations; vitamin K$_1$ concentrates in the liver while vitamin K$_2$ is well distributed to other tissues (Fig.6). Vitamin K$_1$ is derived from dietary intake and vitamin K$_2$ is produced by intestinal bacteria. Thus, vitamin K is not listed among the essential vitamins. Human get most of our dietary vitamin K in the form of phylloquinone (biosynthesized by plants). In prokaryotes, especially in Gram-positive bacteria, vitamin K$_2$ will transfer two electrons in a process of aerobic or anaerobic respiration (electron transport systems). Respiration occurs in the cell membrane of prokaryotic cells. Electron donors will, with the help of another enzyme, transfer two electrons to vitamin K$_2$. Vitamin K$_2$, with the help of another enzyme, will in turn transfer these two electrons to an electron acceptor.
Fig. 6. Structures of phylloquinone and menaquinone

One tablespoon of soybean oil contains about 25 μg of vitamin K₃ (about 47 μg of vitamin K₃ in 100g of soybeans). Green leafy vegetables and some vegetable oils are major contributors of dietary vitamin K. Nattō, a fermented Japanese soybean product, contains large amounts (approximately 870 μg per 100 grams of nattō) of vitamin K₂. Vitamin K₂ is known to be more effective than vitamin K₁ with respect to osteoclastogenesis, hypocholesterolemic effects, and ability to slow atherosclerotic progression. To date, no adverse effects have been reported for higher levels of vitamin K intake from food and/or supplements, there are no documented toxicity symptoms for vitamin K.

3.3.7 Carotene
In the human diet, carotenoids have been shown to have antioxidant activity which may help to prevent certain kinds of cancers, arthritis and atherosclerosis. β-Carotene is a precursor of vitamin A (retinal) which is biosynthesized via the action of β-carotene 15,15'-monooxygenase. There are nearly 600 carotenoids in nature. In humans, four carotenoids (β-carotene, α-carotene, γ-carotene, and β-cryptoxanthin) have vitamin A activity, and they can be converted to retinal. Mature soybean seeds contain about 1 μg of carotenoids. However, concentrations of carotenoids are increased several fold during germination. Due to versatility of soybeans in use, genetic modification of soybeans is a popular subject. Genetically engineered soybean can now produce β-carotene 1,400-fold over non-transgenic soybeans.

3.3.8 Vitamin E
Vitamin E is a lipid-soluble compound and a family of eight related compounds that includes both tocopherols and tocotrienols. α-Tocopherol is most abundant in foods and also dominates in vitamin E supplements; the other leading types are β-, γ-, and δ-tocopherol. α-Tocopherol has become synonymous with vitamin E, and vitamin E is one of the most popular supplements. Vitamin E has antioxidant activities that stop the production of reactive oxygen species formed when unsaturated fatty acids undergo oxidations. Soybeans and corns contain γ-tocopherol (about 12.0 mg of γ-tocopherol in 100g of total soy lipids) (Fig. 5), while α-tocopherol is found in olive oil. In vitro experiments, γ-tocopherol killed animal cells at high concentrations, but α-tocopherol did not show cytotoxicities at the same concentrations. Interestingly, although people in the United State tend to use corn and soybean oil for cooking, most abundant in the body is α-tocopherol. Other than antioxidant activities, α-tocopherol is reported to inhibit protein kinase C activity, which is involved in cell proliferation and differentiation. Vitamin E inhibits platelet aggregation and enhances vasodilation. Vitamin E enrichment of endothelial cells downregulates the expression of cell adhesion molecules, thus decreasing the adhesion of blood cell components to the endothelium.
3.3.9 Vitamin D₂ (Ergocalciferol)
There are only a few food sources (fish, liver, and egg yolk) of vitamin D. These are many fortified foods (i.e. milk, soy drinks, orange juice and margarine) that contain vitamin D. Ergosterol, called provitamin D₂, is found in ergot, yeast, and other fungi. It is converted to vitamin D₂ (ergocalciferol) upon irradiation by ultraviolet (UV) light or electronic bombardment (Scheme 2), whereas, vitamin D₃ (cholecalciferol) is normally synthesized in the human skin from 7-dehydrocholesterol. Vitamins D₂ and D₃ are about equal in activity in all mammals (some literatures described that vitamin D₃ is slightly less bioactive). Deficiency of vitamin D can result in rickets (a softening of bones) in children and osteomalacia in adults. The relationship between ergosterol content in soybeans or soybean oils and soybean fungi was studied. For an example, in the studies of soybeans inoculated with spores of Aspergillus ruber, ergosterol concentrations in seeds increased with time of storage (Dhingra, et al. 1998).

Fig. 7. Representative structures of soy phytoalexins
3.4 Soy phytoalexins

Phytoalexins are antimicrobial substances synthesized by plants that accumulate rapidly at areas of pathogen infection. They are, in general, broad spectrum inhibitors and structurally diverse molecules have been isolated from different plant species. Phytoalexins are known to inhibit bacterial or fungus cell wall biosynthesis, or delay maturation, or disrupt metabolism. To date, several soy phytoalexins have been reported. 6a-Hydroxyphaseollin was the first structurally defined phytoalexin isolated from fungal infected soybeans. Several other hydroxypterocarpan (benzopyrano-furanobenzenes) derivatives are biosynthesized as phytoalexins by soybean tissues on treatment with a variety of biotic or abiotic agents. Glyceollins are a family of pterocarpan found in the Fabaceae family including activated soy. They are biosynthesized from the isoflavone, daidzein (Fig. 1) via 2'-hydroxyisoflavone reductase as illustrated in Scheme 3. The concentration of phytoalexins in soybeans is very low since the compounds are only produced by soy as a defence mechanism from disease or infection. For an example, the accumulation of glyceollins in soybean cotyledon tissue was observed using four species of Aspergillus; 955 µg/g of glyceollins could be isolated from soybean cotyledon tissue inoculated with A. sojae. Representative phytoalexins identified from fungal infected soybeans are summarized in Fig. 7. Besides their antifungal or antibacterial activities, glyceollins have recently been demonstrated to be novel antiestrogens that bind to the estrogen receptor (ER) and inhibit estrogen-induced tumor progression (Zimmermann et al. 2010). Therefore, glyceollins may represent an important component by a phytoalexin-enriched food diet in terms of chemoprevention as well as a novel therapeutic agent for hormone-dependent tumors.

Scheme 3. Biosynthesis of glyceollins

3.5 Coumestrol

Coumestrol is classically categorized as phytoestrogens because this molecule binds to the estrogen receptor (ER). Coumestrol is originally isolated from alfalfa (Bikoff et al. 1957), but later soybeans and clover contain the highest concentrations of this molecule. Biosynthesis
of coumestrol in soy is proposed based on the feeding experiments of labeled precursors to a coumestrol producing bacteria (Berlin et al. 1972). As illustrated in Scheme 4, the isoflavone, daidzein is reduced to form 2’-hydroxy-2,3-dihydrodaidzein which undergoes intramolecular condensation to yield 3,9-dihydroxypterocarp-6a-en. Biological oxidation of the dihydroxypterocarpen furnishes coumestrol.

Scheme 4. Biosynthesis of coumestrol

Coumestrol has less estrogen activities than estrogen and therefore may reduce the risk of developing breast or prostate cancer in humans by preventing estradiol binding to estrogen receptor (ER). Coumestrol was reported to inhibit the enzymes involved in the biosynthesis of steroid hormone (aromatase and hydroxysteroid dehydrogenase), and inhibition of these enzymes results in the modulation of hormone production.

Fig. 8. Representative structures of soyasaponins

3.6 Soyasaponins
Saponins are amphipathic glycosides grouped phenomenologically by the formation of soap-like froth when shaken in aqueous solutions. Structurally, saponins contain one or more glycoside moieties combined with a lipophilic triterpene derivative. Many health
benefits of soybeans are believed to be attributed to their Saponins. Many soy products contain high levels of saponins. Raw soybeans contain between 2 and 5 g saponins per 100 g. Soy saponins are divided in two groups; group A saponins have and undesirable astringent taste, and group B saponins have the health promoting properties. The blood cholesterol-lowering properties of dietary saponins are of particular interest in human nutrition. Saponins bind cholesterol and bile acids in the gut. Cancer cells have more cholesterol-type compounds in their membranes than normal cells. Soy saponins can bind cholesterol in vitro, and thus interfere with cell growth and division. Soy saponins also showed antifungal activities probably due to interference with cholesterol in fungus. Saponins cannot permeate the intestinal wall, but showed effectiveness in binding to cholesterol and making it unavailable for re-absorption within the small and large intestine. Apart from their important role in binding ability to cholesterol, soy saponins showed adjuvant effects for vaccines. An in vitro study demonstrated that soy saponins exhibited potent antiviral effects on the HIV virus. To date, over 26 soyasaponins have been isolated from soybeans and their gross structures were determined via high-magnetic field NMR or X-ray crystallography. Representative soyasaponins are illustrated in Fig. 8.

3.7 Lectins and hemagglutinins

Lectins are plant derived proteins which are capable of binding to carbohydrate moieties of complex glycoconjugates but do not possess immunoglobulin nature. They typically agglutinate certain animal cells and/or precipitate glycoconjugates. Many members of the lectin family agglutinate red blood cells. This particular nature of lectin proteins is classified into hemagglutinin. Lectins are stable proteins that do not degrade easily. For example, some lectins are resistant to stomach acid and digestive enzymes. Unfermented soy products contain high levels of lectins/hemagglutinins. Hemagglutinin renders red blood cells unable to absorb oxygen. However, the soybean fermentation process deactivates soya hemagglutinins, and thus the amounts of lectins present in soybeans have not been considered to be as potentially toxic components. On the other hand, some dried bean products may still contain a large amount of active lectins. These lectins are believed to trigger allergic reactions or toxic reactions in a person’s body. Person’s lectin sensitivity is largely due to 1) genetics, 2) a failure of mucosal immunity (secretory IgA), and 3) bacterial or viral infections that damage human cells, making human body susceptible to lectin antibody/antigen reactions.

![Fig. 9. Water soluble vitamins isolated from soy](www.intechopen.com)
3.8 Soy toxins
Soy contains several naturally occurring compounds that are toxic to humans and animals. The best known of the soy toxins is the trypsin (a serine protease found in the digestive system) inhibitors. In vivo studies using rat, high levels of exposure to trypsin inhibitors isolated from raw soy flour cause pancreatic cancer whereas moderate levels cause the rat pancreas to be more susceptible to cancer-causing agents. However, the US FDA concluded that low levels of soy protease inhibitors pose no threat to human health. Recently, a metalloprotein possessing toxicity to mice (LD_{50} 7-8 mg/kg mouse upon intraperitoneal injection) was identified. This protein has a size of 21 kDa and was named soyatoxin. Some other biological properties of soyatoxin include hemagglutination and trypsin inhibitory activity.

3.9 Water-soluble vitamins
Soybeans are not considered to be very rich sources of any particular vitamin, but they contain a wide variety of vitamins and do contribute to an overall nutritional well-being. Lipid-soluble vitamins (vitamin K, E, D and carotene) in soybeans are discussed above. The water-soluble vitamins in soybeans are thiamine, riboflavin, niacin, pantothenic acid, biotin, folic acid, inositol, choline, and vitamin C. Vitamin B_{6} was also reported to contain in soybeans. Thus, soy includes essential vitamins except for vitamin B_{12} and E. However, soy contains a vitamin E precursor, carotene. Numerous studies have been conducted over the past decades on the relative distribution and concentrations of these vitamins in different portion of soy. The cotyledons contain notably greater amount of all water soluble soy vitamins than those in the hypocotyl. The quantity of all vitamins except thiamine increases through germination (Wai et al., 1947).

4. Conclusion
This chapter summarizes structures of biological active molecules isolated from soy, and their biological activities are briefly reviewed. Since the discovery of soybeans as rich source of protein and oil in 1904, a numerous number of experimental data have been accumulated on chemistry and biochemistry of phytochemicals isolated from soy and soy products. To date, a wide variety of organic compounds have been characterized from soy. The interest of structure elucidation studies of bioactive molecules in soy was to obtain insight into correlation between the reported health benefits, which are associated with soy intake, and soy phytochemicals. Thus, many structural studies on soy have not aimed to discover novel molecules, albeit these efforts resulted in discovery of complex soyasaponins (Fig. 8.). The consumption of soy products has many health benefits, including protection against breast cancer, prostate cancer, menopausal symptoms, heart disease and osteoporosis. These health benefits of soy are believed to be due to part in phytoestrogenic activity of isoflavones, which are stored as glucosyl- and malonyl-glucose conjugates in soybeans. Isoflavon-glucosyl conjugates show very poor oral bioavailability. Recently, the negative aspects of soy have been reported (3.1.8). However, the controversy reports about the adverse effects of soy are not clear. This may be due to the lack of understanding of metabolism and bioavailability of isoflavone-glucosyl conjugates in soy. Some researchers concluded that there is little clinical evidence to suggest that isoflavones will cause the adverse effects (e.g. breast cancer risk in healthy women). It is important to note that all phytochemicals isolated from soy show an array of weak biological activities, and thus, normal consumption of...
foods that contain these phytochemicals should not provide sufficient amounts to elicit a visible physiological response in humans in short-time clinical researches. Unfermented soy products contain high levels of lectins/hemagglutinins, and very low level of soy toxins (protease inhibitors). Most of these proteins will be deactivated through food processing. Remarkably, seeds of soy contain very high levels of protein, carbohydrate conjugates, oil, and minerals. On the other hand, remaining components discussed in this chapter can be isolated minute quantities from soybeans, however, a wide range of health benefit of soy phytochemicals (lipids, phytoestrogens, soy sterols, vitamins, soyasaponins) contributes to the overall nutritional well-being of humans.

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