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10

Minor Constituents and Phytochemicals of Soybeans

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Soybeans are recognized as a storehouse of nutrients. The focus of this chapter is on composition of minor compounds or phytochemicals (Table 10.1), while the major components of the seeds (i.e., proteins and oil) are discussed in other chapters of the book.

Lipid Components

Tocopherols

Tocopherols are important antioxidants present in relatively high concentration in soybeans. The content of tocopherols in raw soybean is shown in Table 10.1 while the compositional comparison of tocopherols in crude soybean oil and wheat germ oil is shown in Table 10.2.

Vitamin E is a mixture of four different forms of tocopherols (Fig. 10.1) and four different forms of tocotrienols (having three double bonds on the side chain), with α-tocopherol being the most effective form of Vitamin E. Soybean only contains tocopherols. Vitamin E protects against the oxidation of polyunsaturated fatty acids in biological membranes and in plasma lipoproteins. The antioxidation mechanism is the termination of the free radical autooxidation of lipids by the reaction of the phenolic ring with the free radical, forming a stable phenoxyl radical. Some tocotrienols may have greater antioxidant activity than their counterpart tocopherols in certain model systems (Serbinova et al., 1991). A good review by White and Xing (1997) describes various investigations on comparisons of relative effectiveness of various forms of tocopherols.

Phytosterols

Phytosterols are sterols in plants, and they are structurally similar to sterols from animal sources, as illustrated in Fig. 10.2. Phytosterols are present at about 300–600 ppm concentrations based on the dry weight of the soybean. The primary soybean

Table 10.1. General Composition (dry seed weight basis) of Soybean Minor Constituents[1]

Constituent	Range	Typical Value	Reference
Lipids			
Tocopherols, ppm			
α-tocopherol	11–28		Guzman & Murphy, 1986
γ-tocopherol	150–90		
δ-tocopherol	25–73		
Phytosterols, ppm	300–600		Rao & Janezic, 1992
Phospholipids, %	0.3–0.6	0.74	Wang et al., 1997
Sphingolipids, ppm	193		Guiterrez & Wang, 2004a
Carotenoids, ppm	0.8–3.7		Kanamaru et al., 2006
Proteins			
Trypsin inhibitors, mg/g	16.7–27.2	22.3	Anderson & Wolf, 1995
Lectin (hemagglutinin) unit/mg protein	1.2–6.0	3.0	Padgette et al., 1996
Lunasin, % in defatted flour	0.33–0.95	0.65	De Mejia et al., 2004
Carbohydrates, %	26–38	34	Liu et al., 1995
Sucrose	2.5–8.2	5.5	Hymowitz et al., 1972
Raffinose	0.1–0.9	0.9	
Stachyose	1.4–4.1	3.5	
Other phytochemicals			
Isoflavones, %	0.1–0.4	2.5	Wang & Murphy, 1994
Saponins, % base on protein	0.1–0.3		Arditi et al., 2000
Phytate, %	1.0–1.5	1.1	Lolas et al., 1976
Water-soluble vitamins, ppm			
Thiamine	6.3–6.9		Fernando & Murphy, 1990
Riboflavin	0.9–1.1		

[1] Adopted from Liu, 2004a.

phytosterols are β-sitosterol, campesterol, and stigmasterol, and their compositions are shown in Table 10.3.

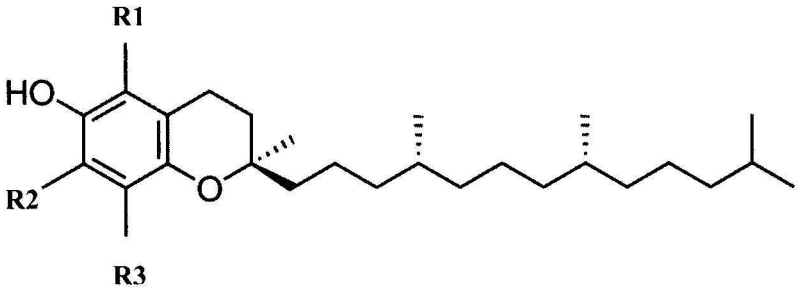
Both phytosterols and tocopherols are co-extracted with oil and partially removed during soybean oil refining. The refining by-product is one of the important sources for commercial phytosterol and tocopherol production.

Health benefits of phytosterols have been a topic of intense research in recent years. The main physiological effect of consuming phytosterols (2–3 g/day for 21–30 days) is their reported lowering of low density lipoprotein (LDL) cholesterol by 10–15%. The Food and Drug Administration (FDA) allows a health claim for food containing phytosterols because of the association with reduced risk of coronary heart disease (Federal Register, 2000).

Table 10.2. Tocopherol Content and Composition of Crude Soybean and Wheat Germ Oils[1]

Tocopherol	Mechanically Pressed Soybean Oil	Solvent-extracted Soybean Oil	Solvent-extracted Wheat Germ Oil
Total tocopherol, ppm	1257	1370	2682
α-Tocopherol, %	9.3	10.5	67.8
β-Tocopherol, %	1.2	1.2	32.2
γ-Tocopherol, %	62.8	63.5	-
δ-Tocopherol, %	26.7	25.0	-

[1]Wang, 2002.



Name	R1	R2	R3
α-tocopherol	$-CH_3$	$-CH_3$	$-CH_3$
β-tocopherol	$-CH_3$	-H	$-CH_3$
γ-tocopherol	-H	$-CH_3$	$-CH_3$
δ-tocopherol	-H	-H	$-CH_3$

Fig. 10.1. Molecular structure of tocopherols present in soybean.

Some phytosterols also are shown to have antioxidant activities. The mechanism of antioxidation is different from the traditional phenolic compounds. They seem to be effective in preventing polymerization reaction in heated oils (Tian & White, 1994), and this effect is due to the structure on the side chain of some specific phytosterols.

Phospholipids (PLs)

Phospholipids are polar membrane lipids that are present in relatively high concentration (about 3% of total lipids) in soybeans, compared to their levels in other oilseeds

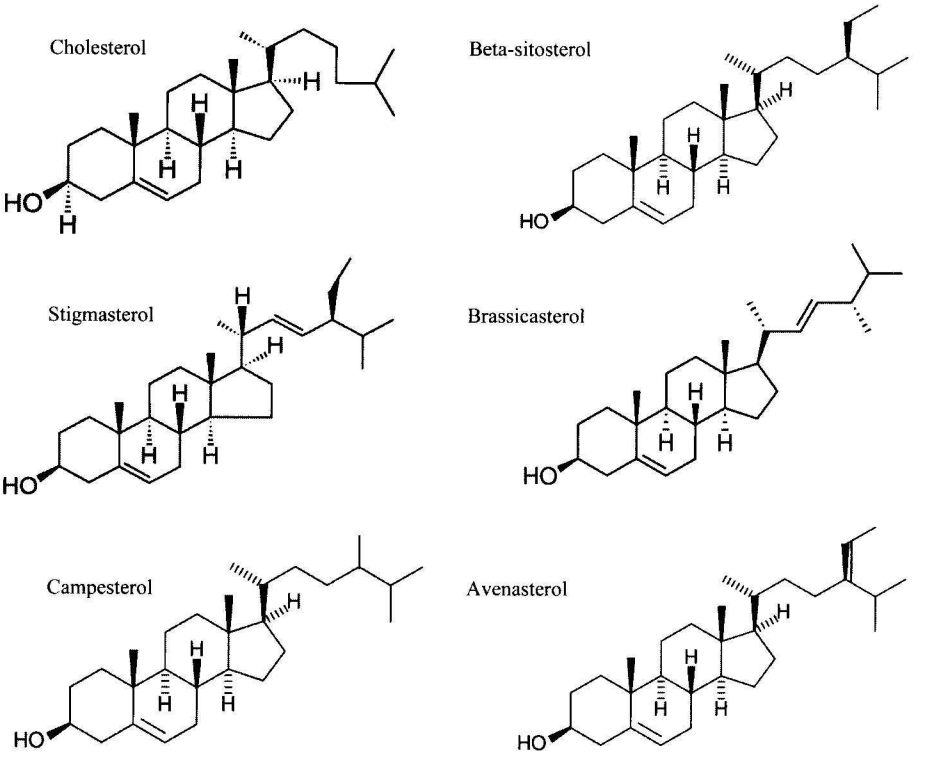


Fig. 10.2. Structure of cholesterol and soybean phytosterols.

Table 10.3. Phytosterol Content (mg/100 g) of Soybean Oils[1]

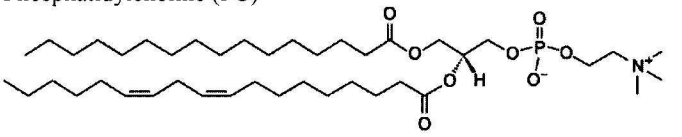
Study	Sterol	Crude	Refined
Study 1			
	β-Sitosterol	183	123
	Campesterol	68	47
	Stigmasterol	64	47
	Δ^5-Avenasterol	5	1
	Δ^7-Stigmasterol	5	1
	Δ^7-Avenasterol	2	<0.5
	Total	327	221
Study 2			
	β-Sitosterol	125–236	
	Campesterol	62–131	
	Stigmasterol	47–77	
	Total	235–405	

[1] Wang, 2002.

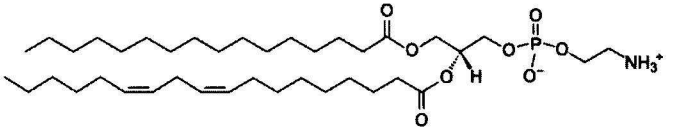
(Wang et al., 1997). On a dry seed-weight basis, soybean contains about 0.74% total PLs (Wang et al., 1997). The three major classes of soybean PLs are phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI), present in relative proportions of 55.3, 26.3 and 18.4%, respectively (Wang et al., 1997). The molecular structures of soybean PLs are shown in Fig. 10.3.

Class composition, fatty acyl stereospecific distribution, and molecular species composition of PLs in normal soybeans and seeds with modified fatty acid composition were studied by Wang et al. (1997) and Wang and Hammond (1999). The PI

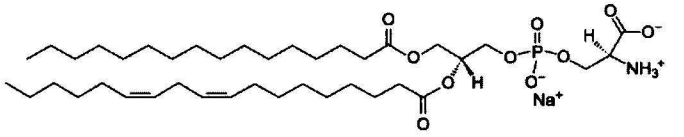
Phosphatidylcholine (PC)



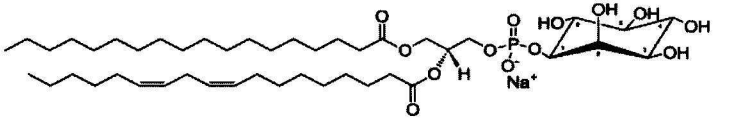
Phosphatidylethanolamine (PE)



Phosphatidylserine (PS)



Phosphatidylinositol (PI)



Phosphatidic acid (PA), sodium salt

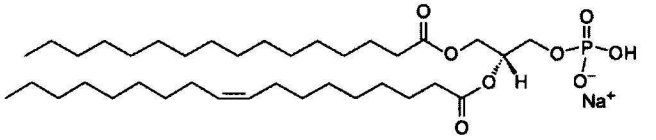


Fig. 10.3. Molecular structure of soybean major (PC, PE, and PI) and minor (PS and PA) phospholipids.

had greater palmitate (~25%) and stearate (~10%) than did PC (~12 and 5%) and PE (~17 and 3%), and PC had the lowest palmitate percentage, whereas PE had the lowest stearate percentage. Stereospecific analysis indicated that saturated fatty acids were concentrated on the *sn*-1 position, and the unsaturated fatty acids preferred the *sn*-2 position on the glycerol backbone.

The mixture of PLs in their commercial form (not highly pure) and applications is referred to as lecithin. Lecithin is an effective antioxidant. The antioxidant property of crude soybean lecithin was studied in various storage tests with sunflower oil and lard (Nasner, 1985). The addition of lecithin after refining improved oxidative stability, and the antioxidant activity depended on the composition of the PLs, and the tocopherol content of the oil. The synergistic effect of tocopherol, ascorbate, and lecithin was clearly shown in fish oil oxidation reduction (Hamilton et al., 1998; Segawa et al., 1995). A better understanding of the effective concentration level (i.e., dose–response relationship) effect of PL polar head group, fatty acid composition of the base oil is still needed. Some studies were performed under very high temperature, such as 100°C (Nwosu et al., 1997) and 180°C (King et al., 1992), where the oxidation mechanism is different than at lower temperatures. The effective concentration of PLs could be as low as 100 ppm in salmon oil, and even at 1% concentration, there was no prooxidant effect observed (King et al., 1992). However, at 0.5% concentration, PLs did not have antioxidant activity in menhaden oil (Nwosu et al., 1997). The need for tocopherols for PLs' antioxidant effect was also shown by Kashima et al. (1991) in a perilla oil model system, but the best synergism conditions for various oil types still need to be determined.

Lecithin's nutritional properties were reviewed by Orthoefer and List (2006). Lecithin is a good source of choline, an essential nutrient that acts as a precursor in the synthesis of the neurotransmitter acetylcholine. Choline is a major source of methyl groups that are involved in the formation of methionine from homocysteine, a sulfur-containing amino acid implicated in cardiovascular disease risk. High homocysteine levels increase the risk of cardiovascular disease, and medical therapy with choline can reduce homocysteine levels (Da Costa et al., 2005; Innis et al., 2007; Zeisel, 2005). Lecithin lowers serum cholesterol levels, and it is a component of lipoproteins, which transport fat and cholesterol (Jimenez et al., 1990). Choline prevents fat accumulation in the liver, and choline deficiency disturbs lecithin synthesis that is needed to export triacylglycerols from the liver as part of lipoproteins. Choline-deficient diets promote liver carcinogenesis because of the disturbance of the protein kinase C (PKC) transmembrane signaling system (Orthoefer & List, 2006). Lecithin and choline are also essential in brain and mental development in fetus and infant. Choline in mother's milk is at a much higher level than that in the maternal bloodstream (Zeisel, 2005), and it is a required nutrient during pregnancy and lactation (Zeisel, 1998). Many vital organs, such as the central nervous system, kidney, and liver, contain high levels of PLs. In several animal and human studies, lecithin

was shown to improve memory and learning. For example, when aged male rats were given lecithin, they showed markedly higher spatial memory as demonstrated in a water maze than untreated rats, and the acetylcholine content in the brains of the treated rats was higher than in those of the control (Masuda et al., 1992). Lecithin supplementation (0.2 g lecithin/kg body mass) prevents the rapid decrease of choline during long-duration intense exercise and improves physical performance (von Allworden et al., 1993).

Sphingolipids (SLs)

SLs are also polar cell membrane lipids, but they are typically present in much lower concentration than PLs. Soybeans are a relatively rich source of SLs (Vesper et al., 1999), and ceramides and cerebrosides are the primary SL classes in soybeans. SLs contain a sphingoid long-chain (C18) dihydroxy base and an α-hydroxy fatty acyl chain that is linked to the base by an amide bond. The main soybean ceramide molecular species is a trihydroxy base (4-hydroxy-trans 8-sphingenine) *N*-acylated with α-hydroxy lignoceric acid (C24:0). The main soybean cerebroside molecular species is a dihydroxy base (*trans* 4-*trans* 8-sphingediene) *N*-acylated with α-hydroxy palmitic acid. The general molecular structures of ceramide and cerebroside are shown in Fig. 10.4.

Significant differences exist in cerebroside concentrations among soybean genotypes, with a range of 142 to 389 nmol/g seed (dry weight basis, equivalent to 102 to 286 ppm) (Table 10.4). The changes in PLs and SLs with seed development are presented in the following section of this chapter.

SLs are highly bioactive in animal cells. They can act as mediators of cell growth, differentiation, and programmed cell death (apoptosis). In vitro studies showed that ceramide and sphingosine were toxic for a variety of transformed cell lines and even inhibited cell transformation during the early events of carcinogenesis (Merill & Schmelz, 2001).

SLs reduced the risk of colon cancer as shown by Dillehay et al. (1994). The occurrence of aberrant colonic crypt foci (ACF, the early biomarker of tumor development) of mice fed sphingomyelin at 0.05% of their diets was reduced by 50% in comparison to the control group. In a longer term study, Schmelz et al. (1996) showed ACF were reduced in the mice fed sphingomyelin (0.1% of diet) by up to 70%. Although the incidence of colonic tumors was not reduced in the sphingomyelin-fed mice, the proportion of benign adenomas versus adenocarcinomas was higher in the sphingomyelin-fed mice than in the control group. It was suggested that sphingomyelin may prevent adenomas from progressing into adenocarcinomas. SLs, such as glucosylceramide and lactosylceramide, also were shown to reduce ACF by 50–80%, indicating that SLs suppress colon carcinogenesis through the release of their metabolites by hydrolysis (Schmelz, 2000).

SLs reduced hepatic cholesterol content in a short-term rat feeding study (Imai-

Table 10.4. Mean Composition and Cerebroside (GlcCer) Content of 10 Soybean Genotypes Grown Near Ames, IA (Guiterrez et al., 2004a)[1]

Genotype and Selectively Modified Trait(s)	GlcCer (nmol/g dry wt basis)
IA1008, Conventional	142
IA2021, low protein (36%)	283
IA2041, high protein (41%)	201
A00-815004, high palmitate (41%)	389
A97-877006, mid palmitate (27%)	221
FA22, high oleate (52%)	306
B0147B013, low palmitate (3.4%)	168
AX7019-12, mid palmitate (21%)/stearate (24%)	246
A97-552013, low linolenate (1.3%)	229
A99-144085, high stearate (28%)	197
MSD	122

[1]MSD = minimum significant difference determined by Tukey Kramer's mean comparison test ($P = 0.05$).

zumi et al, 1997) by decreasing cholesterol absorption and/or increasing fecal excretion. In a long-term feeding study of two generations, rats fed SLs at 1% of their diet had their total plasma cholesterol reduced by 30% (Kobayashi et al., 1997). Dietary SLs may influence plasma and liver lipid levels in humans, but more research is needed to better understand the mechanism of cholesterol reduction.

SLs offer protection against pathogenic microorganisms (bacteria and viruses) and toxins. Synthetic SLs were successfully used to prevent bacterial and viral infections by binding to pathogens and removing them from the intestine (Vesper et al., 1999). The primary compound in human milk that protects against pathogens is assumed to be glycosphingolipids (Vesper et al., 1999). SLs also reduced skin carcinoma development (Birt et al., 1998).

Carotenoids (pro-vitamin A)

Carotenoids are present in soybeans in a very low concentration (0.8–3.7 ppm), and the main forms are lutein and β-carotene. They are co-extracted with oil but are often removed or degraded by oil refining steps designed to remove the undesirable minor components that contribute to physical and chemical instability and undesirable color, such as degumming to remove PLs, neutralization to remove free fatty acids, bleaching to decompose lipid hydroperoxides, and deodorization to remove volatile oxidation products.

Lutein is the major carotenoid in common soybeans with a yellow seed coat, whereas soybeans with a green seed coat contain xanthophylls in addition to lutein.

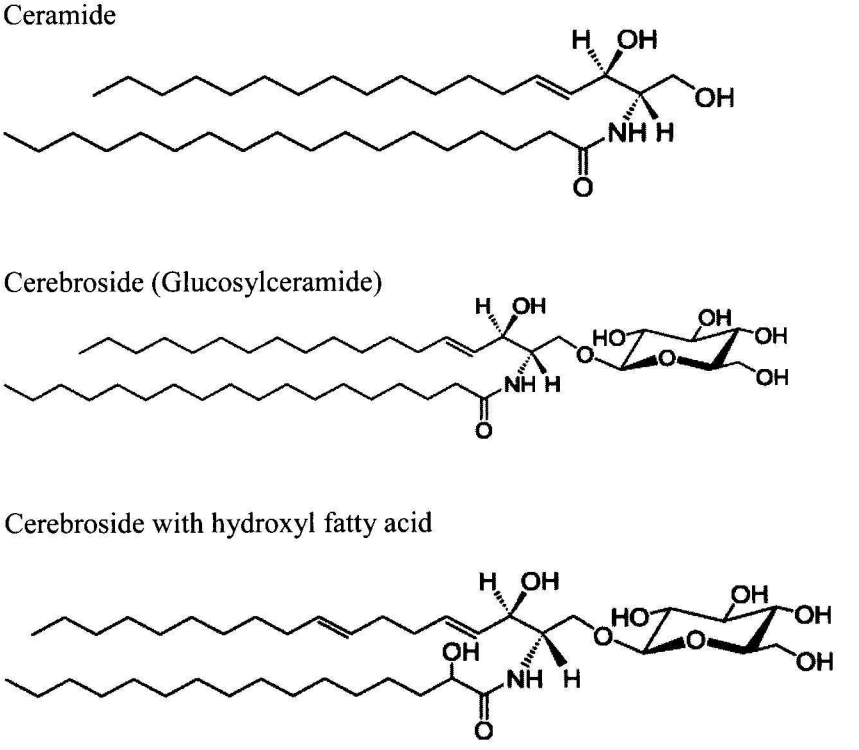


Fig. 10.4. General molecular structure of ceramide and cerebroside (glucosylceramide).

Carotenoid content in immature soybean was affected by genotype, with mean lutein contents ranging from 8.9 to 21.2 ppm and β-carotene from 2.9 to 4.9 ppm based on dried weight (Simonne et al., 2000). The amount of β-carotene decreased more rapidly than that of lutein and chlorophylls during seeds maturation. Mature soybean seeds contained little β-carotene. In commercial mature soybeans, lutein content range was 0.8–3.7 ppm in seed, and no detectable β-carotene was present (Kanamaru et al., 2006).

Protein Components

Trypsin Inhibitors (TI)

TIs are protease-inhibiting factors that bind the protease enzymes to decrease their catalytic power. The two predominant protease inhibitors are Kunitz trypsin inhibitor and Bowman-Birk inhibitor (BBI), and they are both protein in nature.

Kunitz trypsin inhibitor has a molecular weight between 20 and 25 kDa. It consists of 181 amino acid residues and two disulfide bonds (Liu, 1999a). BBI is a pro-

tein with a molecular weight of 8 kDa, and with a single polypeptide chain of 71 amino acids with seven disulfide bonds. BBI is well-characterized for its ability to inhibit trypsin and chymotrypsin.

These protease inhibitors, if not inactivated, are responsible for growth depression for both animals and human by reducing the digestibility of dietary proteins and causing pancreatic hypertrophy (Chernick et al., 1948). They can be denatured, thus deactivated, by proper heating of the proteins or the seeds, such as with live steam, boiling in water, dry roasting, microwave radiation, and extrusion cooking. Chemicals, such as the reducing agents, cysteine, glutathione, and sodium sulfite, can inactivate TIs at relatively low temperatures (Liener, 1994). For example, treatment of raw soy flour at 75°C with 0.03M sodium sulfite for one hour can completely inactivate TIs, leaving no disulfide bonds in the protein. Feeding trials showed the mild treatment is more advantageous than heating treatment regarding nutritional improvement (Liu, 1999a).

The physiological roles of protease inhibitor are controversial, because medical research shows they have anticarcinogenic activities, and they are effective at extremely low concentrations. The soybean-derived BBI was particularly effective in suppressing carcinogenesis (Kennedy, 1998). Purified BBI and BBIC (BBI concentrate) have comparable suppressive effects on the carcinogenic process in a variety of in vivo and in vitro systems, and BBI appeared to be a universal cancer preventive agent (Kennedy, 1998). Purified BBI and BBIC suppressed carcinogenesis in three different animal species (mice, rats, and hamsters), and in organ systems, tissues, and cells of various types (Kennedy, 1998). They had no observed in vivo toxicity. BBIC now has the Investigational New Drug status from the FDA (in April 1992, IND no. 34671; sponsor, A.R. Kennedy).

Soybean BBI showed a significant and dose-dependent growth decrease of human colorectal adenocarcinoma HT29 cells in vitro (Lemente et al., 2005). The mechanism by which BBI suppresses carcinogenesis was studied by Chen et al. (2005). BBI specifically and potently inhibits the proteasomal chymotrypsin-like activity in vitro and in vivo in MCF7 breast cancer cells, leading to accumulation of ubiquitinated proteins. In addition, BBI suppressed cell growth and decreased the activities of phosphorylated extracellular signal-related kinases. Chen's results support a new mechanism of proteasome inhibition by BBI that prevents cancer development. For the first time, soybean TIs induced human leukemia Jurkat cell death, as measured by flow cytometry (Troncoso et al., 2007).

The effect of purified soybean Kunitz and BBI trypsin inhibitors as dietary supplements (5, 15, or 50 g/kg) on spontaneous pulmonary metastasis of lung carcinoma 3LL cells as well as human ovarian cancer HRA cells was investigated in mice (Kobayashi et al., 2004a). Only Kunitz inhibitor inhibited the formation of lung metastasis in a dose-dependent manner. These results suggest that dietary supplementation of Kunitz inhibitor could more efficiently regulate cell metastatic processes than BBI,

and Kunitz inhibitor may also be beneficial for ovarian cancer patients by inhibiting phosphorylation of kinases. Kunitz but not BBI suppressed ovarian cancer cell invasion by blocking urokinase upregulation (Kobayashi et al., 2004b). Inagaki et al. (2005) also showed that Soybean Kunitz inhibitor inhibited signaling pathways in ovarian cancer cell growth.

Lectins

The occurrence of cell-agglutinating and sugar-specific proteins has been known for a long time. Very few lectins had been isolated, and they had attracted little attention until the early 1970s, when it was demonstrated that lectins were extremely useful tools for the study of cell malignant changes. More information is contained in two excellent historical reviews on lectins (Sharon & Lis, 2004; Sharon, 2007).

Lectins, also known as hemagglutinins, are proteins in nature and are composed of a 120 kDa tetrameric glycoprotein, possessing a single oligomannose chain per monomer. Lectins have a strong ability to agglutinate the red blood cells and intestinal mucosa cells by their strong affinity for cell surface carbohydrates. The destruction of the intestinal cell organization has a significant impact on nutrient absorption and utilization; therefore, they are considered as anti-nutritional factors. These proteins can be denatured by moist heat, as can TIs; thus, properly processed soy protein should have a low level of this anti-nutritional factor. However, lectins were reported to be resistant to digestion if not heat-denatured, could survive gut passage but bind to gastrointestinal cells and enter the circulation intact with full biological activity (De Mejia and Prisecaru, 2005). They are useful as cancer therapeutic agents because of their binding to cancer cell membranes, causing cytotoxicity, apoptosis, and inhibition of tumor growth.

Lectins can be mitogenic, non-mitogenic (potato lectin), and anti-mitogenic (tomato lectin). Mitogenic stimulation or mitogenecity is the ability to induce division in a mature quiescent cell that does not normally divide. Prolonged mitogenic activity results in cell proliferation as in cancer. The concentration of lectin seems to be one of the factors that determines proliferation (low concentration) or inhibition (high concentration). A recent review of the literature data concerning the biological activity of plant lectins is given by Abdullaev and de Mejia (1997), where a discussion on toxic, cytotoxic, antitumor, and anticarcinogenic properties of lectins is presented. A brief description of the biological properties of plant lectins, as well as the effect of plant lectins on normal and malignant cells and the antitumor properties of these lectins in vivo and in vitro, are included. These findings are interpreted, and possible mechanisms of the antitumor effect of plant lectins are discussed.

The study of effect of soybean lectin on intestinal morphology and lymphoid organ weight of poultry-fed diets containing soy lectin indicates that lectin up to 0.048% enhanced intestinal development by increasing villus crypt, but might alter the structural integrity of lymphoid organs (Fasina et al., 2006).

Lectins also affect the immune system by altering the production of various interleukins or by activating certain protein kinases. Lectins can bind to ribosomes and inhibit protein synthesis; can modify the cell cycle by inducing non-apoptotic mechanisms, cell cycle arrest and apoptosis; can activate the caspase cascade; and can also downregulate telomerase activity and inhibit angiogenesis (De Mejia & Prisecaru, 2005). The effect of purified soybean lectin on growth and immune function in rats fed diets containing 0, 0.05, 0.10, 0.15, or 0.20% lectin showed growth decline, and decline of the concentrations of interleukin-2, interferon-γ and tumor necrosis factor-α in plasma, spleen, and mesenteric lymph nodes, as well as plasma concentrations of IgA, IgG, and IgM; therefore, dietary soybean lectin has a negative effect on growth and immune function of rats. Although lectins seem to have great potential as anticancer agents, further research is still needed and should include a genomic and proteomic approach.

Lunasin

Lunasin, a naturally occurring peptide in soybeans classified as a 2*S* albumin, has 43 amino acids with a molecular weight of 4.7 kDa and contains nine Asp residues at its carboxyl end, an Arg-Gly-Asp cell adhesion motif, and a predicted helix with structural homology to a conserved region of chromatin-binding proteins.

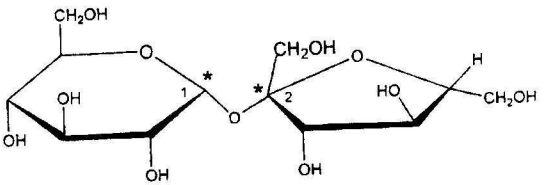
The reduction in cancer risk resulting from soy protein consumption is attributed to the Bowman-Birk protease inhibitor and isoflavones, as well as naturally occurring lunasin peptide, which is cancer preventive (Jeong et al., 2007). Exogenous application of the lunasin peptide inhibited chemical carcinogen-induced transformation of fibroblast cells to cancerous foci in vitro by binding to the deacetylated histones and inhibiting its acetylation. In a mouse skin cancer model, dermal application of lunasin (250 μg/wk) decreased skin tumor incidence by approximately 70%, and delayed the appearance of tumors by 2 weeks relative to a control (Galvez et al., 2001). The results suggest lunasin can be a new chemopreventive agent that functions via a chromatin modification mechanism (Galvez et al., 2001). Lunasin was also shown to upregulate the genes involved in the control of tumor suppression, cell division, DNA repair, and cell death as measured with gene microarray analysis (Magbanua et al., 2004).

The anticancer potential of lunasin and soy hydrolyzates studied on leukemia cells (Wang & de Mejia, 2007) showed that lunasin-enriched soy flour caused cytotoxicity of leukemia cells. Simulated gastrointestinal hydrolysis of soy protein increased topoisomerase inhibitory activities and cytotoxicity. Such hydrolysates contain hydrophilic, small bioactive peptides (<3 kDa), and three novel topoisomerase inhibitory soy peptides that can be isolated.

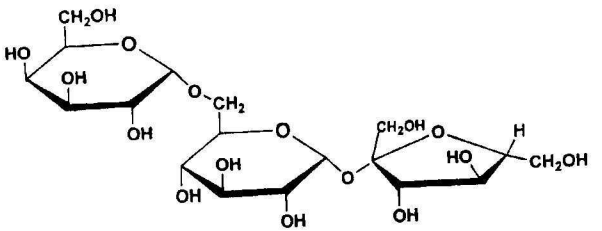
Carbohydrate Components

Sucrose is present in a quantity of about 5.5% in soybean seeds. Oligosaccharides, i.e., raffinose and stachyose, are present at about 0.9 and 3.5% in the seed, respectively. They are soluble sugars with one or two galactose units linked by a α1-6 glycosidic bond to sucrose (Fig. 10.5). Soluble sugars are important for the flavor of certain soy foods, such as tofu. At physiological maturity, soybean has about 12% nonstructural carbohydrate on a dry-seed weight basis, of which, starch accounts for about 2%, and the other 10% is di- or oligosaccharides (sucrose, 41–68%; stachyose, 12–35%; and raffinose, 5–16%). The sugar content tends to be negatively correlated with protein content (Cui et al., 2004).

Sucrose



Raffinose



Stachyose

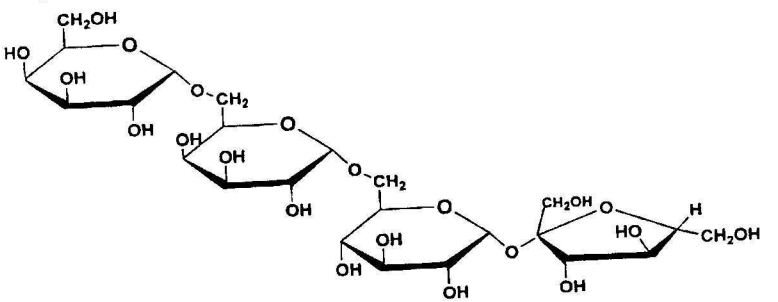


Fig. 10.5. Molecular structures of soluble sugars in soybean.

The raffinose and stachyose oligosaccharides are referred to as flatulence sugars because humans lack the enzyme (α-galactosidase) necessary to break down the molecule for metabolism. The intact sugars travel to the large intestine where the microflora is capable of utilizing them. Gases and acids are produced from microbiological action or fermentation to lead to bloating and diarrhea symptoms. This type of sugar is now used as a prebiotic to encourage the growth of the health-promoting microorganisms in the colon such as bifidobacteria (Bouhnik et al., 2007; Woodmansey, 2007).

Modification of soybeans to produce reduced levels of oligosaccharides for improved feed metabolizable energy is described in a later section.

Other Phytochemicals

Isoflavones and Total Phenolic Compounds

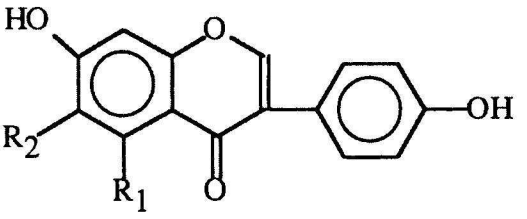
Flavonoids are a group of plant phenolic compounds having a carbon skeleton of C_6-C_3-C_6, with two aromatic rings linked together by a three carbon aliphatic chains, which normally is condensed to form a pyran. Isoflavones differ from the flavones in that the second aromatic ring is attached to position 3 instead of position 2 on the pyran ring. Isoflavones are one type of flavonoid, and the structures of the main soybean isoflavones are shown in Fig. 10.6.

Soybean is one of a few plants that contains high concentrations of isoflavones (Liu, 2004b). The three main types of isoflavones are daidzein, genistein, and glycitein. These are the free aglucone forms, and they can conjugate with glucose and its derivatives to form glycosides. The free and β-glucoside forms of daidzein and genistein, which are referred to as daizin and genistin, are the major soybean isoflavones. The structures of acetylated glucosides are also illustrated in Fig. 10.6. In addition, another glucoside conjugate form exists, which is malonylglucoside. Therefore, a total of 12 isoflavone isomers are in soybeans.

The concentration of isoflavones varies with variety and growing conditions and is reported as 1.2–2.5 mg/g in United States beans, 0.5–2.3 mg/g in Korean beans, and 0.2–3.5 mg/g in Japanese beans (Hammond et al., 2005). Wang and Murphy (1994) reported 12 isoflavones in eight American and three Japanese varieties, with total isoflavone concentrations ranging from 1.2 to 4.2 mg/g in American cultivars, and 1.3 to 2.3 mg/g in Japanese cultivars. Glucosides of genistein and daidzein account for about 90% of the total soybean isoflavones.

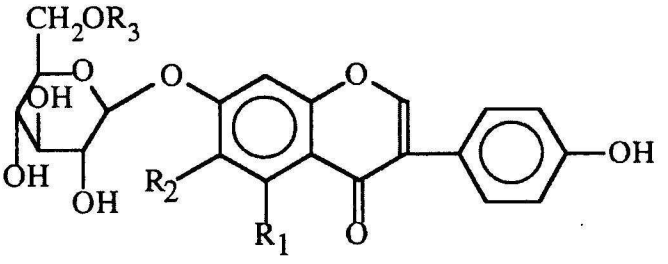
Many of the health benefits of consuming soybeans are attributed to its isoflavones. These compounds have estrogen-like activities and are believed to be beneficial for menopausal women (Messina & Hughes, 2003). Isoflavones also reportedly reduce the risks of coronary heart disease by reducing the degree of oxidation of cholesterol and reducing LDL cholesterol accumulation on the wall of blood vessels, thus enhancing arterial relaxation (Nestel, 2003). Isoflavones play a role in preventing cer-

Aglycones



	R_1	R_2
daidzein	H	H
genistein	OH	H
glycitein	H	OCH_3

Glucosides



	R_1	R_2	R_3
daidzin	H	H	H
genistin	OH	H	H
glycitin	H	OCH_3	H
6″-*O*-Acetyldaidzin	H	H	$COCH_3$
6″-*O*-Acetylgenistin	OH	H	$COCH_3$
6″-*O*-Acetylglycitin	H	OCH_3	$COCH_3$

Fig. 10.6. Structures of soybean isoflavones (White & Xing, 1997).

tain cancers, such as breast cancer (Yan & Spitznagel, 2004; Hirose et al., 2005), and may reduce mortality of prostate cancer by preventing the latent cancer to progress into larger tumors (Griffiths, 2000). The American Cancer Society recommends men eat soyfoods to reduce their risk of prostate cancer. The bone loss following the onset of menopause may be alleviated by isoflavone intake (Cotter & Cashman, 2003).

Other phenolic compounds are in soybeans, mainly acids, such as chlorogenic, isochlorogenic, caffeic, ferulic, *p*-coumaric, syringic, vanillic, *p*-hydroxybenoic, salicylic, and sinapic acids. Some of these acids have strong antioxidant activity (White & Xing, 1997).

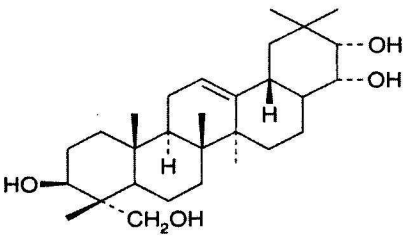
Saponins

Saponins are glycosylated alkaloids, steroid, or triterpenes. They are in low concentrations in soybeans, 0.1–0.3% based on protein content, and legumes are the major source of saponins in the human diet (Lin & Wang, 2004).

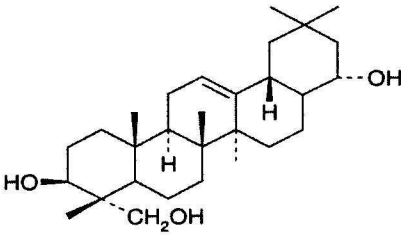
Saponins are amphiphilic in nature because of their hydrophilic sugar groups (galactose, arabinose, rhamnose, glucose, glucuronic acid, and fructose) and the hydrophobic aglycones, referred to as sapogenin. Therefore, they are excellent foaming and emulsifying agents. Five sapogenins were identified in soybeans, and their structures are illustrated in Fig. 10.7. Three groups of soyasaponins are classified: group A, B, and E. Group A saponins have soyasapogenol A as the aglycone and have two sugar chains attached on carbon 3 and 22 (Fig. 10.8). The hydroxyl groups on the sugar may be acetylated. Group B and E saponins have soyasapogenol B and E as the aglycones. Group B saponins, the main group of soy saponins, contain one sugar chain on carbon 3. Group E saponins have a different structural attachment on carbon 22 than that of Group B (Lin & Wang, 2004).

Studies on total content and composition of soy saponins are comprehensively reviewed by Lin and Wang (2004). Saponin composition was not affected by year of cultivation but was affected by soybean seed variety. Seed maturity stage was the most influential factor on content and composition of saponins. In general, saponin content decreases with maturity.

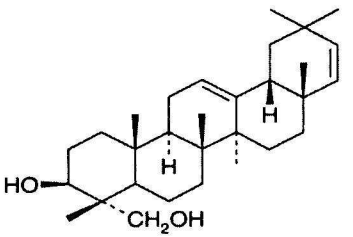
Hu et al. (2002) developed HPLC methods for quantitative determination of the group B soyasaponins. Saponin concentrations in 46 soybean varieties ranged from 2.5 to 5.9 μmol/g. In soy ingredients (soybean flour, toasted soy hypocotyls, soy protein isolates, textured vegetable protein, soy protein concentrates, and Novasoy) and soy foods (commercial soy milk, tofu, and tempeh), the group B soyasaponins ranged from 0.2 to 114 μmol/g (Table 10.5). Soy milk, tempeh, and tofu were low in soyasaponin content compared to the raw soybeans on an "as is" weight basis. However, the soyasaponin concentrations on a dry weight basis were 3.8 μmol/g in tempeh, 4.5 μmol/g in tofu, and 5.1 μmol/g in soy milk, which were greater than that in soy flour (3.3 μmol/g, as-is basis). No apparent correlation existed between isoflavone and soyasaponin concentrations in the soy products examined.



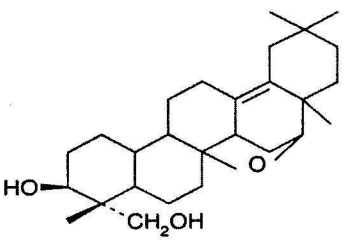
Soyasapogenol A



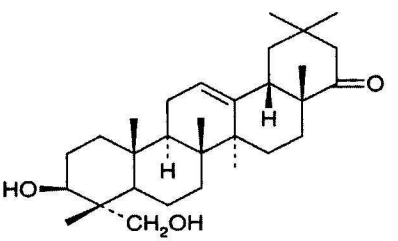
Soyasapogenol B



Soyasapogenol C

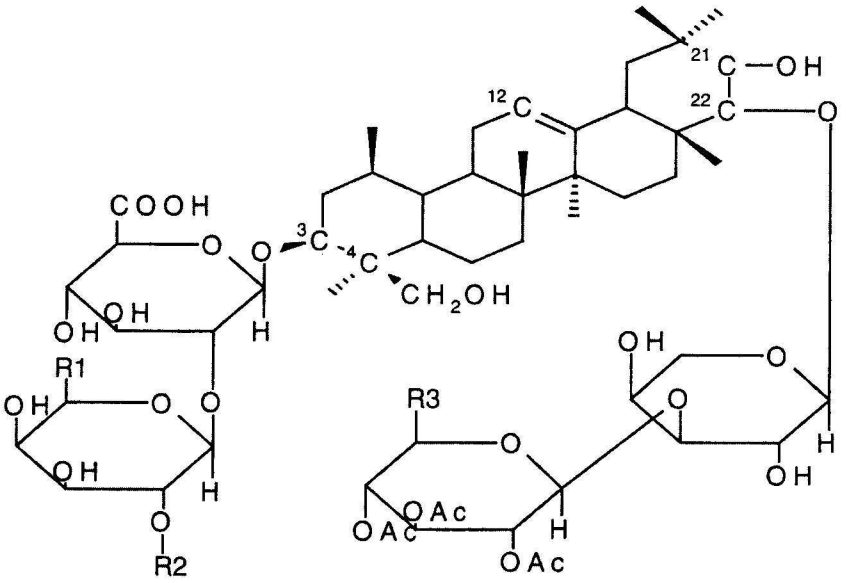


Soyasapogenol D



Soyasapogenol E

Fig. 10.7. Structures of soybean saponin aglycones (sapogenin) (Lin & Wang, 2004).



	R_1	R_2	R_3
Soyasaponin Aa (A4)	CH_2OH	β-D-Glc	H
Soyasaponin Ab (A1)	CH_2OH	β-D-Glc	CH_2Oac
Soyasaponin Ac	CH_2OH	α-L-Rha	CH_2Oac
Soyasaponin Ad	H	β-D-Glc	CH_2Oac
Soyasaponin Ae (A5)	CH_2OH	H	H
Soyasaponin Af (A2)	CH_2OH	H	CH_2Oac
Soyasaponin Ag (A6)	H	H	H
Soyasaponin Ah (A3)	H	H	CH_2Oac

Fig. 10.8. Structures of group A saponins (Lin & Wang, 2004).

Table 10.5. Molecular Weight of Soybean Minor and Bioactive Proteins

Protein	Molecular Weight, kDa
Kunitz trypsin inhibitor	20–25
Bowman-Birk inhibitor	8
Lectins	120
Lunasin	4.7

Saponins are historically considered as anti-nutritional factors, but they are recently regarded as functional food ingredients because of their cholesterol-lowering (Shibayama, 2003; Ueda et al., 1996), cancer-preventative (Jun, 2002; Oh & Sung, 2001), immune-modulating (Kang et al., 2005), and antioxidative properties (Rodrigues et al., 2005). The anti-nutritional properties are because of the hemolytic activity of these compounds, causing lysis of erythrocytes in vitro. Soy saponins did not impair the growth of chicks, rats, and mice; however, they caused slight growth retardation of *Tribolium castaneum* larvae, and were harmful to tadpoles and guppies (Lin & Wang, 2004).

Saponins and foods rich in saponins reduced plasma cholesterols in animal models by increasing the excretion of bile acids and neutral sterols in the feces, as reviewed by Lin and Wang (2004). Saponins are cytotoxic and inhibitory on the growth of tumor cells in cell culture studies. Dietary soy saponins reduced the incidence of aberrant crypt foci (ACF) in the colon of mice and, at 150–600 ppm concentration, there was a dose-dependent growth inhibition effect on human carcinoma cells (Koratkar & Rao, 1997). Soy saponins have antiviral activities for many types of viruses (Lin & Wang, 2004), and they also have inhibitory effect on HIV infection (Nakashima et al., 1989). Soybean saponins, in a dose-dependent manner, suppressed the release of proinflammatory mediators, which play a critical role in tumor development (Kang et al., 2005). Therefore, soy saponins may be useful for ameliorating inflammatory diseases as well as suppressing tumor progression.

Phytate

Phytate is the calcium, magnesium or potassium salt of phytic acid, which is inositol hexaphosphoric acid (Fig. 10.9). More than half of the total phosphorus in soybeans is in the form of phytic acid (Liu, 2004a). Because of its chelating power, phytic acid makes many essential minerals in soybeans or in diets unavailable for absorption and utilization for both human and domestic animals; thus phytic acid is known as an anti-nutritional factor.

Phytic acid or phytate can also bind with protein at the extreme pH ranges. At acidic conditions (pH below pI of soy protein), the positively charged protein binds strongly with phytic acid, and under alkaline conditions the phytate's positively charged mineral binds the negatively charged protein. These complexes make the protein less accessible by the digestive enzymes, thus affecting protein quality.

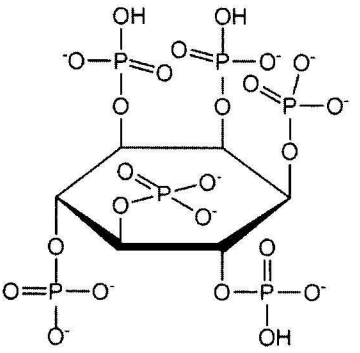


Fig. 10.9. Structure of phytic acid.

The influence of dietary phytate on hepatic activities of lipogenic and drug-metabolizing enzymes was examined in male Wistar rats, and it was found that phytate diminished the increases in hepatic lipids and activities of lipogenic enzymes induced by 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane (DDT) (Okazaki et al., 2003). Therefore, dietary phytate may protect humans from accumulating hepatic lipids by depressing hepatic lipogenesis. It may also improve the function of the liver drug-metabolizing enzyme system. Dietary phytate can also protect sucrose-fed animals against an accumulation of hepatic lipids, as shown in a study on growing rats to compare the effects of dietary phytate on metabolism of hepatic lipids (Katayama, 1997). This study showed that dietary phytate significantly depressed the rises in liver weight and hepatic concentration of total lipids and triacylglycerols.

Phytic acid can chelate transition metal ions and inactivate its pro-oxidant effect. Consumption of diets rich in phytic acid may protect intestinal epithelial cells against iron-induced oxidative damage, as shown in some rat models (Miyamoto et al., 2002). Oral administration of phytic acid also protected large intestinal mucosa against iron-induced lipid peroxidation in male rats. Dietary phytic acid lowers the incidence of colonic cancer and protects against inflammatory bowel diseases (Graf & Eaton, 1990). During digestion, phytic acid is partially dephosphorylated; therefore, its antioxidant properties may decrease as a result of the reduction in chelating power.

Water-soluble Vitamins and Minerals

Soybean contains thiamine, riboflavin, niacin, pantothenic acid, and folic acid. An HPLC method developed to determine thiamin and riboflavin in soy products (Fernando & Murphy, 1990) showed vitamin contents of soy products were less than those reported in the literature for which AOAC methods had been employed. The thiamin and riboflavin contents ranged from 6.3 to 6.9 and 0.9 to 1.1 μg/g in three soybean varieties (Fernando & Murphy, 1990). Processing soybeans into tofu leads to

Table 10.6. Soyasaponin Contents and Compositions an Commercial Soy Products (Hu et al., 2002)

Product	Total Group B Soyasaponin Content[a] (μmol/g)
Soybean flour[b]	3.31
Tofu[c]	0.59
Tempeh[d]	1.53
Soy milk[f]	0.47
Acid-washed soy concentrates[g]	9.41
Ethanol-washed soy concentrates[g]	0.20
Isolated soy protein 500E[h]	10.60
Isolated soy protein Supro 670[h]	9.51
Textured vegetable protein[g]	4.51
Soy hypocotyl[i]	27.46
Novasoy[g]	114.02

[a] Mean value of duplicated analyses. Saponin contents are reported on an "as is" weight basis.
[b] Vinton 81, 1994 crop.
[c] Mori-nu, firm.
[d] Quong Hop and Co.
[f] White Wave, Inc.
[g] Archer Daniels Midland Co.
[h] Protein Technologies International.
[i] Schouten USA Inc., toasted.

retention of thiamin and riboflavin of 7.6–15.7% and 11.7–21.1%, respectively.

Soybeans have an ash content of about 5%, and potassium, phosphorus, magnesium, sulfur, calcium, chloride, and sodium are present in 0.2–2.1% (Liu, 1999a). There are other trace minerals as well.

Compositional Changes during Seed Maturation and Processing

Effect of Seed Development on Content of Minor Components in Soybeans

Soybean seeds of three cultivars (IA1008, IA1010, and IA1014) were harvested at 5-day intervals from 28 days after flowering (DAF) to 68 DAF (mature seed; Wang et al., 2006a). Sphingolipid (SL) and phospholipid (PL) concentrations decreased significantly during seed development (Table 10.7). Averaged across cultivars, ceramide content on a dry-weight basis decreased from 51.4 nmol/g at 28 DAF to 22.2 nmol/g at 68 DAF, whereas cerebroside content decreased from 522.8 nmol/g at 28 DAF to 135.8 nmol/g at 68 DAF. PL percentage of the total lipid decreased from 9.1% at 28 DAF to 3.5% at 68 DAF.

Table 10.7. Lipid Compositions of Developing Soybean Seeds (Wang et al., 2006a)

	Sphingolipid (SL)				Phospholipid (PL)		
DAF	Cer nmol/g	*GlcCer nmol/g*	*Cer mol% of GlcCer*	*SL,mol% in the polar lipid*[a]	*%*[b]	*μmol/g*	Neutral oil [b,c] %
28	51.4	522.8	9.8	2.8	9.1	18.7	81.5
33	43.4	511.5	8.5	2.7	7.8	18.1	86.0
38	44.5	428.0	10.4	2.5	6.3	16.1	87.2
43	33.3	330.0	10.1	1.9	6.4	16.7	88.3
48	32.6	286.9	11.4	1.9	5.8	14.6	89.8
53	27.0	199.5	13.5	1.7	5.1	12.1	90.2
58	20.7	163.1	12.7	1.7	4.0	10.2	91.3
63	22.1	146.7	15.1	1.7	3.8	9.0	92.3
68	22.2	135.8	16.3	1.4	3.5	10.4	92.9
$LSD_{0.05}$	14.0	86.2	5.1	0.6	1.8	4.9	2.2

[a] Polar lipids is the sum of GlcCer (glucosylceramide), Cer (ceramide), SG (steryl glucoside), ESG (esterified steryl glucoside), and PL.
[b] Percentage of PL and neutral oil calculated based on total lipid extract.
[c] Neutral oil was obtained by silica column fractionation and may include triacylglycerols, free fatty acids, mono- and diacylglycerols, tocopherols, and pigments.

Kim et al. (2006) reported changes in soybean composition, such as protein, lipid, free sugars, isoflavones, and saponins during soybean development and maturation in two Korean soybean cultivars. As soybean seed matured, total soy saponin concentration constantly decreased. The ratio of total isoflavone to total soyasaponin in the developing soybean increased from 0.06 to 1.31. Total soy saponin content was negatively correlated with isoflavone content.

During maturation, β-carotene content decreased, reaching its lowest level at maturity. The immature seeds contained 0.46 mg/100 g fresh weight β-carotene, the mature and soaked seeds had 0.12 mg/100 g soaked weight (Bates & Matthews, 1975).

Phytate concentration increased during seed development. Changes in trypsin inhibitors were somewhat controversial, and a general slight increase with seed maturation was demonstrated. Total isoflavone content also generally increased with seed development (Liu, 1999b).

Effect of Processing on Content of Minor Components in Soybeans

The effect of processing on minor lipid components is shown in Table 10.8. Guiterrez and Wang (2004) reported the effect of processing on the sphingolipid content of

Table 10.8. Effect of Processing on Contents of Tocopherols, Sterols, and Squalene in Soybean Oil[1]

	Tocopherols		Sterols		Squalene	
Processing Step	ppm	% Loss	ppm	% Loss	ppm	% Loss
Crude	1132	-	3870	-	143	-
Degummed	1116	1.4	3730	3.6	142	0.7
Neutralized	997	11.9	3010	22.2	140	2.1
Bleached	863	23.8	3050	21.2	137	4.2
Deodorized	726	35.9	2620	32.3	89	37.8

[1] Ramamurthi et al., 1998.

various soybean products. Glucosylceramide (GlcCer), the major sphingolipid type in soybeans, was measured in several processed soybean products to determine partitioning and loss during processing. Whole soybean was processed into full-fat flakes, from which crude oil was extracted. Crude oil was refined by conventional methods, and defatted soy flakes were further processed into alcohol-washed and acid-washed soy protein concentrates (SPC) and soy protein isolate (SPI) by laboratory-scale methods that simulate industrial practices. GlcCer was isolated from the samples by solvent extraction, solvent partition, and TLC, and quantified by HPLC. As shown in Table 10.9, GlcCer mostly remained with the defatted soy flakes (91%) rather than with the oil (9%) after oil extraction. Only 52, 42, and 26% of GlcCer from defatted soy flakes was recovered in the acid-washed SPC, alcohol-washed SPC, and SPI products, respectively. The minor quantity of GlcCer in the crude oil was almost completely removed by water degumming.

Table 10.9. Cerebroside (Glccer) Contents in Soybean Products (Guiterrez & Wang, 2004b)

Soy Product	GlcCer, nmol/g (dry wt basis)	GlcCer, ppm (dry wt basis)
Full-fat soy flakes	268.2	192.5
Defatted soy flakes	311.2	223.3
Soy protein concentrate (SPC, acid washed)	264.4	189.1
SPC (alcohol washed)	216.5	155.3
Soy protein isolate (SPI)	296.9	213.9
Crude oil	Not detected (ND)	ND
Gum	1678.9	1202.8
Soapstock	ND	ND
Alkaline refined oil	ND	ND
MSD[a]	113.4	78.4

[a] MSD = minimum significant differences between means in each column determined by Tukey Kramer's mean comparison ($P \leq 0.05$).

The effect of soybean processing on the distribution of isoflavones was reported by Wang and Murphy (1996). Soybeans (600 g) were used for tofu processing (Table 10.10), and finely ground soybean flour (50 g) was used for soy isolate production (Table 10.11). Isoflavone distribution measured in pilot-plant soymilk and tofu preparation showed no significant loss of isoflavones in soymilk production. However, tofu contained only 33% (based on dry matter) of the isoflavones in the starting raw soybeans. Isoflavone distribution during soy protein isolate preparation indicated a significant loss of 53% of total isoflavone contents in the processing steps between the raw material and the protein isolate (Table 10.11). The alkaline extraction step was the major step for isoflavone loss. In protein isolate processing, alkaline extraction causes the generation of daidzein and genistein, which is attributed to alkaline hydrolysis of the glucosides.

Composition Modification through Plant Breeding and Genetic Engineering

Genetic modification has shown to be an effective means to alter tocopherol contents of soybeans (Mounts et al, 1996; Almonor et al., 1998). The most abundant γ-tocopherol is positively correlated with the most unsaturated linolenic acid content. Therefore, soybean lines with reduced linolenate currently developed to replace some hydrogenated oils containing *trans*-fatty acids will have lower γ-tocopherol contents. However, these lines tend to have higher α-tocopherol contents.

A study by McCord et al. (2004) showed a relationship between reduced palmitate or reduced linolenate and tocopherol content in soybeans. A total of 41 soybean cultivars and lines with palmitate contents ranging from 3.7 to 12.4% and linolenate contents ranging from 1.2 to 8.3% were compared for tocopherol content. Lines with reduced palmitate had significantly greater mean total tocopherol contents than did lines with normal fatty ester contents. No significant difference in total tocopherol was observed between normal and low-linolenate lines containing 1.0 or 2.5% linolenate. In a further study, lines with 1% linolenate and lines with 7% linolenate were compared for tocopherol content. The mean total tocopherol of the 7% linolenate lines was significantly greater than that of the 1% linolenate lines in the three populations tested. However, an overlap in ranges of total tocopherol between the 1% and 7% linolenate lines indicates that it should be possible to develop 1% linolenate cultivars with acceptable contents of individual and total tocopherols compared with normal cultivars.

The relationship between reduced palmitate and high tocopherols was further studied in soybeans having similar genetic backgrounds (Scherder et al., 2006). The mean total tocopherols of the reduced palmitate lines was 15% greater than the normal palmitate lines, and the line with the greatest total tocopherols in each population had reduced palmitate.

Table 10.10. Fraction Weight, Moisture and Isoflavone Contents in Soymilk and Tofu Processing (Wang & Murphy, 1996)[1]

Step	Weight (g)	Moisture (%)	Total Daidzein (mg)	Total Genistein (mg)	*Total Glycitein (mg)*	*Total*
Raw soybeans	600	11.03 ± 0.07	59.3 ± 20.0a	124.0 ± 35.0a	33.9 ± 7.7ab	217.2 ± 63.0a
Soaked soybeans	1297	60.95 ± 0.48	54.2 ± 31.0ab	114.7 ± 47.0a	27.8 ± 4.1b	196.8 ± 82.0a
Soaking water	1063	99.67 ± 0.08	0.5 ± 0.3d	0.3 ± 0.0c	0.2 ± 0.0d	1.0 ± 0.3c
Cooked slurry	5976	91.85 ± 0.09	67.9 ± 17.0a	121.6 ± 22.0a	37.7 ± 2.7a	227.2 ± 41.0a
Soymilk	5581	93.93 ± 0.09	63.6 ± 12.0a	103.7 ± 16.0a	27.6 ± 1.9b	194.8 ± 30.0a
Okara	717	79.08 ± 0.67	4.1 ± 0.8d	14.0 ± 0.6bc	7.8 ± 2.1c	25.9 ± 0.7bc
Tofu	1390	82.11 ± 2.21	16.0 ± 1.5cd	40.2 ± 3.4b	14.6 ± 2.9c	70.8 ± 4.7b
Whey	5140	98.37 ± 0.13	35.9 ± 1.3bc	50.4 ± 1.8b	9.1 ± 0.1c	95.4 ± 3.2b

[1]Values represent the mean ± standard deviation; $n = 3$. Values in a column with different letters were significantly different ($P < 0.05$).

Table 10.11. Fraction Weights and Isoflavone Contents in Soy Protein Isolate Processing (Wang & Murphy, 1996)[1]

Step	Weight (g)	Total Daidzein (mg)	Total Genistein (mg)	Total Glycitein (mg)	Total (mg)
Soybean flour	50	9.1 ± 0.3a	18.4 ± 1.2a	2.4 ± 0.5a	30.0 ± 1.7a
Defatted flour	43	10.0 ± 0.6a	20.0 ± 2.4a	1.7 ± 0.4b	31.7 ± 3.2a
Oil	8	0.7 ± 0.6c	0.0 ± 0.0c	0.0 ± 0.0d	0.7 ± 0.6c
Alkaline soluble	949	7.8 ± 2.6c	8.4 ± 2.6b	1.7 ± 0.1b	17.8 ± 5.3b
Alkaline insoluble	76	6.0 ± 1.8b	9.0 ± 2.9b	0.8 ± 0.2c	15.8 ± 4.9b
Protein isolate	9	6.2 ± 0.6b	7.8 ± 1.0b	0.5 ± 0.1c	14.5 ± 1.5b
Whey	865	1.5 ± 0.1c	0.9 ± 0.1c	0.9 ± 0.1c	3.3 ± 0.1c

[1]Values represent the mean ± standard deviation; $n = 3$. Values in a column with different letters were significantly different ($P < 0.05$).

Isoflavone content in response to genetic modification also was reported. Although little is known about the genetic regulation of isoflavone synthesis, several pathways were studied and the relationship between protein and fatty acid composition and isoflavone content was observed (Tsukamoto et al., 1995). For example, isoflavone content was negatively correlated with linolenic acid content, and also with protein content.

Phospholipid (PL) fatty acid composition and stereospecific distribution of 25 genetically modified soybean lines having a wide range of compositions were determined by GC and phospholipase A_2 hydrolysis (Wang et al., 1997). PL class proportions were affected by changes in overall fatty acid composition. PL fatty acid composition was changed with oil fatty acid modification, especially for palmitate, stearate, and linolenate.

A review by Liu (1999b) summarizes the efforts of plant breeding to eliminate or reduce trypsin inhibitors, oligosaccharides (Table 10.12), and phytate. There is also interest in increasing isoflavone content in soybeans.

Raffinose saccharides are a group of D-galactose-containing oligosaccharides of sucrose that are widely distributed in plants, particularly in legumes. The number of galactose units ranges from 0 to 4, and they are known as *sucrose, raffinose, stachyose, verbascose,* and *ajugose*, respectively. Synthesis of the raffinose saccharide family from sucrose is thought to be catalyzed by distinct galactosyltransferases (Kerr & Sebastian, 2000). Reportedly, removal of raffinose saccharides from soybean meal results in an increase in the metabolizable energy for broilers. Low oligosaccharide soybean meals with raffinose and stachyose levels of 0.08 and 0.42%, compared to 0.58 and 3.23% in regular meal, had 7% higher energy value (Parsons et al., 2000).

Efforts have been made to identify soybean germplasm that may contain genes giving a low seed oligosaccharide content. Low oligosaccharide content is typically related to high sucrose content in soybeans, and a sucrose content range of 6.1% to 12.4% and genomic regions associated with sucrose synthesis were identified with

Table 10.12. Modification of Soybean Soluble Sugars by Traditional Plant Breeding, % Dry Weight Basis[1]

Lines	Sucrose	Raffinose	Stachyose
Normal	5.1	1.0	4.7
Modified 1	6.0	0.4	1.3
Modified 2	7.0	0.1	0.5
Modified 3	11.5	0.1	0.0

[1]Adopted from Liu, 1999c.

molecular markers (Maughan et al., 2000). A cDNA coding for a variant raffinose synthase from a Japanese soybean was found, and low raffinose content plants were obtained by Watanabe and Oeda (2001). However, surveys of the soybean germplasm suggested instability of the low oligosaccharide phenotype. Therefore, the practical means to achieve a desirable oligosaccharide content was to physically and/or chemically treat the soybean to cause mutation, and patents by Kerr and Sebastian (1998, 2000, and 2003) described soybeans with stachyose content as low as 1% in the seeds.

In summary, soybeans contain many bioactive minor components that are lipids, proteins, or carbohydrate in nature, or that are low-molecular weight phenolics or saponins, etcetera. Although much research has been conducted on isolated components, and many health benefits have been demonstrated, the interactions, synergism, or antagonism among various minor compounds and interactions between the minor and major components in soybeans are not yet fully understood. Extracting and concentrating these minor compounds may provide a convenient source for expected beneficial effects; however, the cost associated with the processing may be prohibitive. Increasing the consumption of whole soy foods may allow us to gain not only the health, but also the economic, benefits of this relatively inexpensive food.

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