Agric. Chem. Biotechnol. **48**(2), 49-57 (2005)

Review

Soy Isoflavones and Soyasaponins: Characteristics and Physiological Functions

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Received January 18, 2005; Accepted April 29, 2005

Soy is an important food in Asia and many studies have suggested that the low incidences of chronic diseases in Asian countries are associated with diets that are rich in soy. Soy contains many kinds of phytochemicals, and soy isoflavones and soyasaponins have received considerable attention. Twelve isoflavone components have been isolated from soy: three aglycones (daidzein, genistein, and glycitein), and their respective nine glucosidic conjugates. Soy isoflavones are similar in structure to estrogen and exhibit both estrogenic and antiestrogenic activities. Soy isoflavones exhibit anticancer activity, can reduce the risk of cardiovascular disease, and are beneficial to brain and bone health. Soyasaponins are divided into three groups (A, B, and E saponins), and they exhibit hypocholesterolemic, anticancer, hepatoprotective, antioxidative, and anti-human-immunodeficiency-virus effects. Despite the abundant literature suggesting that soy isoflavones and soyasaponins have potential applications in preventive medicine, further research is needed to standardize dosages and ensure their efficacy.

Key words: *soy, chronic diseases, phytochemicals, isoflavones, soyasaponins, preventive medicine*

For more than 1,000 years, Asian people have been consuming soy in a variety of traditional soy food products. In contrast, soy has become a significant food in Western countries only within the past 20 years, and still plays a minor dietary role despite a good source of protein, dietary fiber, and a variety of phytochemicals.

Soy contains numerous phytochemicals including isoflavones, phytic acid, phytosterols and saponins[1]. Among them, soy isoflavones now receive considerable attention due to their anticancer and antiosteoporosis activities[2]. Phytic acid has long been recognized as interfering with the absorption of minerals[3], and recent work suggests that it also has anticancer properties[4]. Phytosterol was reported to have an hypocholesterolemic effect[5]. Soyasaponins were originally considered to be toxic because of structural analogy with saponins from other sources that are toxic[6]. However, soyasaponins have been shown to exert no toxic effects, and indeed have been reported to exert hypocholsterolemic[7] and anticacncer[8] effects.

Here the structure, content, and physiological functions of soy isoflavones and soyasaponins will be reviewed.

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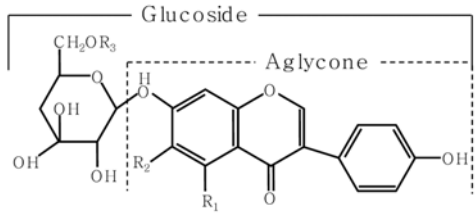
The Structures of Soy Isoflavones

Soy contains relatively high concentrations of isoflavones[9], and genistin was first isolated from soy 60 years ago[10]. Twelve isoflavone components have been isolated from soy: three aglycones (daidzein, genistein, and glycitein), and their respective nine glucosidic conjugates[9-12]. The glucosides comprise three 7-*O*-glucosides (daidzin, genistin, and glycitin), three 6"-*O*-acetyl glucosides (6"-*O*-acetyl-daidzin, 6"-*O*-acetyl-genistin, and 6"-*O*-acetyl-glycitin), and three 6"-*O*-malonyl-glucosides (6"-*O*-malonyl-daidzin, 6"-*O*-malonyl-genistin, and 6"-*O*-malonyl-glycitin) (Fig. 1). The soy isoflavones are similar in chemical structure to mammalian estrogens [13], and their phenolic ring is a key structural element that binds to estrogen receptor (ER)[14].

Soy Isoflavone Contents

Soy contains two major isoflavones, genistein and daidzein, and a minor one, glycitein. In the seed, the soy isoflavones exist primarily as β-glucosides and their contents are higher in the hypocotyls[12]. The soy germ or hypocotyl is the part of the soy that contains the highest concentration of isoflavones, which approximately reaches 2%.

The isoflavone content of soy can vary by 3- to 5-fold with the year of cultivation and variety, and this variation will be



R_1	R_2	R_3	Aglycone	Glucoside
H	H		Daidzein	
OH	H		Genistein	
H	OCH_3		Glycitein	
H	H	H		Daidzin
OH	H	H		Genistin
H	OCH_3	H		Glycitin
H	H	$COCH_3$		6"-O-Acetyldaidzin
OH	H	$COCH_3$		6"-O-Acetylgenistin
H	OCH_3	$COCH_3$		6"-O-Acetylglycitin
H	H	$COCH_2COOH$		6"-O-malonyldaidzin
OH	H	$COCH_2COO$		6"-O-malonylgenistin
H	OCH_3	$COCH_2COO$		6"-O-malonylglycitin

Fig. 1. Structures of soy isoflavones.

reflected in soy products produced therefrom. Processing can also significantly affect isoflavone concentrations[15]. Heat processing of soy reduces the concentration of malonylglucosides and increases the concentrations of glucosides and acetylglucosides compared with concentrations in raw soy (Table 1). Aqueous processing affects isoflavone compositions in soy products, and tofu and tempeh have a greater proportion of isoflavones as aglycones than raw soy[16].

The Physiological Functions of Soy Isoflavones

Hormonal effects. Soy isoflavones bind to the ERs, affecting an estrogen-mediated pathway, and are therefore referred to as phytoestrogens[17]. The binding affinity of soy isoflavones for ERs has been studied in the uterus of sheep[18], and also studied in neoplastic cells[19]. Although soy isoflavones bind nuclear ERs and the resulting messages are transported to the cells via RNA[20], they are not the same as those observed when natural estrogens bind to the same receptors. Only one ER was originally thought to exist: ERα. However, a separate subtype, termed ERβ, has subsequently been identified in cDNA libraries from rat prostate and ovary tissues[21]. The tissue distribution and relative ligand-binding affinities of the ERβ and ERα differ. ERβ and ERα appear to be expressed at different sites in the brain[22]. ERβ has been found in normal human breast tissue[23], and is also expressed in both bone[24] and the cardiovascular system[25]. Genistein exhibits higher binding affinities for ERβ than ERα[23], and more potent in transcriptional activity with ERß than with ERα[26]. The different tissue distributions of the α- and β-receptors indicates the possibility of tissue-selective effects of the isoflavones.

Cancer prevention. Several studies have investigated the relationships between soy isoflavones and the prevention and/or incidence of several types of cancer, including the breast and prostate.

In the 1980s it was suggested that isoflavones can prevent breast cancer. High excretion of isoflavones in plasma and urine, indicating a high intake thereof, was associated with a

Table 1. Isoflavone content of representative soyfoods[a]

Product	Aglycone			Glucoside			Malonyl-			Acetyl-			Total[b]
	Dein	Gein	Glei	Din	Gin	Glin	Din	Gin	Glin	Din	Gin	Glin	
		μg/g			μg/g			μg/g			μg/g		μg/g
Raw soybeans[1]	21	22	0	163	178	50	1364	1467	137	0	29	0	1830
Roasted soybeans[1]	44	77	58	474	568	70	46	65	74	40	71	54	1059
Tofu[1]	116	140	26	453	562	130	753	788	131	54	66	0	1924
Fried tofu[1]	63	90	18	340	523	87	338	410	60[e]	85	110	0	1289
Tempeh[1]	318	518	31	117	346	34	404	750	34	68	76	65	1910
Miso[1]	61	39	11	157	122	37	1180	979	217	0	0	0	1534
Soy milk[1]	18	19	10	410	710	65	690	871	39	22	820	89	2140
Soygerm[1]	303	114	684	5208	1664	6505	3466	857	3313	10830	2616	3952	23201
Soy flour[2]	29	34.5	5.3	348	507	23.2	1630	2229	36	25.1	23.8	0.4	2646
Isolated soy protein[2]	12.2	22.9	6.8	171	406	39.9	107	256	22.1	24.4	43.1	3	665
Soy paste[2]	151	197	73.6	113	126	12.8	9.6	8.2	9	29.2	28.9	18.6	635
Natto[2]	27.6	70.7	21.7	1203	1321	366	5.3	5.8	2.9	18.7	30.5	7.1	1952
Fried Tempeh[2]	42.8	55.6	8.7	10.8	29.6	2.4	3.5	8.3	8.7	35	51.9	1.9	195

[a]Dein, daidzein; Gein, genistein; Glei, glycitein; Din, daidzin; Gin, genistin; Glin, glycitin; tr, traced; nd, not detected.
[b]To calculate total soy isoflavones, individual isoflavone glucosides and aglycones are adjusted for their molecular weight differences and summed.
[1]Data from reference 15.
[2]Data from reference 16.

low incidence of breast cancer in two case-control studies[27,28]. It was reported that urinary daidzein excretion was much lower in breast cancer patients (31 nmol/24 h) than in controls (427 nmol/24 h)[28]. Several studies have examined the effects of isoflavones or isoflavone-rich extracts on the development of chemically induced mammary cancer in adult animals. Daidzein and genistein reduced *N*-methyl-*N*-nitrosourea (MNU)-induced mammary carcinogenesis (number of tumors per rat) by about 20%[29]. It was reported that the number of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine(PhiP)-induced mammary tumors per rat was significantly lower in rats fed diets containing 0.02% or 0.04% isoflavone than in rats fed an isoflavone-free diet[30]. Decreases in tumor incidence were also noted in F344 rats in response to the consumption of diets containing 1.5% or 5% soy hypocotyls[31].

A prospective study demonstrated a negative relationship between soy intake and risk of prostate cancer[32], and a recent epidemiological study of men in 59 countries revealed that the intake of soy products reduces prostate cancer mortality[33]. In a study involving in severe combined immune deficiency (SCID) mice, the growth of prostate cancer cells was significantly reduced in the group that received a low-fat diet containing casein, soy, and isoflavone compared the other groups that received a low- or high-fat diet containing casein[34]. In addition, genistein has been shown to induce apoptosis in prostate cancer cells[35], and decrease prostate specific antigen mRNA and protein levels in these cells[36]. In addition, Mentor-Marcel *et al.*[37] demonstrated that genistein reduced the incidence of high-grade, poorly differentiated tumors in a transgenic mouse model of prostate cancer in a dose-dependent manner. Genistein also downregulates the expression of androgen and ERs in rat prostate glands when given at concentrations that are comparable to those found in humans consuming a soy-based diet[38].

Soy isoflavones can exert both agonistic and antagonistic estrogenic effects[39], and these hormonal effects may reduce the risk of cancer[40]. Soy isoflavones are able to inhibit angiogenesis[41], and daidzein may enhance immune function[42]. Moreover, soy isoflavones can inhibit the key enzymes 5α-reductase[43] and 17β-hydroxysteroid dehydrogenase[44], and thereby alter the balance between estrogen and androgen levels[45]. Genistein enhances activities of several antioxidative enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and reductase, and it induces tumor-cell differentiation[46]; this may contribute substantially to its beneficial effects on health.

Reduction of risk factors for cardiovascular diseases. Soy foods have been reported to have several beneficial effects on cardiovascular health. Monkeys fed soy protein containing isoflavones had lower serum levels of total and low-density lipoprotein (LDL) cholesterol compared with those fed an isoflavone-free soy protein[47]. The consumption of soy protein containing isoflavones significantly reduced the levels of total and LDL cholesterol in hypercholesterolemic patients (by 4% and 6%, respectively)[48]. However, there are also reports that isoflavone supplements rich in genistein[49] and genistein plus daidzein[50] do not affect plasma cholesterol levels in normocholesterolemic subjects and postmenopausal women[51]. Although the effects of soy protein on plasma lipids have been variable and highly dependent upon the initial serum cholesterol level, a meta-analysis found that total cholesterol, LDL cholesterol, and triglycerides were decreased by 9 %, 13 %, and 11%, respectively when 47 g of soy protein was consumed daily[52]. Moreover, preparations containing 25 g of soy protein were allowed to make health claims for cholesterol lowering by the U.S. Food and Drug Administration in October, 1999[53].

The protein component seems to play a crucial role in the cardioprotective effect of soy. When soy isoflavones are added to casein-lactalbumin protein, the beneficial effects on plasma lipids seen with intact soy protein don't observed[54]; but when both the soy proteins and isoflavones are present, the hypocholesterolemic effect is directly proportional to the soy isoflavone concentration[48]. Genistein reduces the extension of ischaemic lesions in murine models of stroke[55] and myocardial infarction[56]. This cardioprotective effect of genistein appears to be mediated via its antioxidative properties. In humans, soy isoflavones reduce the susceptibility of LDL cholesterol to oxidation in both normal[57] and hypercholesterolemic[58] individuals via a similar mechanism. Soy isoflavones also decrease the extent of atherosclerotic lesion formation in nonhuman primates[59,60], decrease the formation of thrombin[61], and improve the compliance of systemic arteries[62]. There is also a report that isoflavone-containing soy foods have a modest hypotensive effect[63].

Effects of the central nervous system. Soy isoflavones have been studied for their potential beneficial effects against hormone-dependent cancers and age-related diseases. However, little is known about the influence of soy isoflavones on the cognitive functions. The incidence of dementia is lower in Japan[64], which has led to suggestions that soy isoflavones can prevent dementia and improve memory. Several case-control studies have shown that a diet high in soy isoflavones improves memory in postmenopausal women. In animal experiments, it was found that a high-soy diet increased brain-derived neurotrophic factor (BDNF) mRNA in the frontal cortex of ovariectomized, retired breeder rats and improved their performance in the radial arm maze[65-67]. In another study, female rats fed a high-isoflavone diet (600 μg/g diet) acquired the maze test significantly faster than females fed an isoflavone-free diet[68]. However, the recent Honolulu-Asia Aging Study found a negative correlation between midlife tofu consumption and cognition scores and brain weight in later life in Japanese-American men[69]. It was also reported that the outcome in a visual spatial memory test was reversed in rats after lifelong exposure to an isoflavones-rich diet (600 μg/g), with male rats showing reduced outcome and females showing an increased outcome[70,71]. These studies suggest that a diet high in soy isoflavones improves memory in women but impairs it in men[72]. However, a high-soy diet was found to

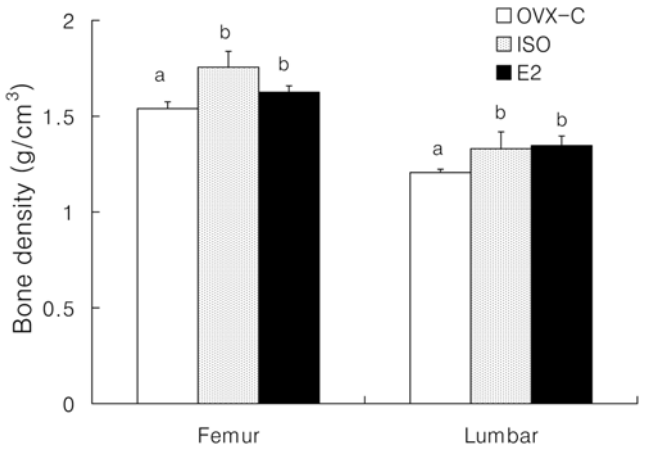
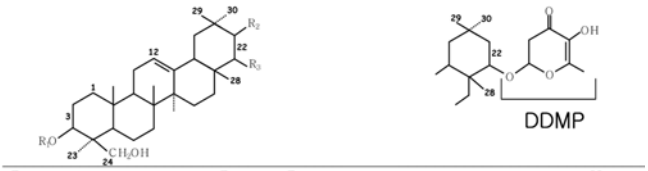


Fig. 2. Bone densities of right femur and fourth-lumbar vertebra in ovariectomized rats fed the control diet (OVX-C) and the diets containing soy isoflavones (ISO, 521.9 mg/kg diet) and 17β-estradiol (E2, 3.9 mg/kg diet) for 16 weeks. Values are means ± SD and bars without a common letter are significantly different ($p < 0.01$). Data are adopted from reference 81.



R_1	R_2	R_3	Name
GleU(1→2)Gal (1→2)Glc	OH	Ara(1→3)Xyl (2,3,4,-tri-O-acetyl)	Aa (A4)
GleU (1→2)Gal (1→2)Glc	OH	Ara(1→3)Glc (2,3,4,6,-tetra-O-acetyl)	Ab
GleU (1→2)Gal (1→2)Rha	OH	Ara(1→3)Glc (2,3,4,6,-tetra-O-acetyl)	Ac
GleU (1→2)Ara (1→2)Glc	OH	Ara(1→3)Glc (2,3,4,6,-tetra-O-acetyl)	Ad
GleU (1→2)Gal	OH	Ara(1→3)Xyl (2,3,4,-tri-O-acetyl)	Ae (A5)
GleU (1→2)Gal	OH	Ara(1→3)Glc (2,3,4,6,-tetra-O-acetyl)	Af (A2)
GleU (1→2)Ara	OH	Ara(1→3)Xyl (2,3,4,-tri-O-acetyl)	Ag (A6)
GleU (1→2)Ara	OH	Ara(1→3)Glc (2,3,4,6,-tetra-O-acetyl)	Ah (A3)
GleU (1→2)Gal (1→2)Glc	H	OH	Ba (V)
GleU (1→2)Gal (1→2)Rha	H	OH	Bb (I)
GleU (1→2)Ara (1→2)Rha	H	OH	Bc (II)
GleU (1→2)Gal	H	OH	Bb' (III)
GleU (1→2)Ara	H	OH	Bc' (IV)
GleU (1→2)Gal (1→2)Glc	H	DDMP	αg
GleU (1→2)Gal (1→2)Rha	H	DDMP	βg
GleU (1→2)Ara (1→2)Rha	H	DDMP	βa
GleU (1→2)Gal	H	DDMP	γg
GleU (1→2)Ara	H	DDMP	γa
GleU (1→2)Gal (1→2)Glc	H	O	Bd
GleU (1→2)Gal (1→2)Rha	H	O	Be

Fig. 3. Structures of soyasaponins. Ara, α-L-arabinopyranosyl; DDMP, 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one; Gal, β-D-galactopyranosyl; Glc, β-D-glucopyranosyl; glcU, β-D-glucuronopyranosyl; Rha, α-L-rhamnopyranosyl; xyl, β-D-xylopyranosyl.

improve cognition in both male and female young adults[73]. These contradictory results indicate that further animal experiments and case control, and prospective studies are needed.

The exact mechanisms underlying the soy-isoflavone-induced memory improvement are not known, but one possibility is that it mimics the effect of estrogen. Estrogen was found to increase spine density and synapse formation in the adult hippocampus[74]. Estrogen also regulates the cholinergic neurones that project to the cerebral cortex and hippocampus. The regulation of these neurones by neurotrophins and estrogen might directly regulate the transcription of neurotrophin genes. Neurotrophins such as BDNF and nerve growth factor have been shown to promote cell survival[75,76] and are involved in synaptic plasticity[77], which could improve cognitive function.

Osteoporosis prevention. The Study of Womens Health Across the Nation showed that there was a positive and dose-dependent relation between bone mineral density (BMD) and genistein intake in the premenopausal Japanese women[78]. In a case-control study involving 66 postmenopausal women, the lumbar spine BMD increased significantly after consuming 40 g of soy protein (2.25 mg isoflavones/g protein) daily for 6 months, whereas it was unaffected when the same amount of soy protein but containing only 1.39 mg isoflavones/g protein was consumed[79]. In a similar study performed in 69 perimenopausal women, 80 mg of isoflavone in the daily diet prevented lumbar spine bone loss while a 1.28% loss was observed in the placebo group[80]. Animal studies have shown the antiosteoporosis effects of soy isoflavones. Lee *et al.*[81] reported that soy isoflavone extracts prevented the decrease in bone density in ovariectomized rats (Fig. 2). There are reports that genistein particularly affects bone density. Ovariectomized rats fed 30 μmol genistein per day for 4 weeks showed a 12% higher femoral mass than control rats[82]. In a study by Fanti *et al.*[83], subcutaneous injection of 5 and 25 μg genistein significantly reduced ovariectomized tibial bone mineral loss. Soy isoflavones appear to participate in both the stimulation and inhibition of bone formation. Genistein increases the number of osteoblasts and serum osteocalcin concentration, but have no effect on the number of osteoclasts[83]. Genistein inhibits osteoclast protein synthesis, an effect that might be due to the inhibitory effects of genistein on tyrosine phosphorylation[82].

The Structures of Soyasaponins

Soyasaponins are triterpenoid saponins of the oleanene-glucuronide type, i.e., olean-12-ene triterpenes with a C-28 methyl group and a glucuronic acid moiety linked at the C-3 of the triterpene[84]. Soyasaponins are divided into three groups according to their structure: A, B, and E saponins[85].

Soyasapogenols A, B, and E are conjugated as glycosides in soy (Fig. 3). Group A soyasaponins are bisdesmoside saponins and contains the aglycone soyasapogenol A (olean-12-en-3β, 21β, 22β, 24-tetraol). This aglycone contains two ether-linked sugar chains attached to positions 3 and 22. Group B and E soyasaponins are monodesmoside saponins and contain aglycone soyasapogenol B (olean-12-en-3β, 22β, 24-triol) and aglycone soyasapogenol E (olean-12-en-3β, 24-diol-22-one), respectively. Group B soyasaponins include soyasaponins I-V, and the predominant soyasaponins in group B soyasaponins have characteristic structures with a 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) moiety attached via an ether linkage to the C-22 hydroxyl of soyasaponin.

Table 2. Soyasaponin contents in soy and processed soy products

Product	Saponin content
	g/100 g dry matter
Soy[1]	0.220-0.326
Cotyledons[2]	0.22-0.27
Hypocotyl[2]	1.67-1.98
Full-fat flour, unheated[3]	0.47
Full-fat flour, heated[3]	0.53
Protein isolate[3]	0.81
Soymilk[1]	0.39
Natto[1]	0.246
Miso[1]	0.148
Tofu[1]	0.301
Yuba[1]	0.407
Okara[1]	0.103

[1]Data from reference 90.
[2]Data from reference 86.
[3]Data from reference 89.

The Soyasaponin Contents

Age, and environmental and agronomic factors have been shown to affect saponin content. Soy contains 0.1-0.5% soyasaponins. The cotyledons contain 0.2-0.3% soyasaponins[86], whereas the content in the hypocotyl can be as high as 6%[87].

Group A soyasaponins are confined to the hypocotyls[88], and their contents are not affected by cultivation conditions. Soyasaponins Aa and Ab are the major constituents of group A soyasaponins. Soyasaponin Aa has a xylose residue at the C-22 position of soyasapogenol A, while soyasaponin Ab has a glucose residue at this position. The group B soyasaponin content is 0.5-1.9% in hypocotyls and 0.03-0.3% in cotyledons[87,88]. Both unheated and heated full-fat flours contain approximately 0.5% soyasaponins[89]. Protein isolates have been reported to contain 0.8% soyasaponins. The soyasaponin contents of soymilk, yuba, and tofu are in the range of 0.3-0.4%, whereas those of okara, miso, and natto tend to be somewhat lower, at 0.1-0.3%[90] (Table 2).

The Physiological Functions of Soyasaponins

Soyasaponins are amphiphilic compounds containing a lipid-soluble aglycone moiety attached to water-soluble glycosidic chains, which enable saponins to interact with cell membranes[91]. Saponins have been shown to form large micelles (1×10^8 Da) with bile acids *in vitro*[92]. These micelles reduced the rate of absorption of bile salts from perfused small intestine in rats. It has been proposed that the formation of the micelles contributes to the hypocholesterolemic effects of soyasaponins.

Soyasaponins prevent liver injury induced by highly peroxidized fat in rats[93]. Increases in glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were prevented by the oral administration of soyasaponins, and *in vitro* application of soyasaponins also prevented liver injury induced by CCl_4 in primary cultured rat hepatocytes[94]. In addition, soyasaponin B showed hepatoprotective actions against induced liver injury on primary cultured rat hepatocytes[95].

Soyasaponins suppress the growth of colon tumor cells *in vitro*[96], and a 2% crude soyasaponin diet inhibited the formation of carcinogen-induced colonic aberrants in rats[97]. A recent study investigated the effects of soyasaponins on aflatoxin B (1) (AFB (1))-induced mutagenicity and AFB (1)-DNA adduct formation using *Salmonella typhimurium* and human liver hepatoma cells[98]. Soyasaponins at a concentration of 30 μg/m*l* showed high antimutagenic activity and reduced the formation of AFB (1)-DNA adducts by 50.1%. It was reported that inhibition of the growth of colon adenocarcinoma cells by crude soyasaponin extract is partly mediated by the blocking of protein kinase C activity[99]. Crude soyasaponin extract also significantly reduced colon adenocarcinoma cell growth by downregulating the expression of COX-2 and protein kinase C[100].

Soyasaponins, especially DDMP saponins, exhibit antioxidative activity. DDMP saponins scavenge superoxide and hydrogen peroxide[101], and inhibit hydrogen peroxide damage in mouse fibroblast cells[102].

Soyasaponin B1 at concentrations greater than 0.5 mg/m*l* was found to completely block human-immunodeficiency-virus-induced cytopathic effects and virus-specific antigen expression *in vitro*[103]. Soyasaponins also show potent inhibiting activities against α-glucosidase[104]. Group B, group E, and DDMP soyasaponins are potent noncompetitive inhibitors of α-glucosidase. Their *in vivo* efficacies are currently under investigation.

Concluding Remarks

The above experimental and epidemiological data on the beneficial effects of soy isoflavones and soyasaponins indicate that these compounds have potential applications in preventive medicine. Although there is abundant literature on the physiological functions of soy isoflavones and soyasaponins, much work is still needed. In particular, large population-based studies should be carried out and randomized controlled clinical studies are needed to standardize dosages and ensure efficacy.

Acknowledgements. This study was supported by technology development program for agriculture and forestry, Ministry of Agriculture and Forestry, Republic of Korea (No.203022-3).

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