Journal of Scientific & Industrial Research
Vol. 66, January 2007, pp. 11-18

Soybean isoflavones: Remedial nutraceuticals in Indian perspective

J R Udgata and S N Naik*
Center for Rural Development and Technology, Indian Institute of Technology, New Delhi 110 016

Received 15 May 2006; revised 29 August 2006; accepted 06 November 2006

Soybeans flour is a rich source of isoflavones, which are a subclass of ubiquitous flavonoids. Isoflavones, which have potent antioxidant properties comparable to that of vitamin E, can reduce the long-term risk of cancer by preventing free radical damage to DNA. Genistein followed by daidzein is the most predominant antioxidant among soybean isoflavones. This review describes extraction, purification and health care properties of soy isoflavones.

Keywords: Antioxidant, HPLC, Isoflavones, Soybeans

Introduction

Soybeans have been a favorite food in Asian countries and some other parts of the world for more than 5,000 years[1]. Even, the people living in ancient China were growing soybeans in 2800 BC. In India, Madhya Pradesh, followed by Maharashtra, Rajasthan and Andhra Pradesh is the major producer of soybeans. During 2004-05, total soybean production in Madhya Pradesh was 44.439 tons (62% of the total), whereas the total produce from India was 72.083 tons.

Isoflavones

Soybean (main varieties: Braig, Ankur, Gaurav, Jawahar and Panjab-1) contains isoflavones, which are heterocyclic phenols that structurally closely relate to estrogenic steroids of animals, called phytoestrogens, which activate mammalian estrogen receptors and also they are like a substitution of vitamin E[2,3]. Phytoestrogens are circulated in plasma primarily in the conjugated form (< 3% circulate in free form[4]), mostly bound to glucoronic acid. Hydroxylation of the B-ring of isoflavones is an important factor for the antioxidative activity[5]. Genistein and daidzein are the most predominant antioxidants among soybean isoflavones (Fig. 1).

Isoflavones in plant are mostly in the form of glycosides (Fig. 2), which are conjugated with glucose, biologically inactive, and not absorbed through the intestinal wall[6] until or unless fermented. Soy isoflavones reduce serum testosterone and improve markers of oxidative stress. These compounds mainly possess anti-inflammatory, antiallergic, antiviral, anticarcinogenic, antineoplastic, antimicrobial, antihemninthic, liver protective, antithrombotic, antioxidant, metal chelation and antihormonal effects[7]. Stabilization of vitamin C and its increased absorption is also noticed[8]. Isoflavones also affect enzyme systems such as protein kinase C, tyrosine kinases, ornithine decarboxylase; nucleotide phosphodiesterases, lipoxygenases and glutathione S transferase[9]. These enzymes play a role in the pathogenesis of diseases such as cancer, arthritis, heart disease, inflammatory diseases and in antimicrobial activity. Kenneth Setchell[10] first identified the isoflavones in the urine of laboratory animals.

Forms and Sources of Isoflavones

Except soy sauce and soy oil, basic sources of isoflavones are soybeans, soy nuts, tofu, tempeh, whole soymilk, soy powders or capsules. Raw soybeans[11] contain isoflavones (2-4 mg/g, dry wt), which vary (0.5-2.0 mg) in soy protein. Isoflavones are both with and without a sugar molecule attached. There are 12 different soybean isoflavone isomers[12] (Fig. 2).

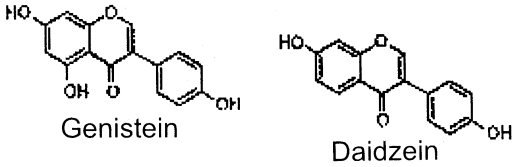
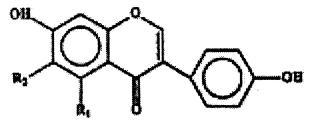


Fig. 1— Genistein and daidzein

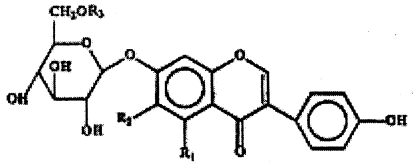
*Author for correspondence
Tel: +91-11-26591162; Fax: 26591121
E-mail: snn@rdat.iitd.ernet.in

Aglycones:



Compounds	R_1	R_2
Daidzein	H	H
Genistein	OH	H
Glycitein	H	OCH_3

Glucosides:



Compounds	R_1	R_2	R_3
Daidzin	H	H	H
Genistin	OH	H	H
Glycitin	H	OCH_3	H
Acetyldaidzin	H	H	$COCH_3$
Acetylgenistin	OH	H	$COCH_3$
Acetylglycitin	H	OCH_3	$COCH_3$
Malonyldaidzin	H	H	$COCH_2COOH$
Malonylgenistin	OH	H	$COCH_2COOH$
Malonylglycitin	H	OCH_3	$COCH_2COOH$

Fig. 2— Isoflavones from soybeans

Nutrient Content of Soybeans

Isoflavones are known antioxidants and genestin influences signal transduction through its effect on several enzymes[13]. Soy foods typically contain more genistein than daidzein, although this ratio varies among different soy products[14] (Table 1). Soy foods are good source of protein[15] (Table 2) and minerals[16] (Table 3).

Methods of Extraction and Purification of Isoflavones from Soybeans

Supercritical fluid extraction (SFE) method offers advantages over conventional methods such as increased selectivity, expeditiousness, automaticity, environmental safety, dramatically decreased use of organic solvents, higher speed and better reproducibility[17]. HPCL method is suitable for extracting all the 12 forms of isoflavones. All the methods are as follows.

Superheated Water Extraction (SWE) Method

SWE[18] is a viable alternative means of extracting five important isoflavones from defatted soybean flakes (DSF). For SWE, optimum conditions are: temperature, 110 C; and pressure, 641 psig (4520 kPa) over 2.3 h of extraction. Under optimum conditions, 3937 total isoflavones (TIF) μg/g $_{DSF}$ was produced. It was an efficient way of recovering and purifying the resulting samples by using solid-phase Amberlite XAD16-HP resin adsorption.

Table 1— Total isoflavone, genistein and daidzein content of selected foods[14]

Food	Serving g	Genistein mg	Daidzein mg	Total isoflavones mg
Soy protein concentrate, aqueous washed	100	56	43	102
Soy protein concentrate, alcohol washed	100	5	7	12
Miso	138	34	22	59
Soybeans, boiled	86	24	23	47
Tempeh	85.5	21	15	37
Soybeans, dry roasted	28.5	19	15	37
Soy milk	275	17	12	30
Tofu yogurt	131	12	7	21
Tofu	85.5	12	8	20

Table 2— Total protein, carbohydrate, fat and saturated fat content of selected foods[15]

Food 1/2 cup	Calories	Protein g	Carbohydrate g	Fat g	Saturated fat g
Soybeans, cooked	149	14.3	8.5	7.7	1.1
Tempeh	165	15.7	14.1	6.4	1.1
Textured soy protein, cooked	59	11	7	0.2	Fat free
Soynuts	404	30	29	20.0	3.2
Tofu	94	10	2.3	5.9	0.9
Soy flour, defatted	163.4	25.6	16.8	0.6	.04
Soy milk, plain	39.5	3.3	2.15	2.3	0.25

Table-3— Mineral content of soybeans[16]

Food	Niacin mg	B6 mg	Folic acid mg	Calcium mg	Iron mg	Magnesium mg	Copper mg	Zinc mg
Soybeans cooked, 1/2 cup,	1.35	0.20	47	88	4.42	74	0.35	0.99
Tempeh, 1/2 cup	3.8	0.25	43	77	1.9	58	.55	1.5
Textured cooked soy protein, 1/2 cup	.75	0.12	-	85	2	86	0.32	1.37
Soynuts, 1/2 cup	1.2	0.18	182	118	2.3	126	0.7	2.7
Tofu, 1/2 cup	0.2	0.06	19	130*	-	127	0.24	1.00
Soyflour defatted, 1/2 cup	130	0.28	156	120	4.6	144	2	1.22
Soy milk plain, 1/2 cup	0.7	0.05	2	5	0.69	22.5	0.144	0.27
Miso, 2 Tbsp	0.3	-	10.1	23	0.95	14.5	0.15	1
Adult recommended daily allowance	15	1.6-2.0	180-200	800	10-15	280-350	1.5-3.0	12-15

Ultrasound-assisted Extraction (UAE) Method

Efficiency in extracting four isoflavone derivatives (daidzin, glycitin, genistin and malonyl genistin) from freeze–dried ground soybeans using UAE[19] is more comparing than other methods, particularly mix-stirring extraction. UAE improved extraction efficiency of soy isoflavones but this method was dependent on the solvent employed, ratios of sample quantity to solvent volume and length of extraction time. Isoflavones can be quantitatively extracted from soybeans with 50% ethanol at 60 C using UAE in 20 min.

Pressurized Liquid Extraction (PLE) Method

Several researchers have successfully used PLE to extract chemically similar natural products from different matrices, like phenolic compounds from grape seeds and apple[20,21] and proanthocyanidins from malt[22]. Under PLE conditions, degradation of malonyl glucoside forms of the isoflavones takes place if temperature is higher than 100 C whereas degradation of glucosides takes place above 150 C. So using the optimized protocol (sample, 0.1 g; temp, 100 C; three static extraction cycles, 7 min; and ethanol 70% as extracting solvent), isoflavones can be extracted from freeze-dried soy matter[23].

Solid-phase Extraction (SPE) Method

SPE[24]method basically is used to evaluate the accuracy of isoflavones from soybeans. It can be obtained by solid–liquid extraction of ground soybeans on an ultrasonic bath of 360 W. Analytical protocol was scaled up to obtain a large amount of extract and consists of extracting approx 10 g of ground soybeans in 250 ml of 50% ethanol for 30 min at 60 C. The extract was centrifuged for 10 min, filtered through filter paper and freeze-dried. The resulting fine powder was stored at -32 C. The standardized extract used throughout the development of SPE method consists of 0.1 g of this freeze-dried extract dissolved in 25 ml of water[25].

Supercritical Fluid Extraction (SFE) Method

Under SFE, CO_2 is most commonly used as solvent. The comparative study of supercritical carbon dioxide (SC-CO_2), with conventional extraction methods (soxhlet and ultra-sonification), revealed that extraction of genistin and genistein at 70 C/200 bar using a mixture of CO_2 (55.2 g) and modifier (10 mol%), resulted in lower values than conventional methods. Analytical results additionally showed a predominant effect of temperature in the amount of genistin and genistein extracted by SC-CO_2. Extraction of daidzein at 50 C/360 bar resulted in higher values than conventional methods and a predominant effect of the pressure was observed. Maximum amount of total isoflavonoids[26] extracted by each methods was: ultra-sonification, 311.55; soxhlet, 212.86; and SC-CO_2, 86.28 $\mu g \cdot g^{-1}$.

Soy protein isolate-ProFam 873 (SPI) Method

Using SPI method[27, 28], nine different isoflavones (daidzein, glycitein, genistein, daidzin, glycitin, genistin, malonyl-daidzin, malonyl-glycitin and malonyl-genistin) were isolated using three different solvents [80% acetonitrile–HCl, 0.1 *N* methanol (80%) and 80% ethanol]. Samples (2 g) were dispersed in each solvent (10 ml) and vigorously mixed at room temperature for 2 h using a VWR-Rocking Platform Shaker (Model 200). The dispersions were then centrifuged for 30 min at 3000 rpm, using a Backman CS-6 centrifuge. The extracts were taken to dryness under a flow of nitrogen, re-dissolved in 80% methanol and filtered through a 0.45 μm filter unit prior to HPLC analysis. The precipitates from first extractions were subjected

Table 4— HPLC analysis methods of isoflaovnes

Sl.No.	Column size	Solvent system	Flow rate ml/min	Temp, of column°C	Detector	Constituents identified	Reference
1	ODS-3 (150*3 mm)	Methanol (A) Acetonitrile (B)	0.3	37	*CEAD	All 12 isoflavones	17
2	RP-C8 (250*4.6 mm)	TFA# (A) Acetonitrile (B)	1.5	40	UV (254 nm)	daidzein,glycitein, genistein, daidzin, genistin.	18
3	RP-18 (5 μm)	Acetonitrile (A) Acetic acid (B)	0.3	-	UV (254 nm) & PAD	daidzin, glycitin, genistin and malonyl genistin	20
4	RP-18 (250 μm * 4 μm)	Acetic acid (A) Methanol (B)	0.5	-	UV (254 nm) & PAD	daidzin, glycitin, genistin and malonyl genistin	24
5	RP-18e (4.6 mm, 100 mm)	Acetic acid (A) Methanol (B)	0.8	-	UV (200-400 nm)	Total isoflavonoids	26
6	C18 (20 cm*4.6 mm i.d.*5 μm)	Acetonitrile (A) TFA# (B)	1.0	Ambient	UV (262 nm)	Total isoflavonoids	27
7	C18-YMC –pack ODS AM (5 μm, 25 cm * 4.6 mm)	Acetonitrile (A) TFA# (B)	1.0	22	UV (254 nm)	daidzein,glycitein, genistein, daidzin, glycitin, genistin, malonyl-daidzin, malonyl-glycitin and malonyl-genistin	28

*Coulometric electrode array detection; #Trifluoroacetic acid

to repeated extractions (total of 5 extractions), with each extraction lasting 2 h. A second set of samples, dispersed in the same solvents, was subjected to sonication in a VWR-Aquasonic (Model 750D) at ultrasonic frequency of 50-60 Hz. Samples were maintained at 22°C (with a thermostatic controller) and sonicated for 15, 30 and 60 min. All extracts were then centrifuged for 30 min at 3000 rpm, dried with nitrogen, re-dissolved in 80% methanol and filtered prior to HPLC analysis.

HPLC Analysis Method

Simplified HPCL method is comparatively better than other conventional methods for separating isoflavones (Table 4).

Physiological Importance of Soybean Isoflavones

Soybean isoflavones show about 1/1,000 to 1/100,000 the activity of estradiol[29]. Isoflavones as wonder neutraceuticals are fueled by three important therapeutic effects: i) Estrogenic; ii) anticarcinogenic; and iii) anticholesterolemic. Following are specific:

Reduced Menopause Symptoms

Isoflavones reduce menopausal problems without any hormone related complexity. Women fed with soy flour (45 g) daily show reduction (40%) in menopause symptoms[29].

Prevent Osteoporosis

Isoflavones are structurally similar to ipriflavones, which inhibit bone resorption in humans. They inhibit proliferation of osteoblast cell line G292 stimulated by EGF[30] and inhibit Ca^{2+} influx mediated by thapsigargin[31].

Reduce Bone Loss

Isoflavones prevent and reverse bone loss caused by estrogen deficiency[32].

Reduce Cancer Risk

By competing with the more potent estrogen for binding to the estrogen receptor, weak estrogens are thought to be able to function as antiestrogens[33]. Genistein and glycetin inhibit angiogenesis, the growth of new blood vessels that, when abnormal, can contribute to the development of cancer[34,35]. *In vitro* work has shown that genistein has a greater antimutagenic property than daidzein[36,37], which inhibits the growth of a wide range of cancer cells including those that are not hormone-dependent. The proposed explanation for these anticancer effects is the ability of genistein to markedly inhibit the activity of enzymes (tyrosine protein kinase, ribosomal S6 kinase, DNA topoisomerase) that control cell growth and regulation. Although only limited data are available, animal work indicates that genistein inhibits tumor development[38].

Inhibit Angiogenesis

Soy isoflavones inhibit angiogenesis, an important step in tumor promotion, and may also provide protection against synthetic estrogen-like compounds, found in pesticides[39].

Protection against Prostate Problems

Eating isoflavones rich products may protect against enlargement of the male prostate gland. Isoflavones slow prostate cancer growth and cause prostate cancer cells to die[40]. Soy isoflavones also inhibit 5α-reductase, the enzyme that activates testosterone in the prostate gland and other tissues. 5α-Reductase inhibitions represent a potentially effective therapeutic approach to benign prostate enlargement and male pattern baldness. However, isoflavones also exhibit effects that are not related to estrogen activity.

Reduce Heart Disease Risk

Isoflavones inhibit the growth of cells that form artery-clogging plaque that usually form blood clots, which can lead to a heart attack. A review on soy and heart disease concluded that soy as isoflavones is definitely effective for improving cholesterol profile[41]. Isoflavones help to reduce cholesterol levels and prevent gallstone. Soy protein is hypocholesterolemic in individuals with elevated cholesterol. Soy product, from which isoflavones had been removed, had little effect on cholesterol levels in animals compared to the isoflavone-containing soy product[42]. Soy isoflavinoids (genistein and equol) have many positive effects in the treatment of heart and kidney disease, and also replacements for loop-diuretics[43,44].

Inhibit Growth Factor

It inhibits transforming growth factor β_1-mediated signal transduction[38]. Genistein inhibits platelet aggregation and smooth muscle cell proliferation. Smooth muscle cells are one of the primary cell types comprising plaques[45].

Effects on Alcohol

Daidzin and daidzein shortened alcohol-induced sleep-time, suppression of appetite for alcohol and also consumption without accelerating the liver enzyme[41, 46].

Inhibit Lipid Peroxidation

Tempe, a fermented soybean product, has the capacity to inhibit the lipid peroxidation due to presence of isoflavones as well as decreased the total amount of aldehyde excretion[47].

Acting as a Bio-monitor

Total isoflavone content of soybean seeds increases, if it grows under elevated CO_2 experienced ($p<0.005$), while total isoflavone content decreases, if the elevated O_3 experienced ($p<0.05$).Thus CO_2 or O_3 can cause changes in isoflavone concentration[48].

Prevents Wilson's disease and Hemochromatosis

Isoflavonoids may play an important role in metal-overload diseases [Wilson's disease (copper overload) and hemochromatosis (iron overload)] and also in all oxidative stress conditions involving a transition metal ion[37,39].

Other Physiological Importance of Isoflavones

Antioxidants also play an important role in brain aging[49] and may prevent progressive cognitive impairments. Vitamin E supplementation showed an intracellular effect in monocytes that are antiatherogenic. Genistein is known to influence mitogensis, cell-cycle regulation, cell survival, cell death, antifungal activity and cellular transformation[50]. Flavonoids in plants participate in light-dependent phase of photosynthesis when they catalyze electron transport[51]. Flavonoids function as antioxidants and UV light filters in higher plants[52].

Adverse Effects of Isoflavones

Soy isoflavones, genistein and daidzein, inhibit thyroid peroxidase[53] and 5'-deiodinase[54], key enzymes involved in thyroid hormone biosynthesis. The inhibition of these enzymes results in decreased levels of circulating thyroid hormones that leads to increased secretion of thyroid stimulating hormone (TSH) by the anterior pituitary. The increased levels of TSH provide a growth stimulus to the thyroid, resulting in goiter. Genistein, at high concentrations *in vitro* induces chromatid breaks, gaps, and interchanges in human lymphocytes[55] (chromatid). Also, genistein inhibits cyclic AMP–mediated release of lipoprotein lipase activity from fat pads[56]. Reproductive problems, infertility, thyroid disease and liver disease due to dietary intake of isoflavones have been observed[57,58]. Infants fed soy formula may experience negative effects on general health or reproduction later in life, because isoflavones influence the activity of certain hormones. A recent study of over 800 young adults who (as infants) were fed either soy formula or cow's milk formula; however, found that

Table 5—Isoflavone dosage of different ailments[61]

Dosage of Isoflavones	Ailments
1-2 scoops twice a day mixed with juice	Alcoholism
Follow package instructions (usually 2 scoops twice a day, mixed with juice)	Cancer
50-100 mg once or twice a day	Menopause
50-100 mg once or twice a day	Perimenopause
2 500 mg capsules (standardized to contain 5% soy Isoflavones) each morning	Prostate problems
50 mg of once a day	Cholesterol problem
40-80 mg once a day	Cardiovascular health

Table 6—Commercial Products of soybean isoflavones

Sl No	Company	Products	Comments
1	Viva Herba Private Ltd	SFS soya deoiled cake & saponin free protein isolate	Animal feed ingredients
2	Alembic Ltd [62]	Isovon capsules	For menopausal health
3	ProSoya Foods (India) Pvt Ltd	Staeta Soya Milk	Health drink
4	Sabinsa Corporation[63]	Policosanol, Galanga extract, SabiWhite™ and soy isoflavones	Dietary supplement and cosmetic products

there were no differences between the two groups in over 30 different parameters measured, including fertility and general health[59].

Intake

Daily exposure of infants to isoflavones in soy infant formula is 6-11 times higher on a body weight basis than the dose that has hormonal effects in adults consuming soy foods[58]. Isoflavones concentration in infants fed soy-based formula was 13,000 -22,000 times higher than plasma estradiol concentrations in infants on cows' milk formula. Using food consumption data in Japan[60], intakes of soy products and isoflavones were reported positively correlated with mortality from colorectal cancer. There is a significant effect of soy diet or isoflavone supplement (Table 5) inhibiting the formation of Colonic Aberrant Crypt Foci (ACF)[61].

Deodourization and Detoxification of Soybeans

A number of soybean isoflavone products[62,63] have been commercialized (Table 6). Unprocessed soybeans contain unfavorable compounds and unpleasant odour. Certain compounds lock or inhibit digestion of nutrients. Soy protein isolate (SPI) is a convenient and effective method for deodourizing the typical odour of soybeans. In SPI deodorizing method, various types of solid adsorbents made of polystyrene, polymethacrylate, and zeolite, as well as charcoal are used. Treatment of the SPI solution decreased hexanal content in the solution, whereas linoleic acid was not decreased much. Hexanal in the SPI solution can be classified into two types. Hexanal of type I may be free or bound weakly on the surface of proteins and is removable by the adsorbents, whereas hexanal of type II may be bound tightly inside proteins and is unremovable by the adsorbents. Type I hexanal may be closely related to the soybean odor. Removal of hexanal by the adsorbents was not much improved by chymotryptic digestion of SPI. Type II hexanal might be in similar states even in the chymotryptic digests[64].

Conclusions

Soy isoflavones are important to cure breast cancer, menopausal syndromes, heart diseases, LDL - cholesterol, osteoporosis, kidney diseases, angiogenesis problem, prostate problem, tumor risk etc. Except this, it has some antimicrobial properties. But the infants and persons allergic to soy foods are not advisable to take isoflavones through different forms of soy foods. Excess intake of isoflavones (> 600 mg in daily diet) is toxic.

Precise metabolic pathways of isoflavones in human system need further study. Most of the research on isoflavones targeted to purification and isolation of isoflavone compound. The disease control activity of naturally occurring isoflavone delivered by a food product has not been well studied in India. The investigations of impact of cooking and processing of soy foods on these bioactive constituents is badly lacking especially harsh cooking practices in India or widely emerging microwave cooking.

References

1 *Soybeans*, http://chinese-school.netfirms.com/soybeans.html.
2 Isoflavones, http://www.isoflavones.info.
3 Barton D L, Loprinzi C L, Quella S K, Sloan J A, Veeder M H, Egner J R, Fidler P, Stella P J, Swan D K, Vaught N L, Novotny P, Prospective evaluation of vitamin E for hot flashes in breast cancer survivors, *J Clin Oncology*; **16** (1998) 495-500.
4 Setchell K D, Brown N M, Desai P, Zimmer-Nechemias L, Wolfe B E, Brashear W T, Kirschner A S, Cassidy A & Heubi J E, Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavones supplements, *J Nutr*, **131** (2000) 1362-1375.
5 Hughes R E & Wilson H K, Flavonoids: Some physiological and nutritional consideration, *Prog Med Chem,* **14** (1977) 285-301[Medline].

6 Miksicek R S J, Esstrogenic flavonoids: Structural requirements for biological activity, *Proc Soc Exp Biol Med,* **208** (1995) 44-50.

7 Mira L, Fernandez M T, Santos M, Rocha R, Florencio M H & Jennings K R, Interactions of flavonoids with iron and copper ions: a mechanism for their antioxidant activity, *Free Radic Res*, **36** (2002) 1199-1208.

8 Clemetson C A B & Anderson L, Plant polyphenols as antioxidants for ascorbic acid, *Ann NY Acad Sci,* **136** (1966) 341-376.

9 Middleton E Jr. & Kandaswami C, The impact of plant flavonoids on mammalian biology: Implications for immunity, inflammation and cancer, in *The flavonoids: Advances in Research since 1986*, edited by J B Harbourne (Chapman & Hall, London) 1993, 619-652.

10 Messina S M & Bennink M, Soyfoods, isoflavones & risk of colonic cancer: A review of the *in vitro* and *in vivo* data, *Baillieres Clin Endocrinol Metab*, **12** (1998) 707-728.

11 Coward L, Barnes N C, Setchell K D R & Barnes S, Genistein, daidzein and their β- glycoside conjugates; Antitumor isoflavones in soybean foods from American and Asian diets, *J Agric Food Chem,* **41** (1993) 1961-1967.

12 Murphy P A, Song T, Buseman G, Barua K, Beecher G R, Trainer D & Holden S J, Isoflavones in retail & institutional soy foods, *J Agric Food Chem*, **47** (1999*)* 2697-2704.

13 USDA-Iowa State University Isoflavones Database, 1999.

14 Jinsart W, Ternai B & Polya G M, Inhibition of rat liver cyclic AMP-dependent protein kinase by flavonoids, *Biol Chem Hoppe-Seyler,* **373** (1992) 205-211.

15 Liu K S, *Soybeans: Chemistry, Technology, and Utilization* (International Thompson Publishing, Tokyo, Japan), 1997.

16 *Soy Nutritive Content***,** http://www.alsnetwork.com/soy.htm**.**

17 Araújo J M A, Carlos J C S & Sedyama C S, Isoflavonas em Grãos de Soja: Importância da Atividade de β-glicosidase na Formação do Sabor Amargo e Adstringente, *Ciência e Tecnologia de Alimentos,* **172** (1997) 137-141.

18 José L, Peñalvo, Tarja Nurmi & Herman Adlercreutz, A simplified HPLC method for total isoflavones in soy productes, *Food Chem*, **87** (2004) 297-305.

19 Chang Li-Hsun, Cheng Ya-Chuan & Chang Chieh-Ming, Extracting and purifying isoflavones from defatted soybean flakes using superheated water at elevated pressures, *Food Chemistry,* 84 (2004) 279-285.

20 Mauricio A, Rostagno M P & Carmelo G B, Ultrasound-assisted extraction of soy isoflavones, *J Chromatogr A,* **1012** (2003) 119-128.

21 Alonso-Salces R M, Korta E, Barranco A, Berrueta L A, Gallo B & Vicente F, Pressurized liquid extraction for the determination of polyphenols in apple, *J Chromatogr A,* **993** (2001) 37-43.

22 Palma M & Barroso C G, Ultrasound-assisted extraction and determination of tartaric and malic acids from grapes and winemaking by-products Anal, *Chim Acta,* **458** (2002) 119.

23 Papagiannopoulos M, Zimmermann B, Mellenthin A, Krappe M, Maio G & Galensa R, Online coupling of pressurized liquid extraction, solid-phase extraction and high-performance liquid chromatography for automated analysis of proanthocyanidins in malt, *J Chromatogr A,* **958** (2002) 9-16.

24 Rostagno M A, Palma M & Barroso C G, Pressurized liquid extraction of isoflavones from soybeans, *Anal Chim Acta*, **522** (2004) 169-177.

25 Rostagno M A, Palma M & Barroso C G, Ultrasound-assisted extraction of soy isoflavones*, J Chromatogr A,* **1012** (2003) 119-128.

26 Mauricio A R, Miguel P & Carmelo G B, Solid-phase extraction of soy isoflavones, *J Chromatogr A,* **1076** (2005), 110-117.

27 Maurício A, Rostagno M A, Júlio A & Delcio S, Supercritical fluid extraction of isoflavones from soybean flour, *Food Chem*, **78** (2002) 111-117.

28 Murphy P A, Song T, Buseman G & Barua K, Isoflavones in soy-based infant Formulas, *J Agric Food Chem,* **45** (1997) 4635-4638.

29 Allaoua A, Joyce I B & Denis B, Soybean isoflavones efficacy of extraction conditions and effect of food type on extractability, *Food Res Int,* **38** (2005) 1199-1204.

30 USDA-Iowa State University Isoflavones Database (United States Department of Agriculture, USA). http://www.nal.usda.gov/fnic/foodcomp/Data/isoflav/isoflav.html. Accessed, 14 April 2004.

31 Cassidy A, Bingham S & Setchell K D, Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women, *Am J Clin Nutr,* **60** (1994) 333-340.

32 Stephan E B & Dziak R, Effects of genistein, tyrphostin, and pertussis toxin on EGF-induced mitogenesis in primary culture and clonal osteoblastic cells, *Calcif Tissue Int,* **54** (1994) 409-413.

33 Yule D L, Kim E T & Williams J A, Tyrosine kinase inhibitors attenuate "capacitative" Ca^{2+} influx in rat pancreatic acinar cells, *Biochem Biophys Res Commun,* **2002** (1994) 1697-1704.

34 Arjmandi B H, Birnbaum R, Goyal N V & Getlinger M J, Bone-sparing effect of soy protein in ovarian hormone-deficient rats are related to its isoflavone content, *Am J Clin Nutr,* **68** (1998) 1364-1368.

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

36 Tong T S, Suzanne H & Patricia A M, Estrogenic activity of glycetin, a soy isoflavone, *J Agric Food Chem,* **47** (1999) 1607-1610.

37 Miyazawa M, Sakano K, Nakamura S-I & Kosaka H, Antimutagenic activity of isoflavones from soybean seeds (*Glycine max* Merrill), *J Agric Food Chem,* **47** (1999) 1346-1349.

38 Zhou J R, Gugger E T, Tanaka T, Guo Y, Blackburn G L, Clinton S K, Soybean phytochemicals inhibit the growth of trans-plantable human prostate carcinoma and tumor angiogenesis in mice, *J Nutr*, **129** (1999) 1628-1635.

39 Kim H, Peterson T G & Barnes S, Mechanisms of action of the soy isoflavone genistein: Emerging role for its effects via transforming growth factor β signaling pathways, *Am J Clin Nutr,* **68** (1998) 1418-1425.

40 Fotsis T, Pepper M, Adlercreutz H, Fleischmann G, Hase T, Montesano R & Schweigerer L., Genistein, a dietary-derived inhibitor of *in vitro* angiogenesis, *Proc Natl Acad Sci*, **90** (1993) 2690-2694.

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

42 Candy O, The role of soy in medicine, Part 1. *Palais des Congress,* 15-17 Sept 1996.

43 Beretz A & Cazenave J-P, The effect of flavonoids on blood-vessel wall interactions, in *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular and Medicinal Properties*, edited by V Cody, E Middleton, J B Harborne & A Beretz (Alan R Liss, Inc., New York) 1988, 187-200.

44 Verbruggen M, Soybeans protein and heart disease, *Chemical Weekly*, (2001) 185.

45 Keung W-M & Vallee B L, Daidzin & daidzein suppress free-choice ethanol intake by Syrian golden hamsters, *Proc Natl Acad Sci USA*, **90** (1993) 10008-10012.

46 Comporti M, Lipid peroxidation and biogenic aldehydes from the identification of 4-hydroxynonenal to further achievements in biopathology, *Free Rad Res,* **28** (1998) 623-635.

47 Bordignon J R, *Food Science* (University of Illinois, Urbana-Champaign)

48 Helen A, Too much tofu induces 'brain aging,' study shows, *Honolulu Star- Bull*, 19 November 1999.

49 Naim M, Gestetner B, Zilkah S, Birk Y & Bondi A, Soybeans isoflavones, characterization determination and anti fungal activity, *J Agric Food Chem,* **22** (1974) 806-810.

50 Das D K, Naturally occurring flavonoids: Structure, chemistry, and high- performance liquid chromatography methods for separation and characterization, *Methods Enzymol,* **234** (1994) 410-420.

51 McClure J W, Physiology and functions of flavonoids, in *The Flavonoids*, edited by J B Harborne, T J Mabry & H Mabry (Academic Press, New York) 1975, 970-1055.

52 Divi R L, Chang H C & Doerge D R, Anti-thyroid isoflavones from soybean, *Biochem Pharmacol*, **54** (1997) 1087-1096.

53 Cody V, Koehrle J & Hesch R D, Structure-activity relationships of flavonoids as inhibitors of iodothyronine deiodinase, in, *Environmental Goitrogenesis,* edited by Gaitan, E. (CRC Press, Boca Raton, FL), 1989, 57-69.

54 Kulling S E, Rosenberg B, Jacobs E & Metzler M, The phytoestrogens coumoestrol and genistein induce structural chromosomal aberrations in cultured human peripheral blood lymphocytes, *Arch Toxicol*, **73** (1999) 50-54.

55 Motoyashiki T, Miyake M, Yoshida A, Morita T & Ueki H, A vanadyl sulfate-bovine serum albumin complex stimulates the release of lipoprotein lipase activity from isolated rat fat pads through an increase in the cellular content of cAMP and myo- inositol 1,4,5-trisphosphate, *Biol Pharm*, **22** (1999) 780–786.

56 Leopold A S, Phytoestrogens: Adverse effects on reproduction in California Quail, *Science,* **191** (1976) 98-100.

57 Chorazy P A, Himelhoch S, Hopwood N J, Greger N G, Postellon D C, Persistent Hypothyroidism in an infant receiving a soy formula: case report and review of the Literature, *Pediatrics*, **96** (1995) 148-150.

58 Setchell K D R *et al*, Dietary estrogens - A probable cause of infertility and liver disease in captive cheetahs, *Gastroenterology,* **93** (1987) 225-233.

59 Nagata C, Ecological study of the association between soy product intake and mortality from cancer and heart disease in Japan, *Int J Epidemiol*, **29** (2000) 832-836.

60 Thiagarajan D G, Bennink M R, Bourquin L D & Kavas F A, Prevention of precancerous colonic lesions in rats by soy flakes, soy flour, genistein and calcium, *Am J Clin Nutr;* **68** (1998) 1394-1399.

61 Murphy P A, Phytoestrogen content of processed soybean foods, *Food Technology*, January (1982) 60-64.

62 http://www.pharmabiz.com/article/detnews.asp?articleid=8687&sectionid=45.

63 http://www.wholehealthmd.com/refshelf/substances_view/1,1525,10057,00.html.

64 Kuniyo Inouye, Misa Shiihara, Tadashi Uno & Teisuke Takita, Deodorization of soybean proteins by enzymatic and physicochemical treatments, *J Agric Food Chem,* **50** (2002) 1652 –1658.