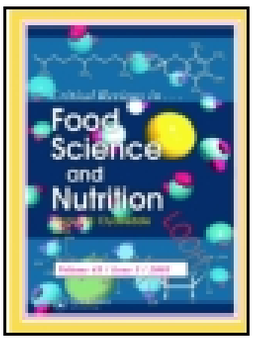

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REVIEW

Biochemistry and use of soybean isoflavones in functional food development

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ABSTRACT

Soybeans and their food products exist in the market in various forms, ranging from crude oils and bean meals to nutritious products (e.g. soy milk powers). With the availability of technologies for mass production of soy products and for enrichment of soy components (e.g. phospholipids, saponins, isoflavones, oligosaccharides and edible fiber), the nutritional values of soy products have been enhanced remarkably, offering the potential for functional food development. Among different bioactive components in soybeans, one important component is isoflavones, which have been widely exploited for health implications. While there are studies supporting the health benefits of isoflavones, concerns on adverse effects have been raised in the literature. The objective of this article is to review the recent understanding of the biological activities, adverse effects, and use of isoflavones in functional food development.

KEYWORDS

Soybeans; phytochemicals; isoflavones; functional food; natural product

Introduction

Soybeans derive from the family of Fabaceae, and belong to the genus of *Glycine* Willd. *Glycine Soja* is the annual wild soybeans. It is a close relative to *Glycine max* (L.) Merrill. Soybeans are rich in diverse nutrients, including proteins, lipids, carbohydrates, vitamins, and minerals (USDA Nutrient Data Laboratory 2018). Over the years, soybean consumption as part of the regular diet is common in Asian countries, and is also gaining popularity in the West. Soybeans are seldom eaten raw, but are often processed before consumption. Processing methods can be non-fermentative or fermentative. Different processing methods can lead to variations in tastes and textures of the end products. Representative examples of soy products are given in Table 1.

As far as active components in soybeans are concerned, one important class is isoflavones. Among different types of isoflavones, the free and conjugate forms of daidzein and genistein account for up to 30% and 60% of the total isoflavones in soybeans, respectively. During synthesis, 4-hydroxycinnamoy CoA is first produced by a reaction between phenylalanine and malonyl CoA. After that, chalcone synthase, along with malonyl CoA, involves in the formation of isoliquiritigenin and naringenin chalcone before daidzein and genistein are finally generated. Besides daidzein and genistein, glycitein is a major isoflavone in soy germ; however, the biosynthetic process is poorly understood. These, along with other forms of isoflavones [including glucosides (glycitin, daidzin, and genistin), malonylglucosides (malonylglycitin, malonylgenistin, and malonyldaidzin), and acetylglucosides (acetylglycitin, acetylgenistin, and acetyldaidzin)] have partly contributed to the biological activities of soybeans, and have attracted extensive interests in functional food development.

Biological activities of isoflavones

Over the years, isoflavones have been reported to be beneficial to health (Table 2). Because of this, isoflavones (and even soybeans *per se*) have emerged as one of the favorite candidates for use in functional food development, with the potential health benefits heavily emphasized for marketing purposes (Table 3). Examples of health benefits of isoflavones include the protective effect on radiation-induced tissue damage (Hillman et al. 2013), the capacity of improving glucose/lipid metabolism to reduce adiposity (Silva et al. 2018), and the anti-oxidative effects (Ogawara et al. 1986; Akiyama et al. 1987). The latter has partly been evidenced in genistein, which can retard the process of lipid peroxidation by reacting with lipid radicals (Patel et al. 2001). However, as the genistein radical can react with a polyunsaturated lipid to restart the oxidation cycle, genistein is not a strong antioxidant (Patel et al. 2001). Apart from genistein, daidzein and other isoflavones have been reported to possess anti-oxidative properties by not only boosting the activity and content of glutathione S-transferase (GST), but also reducing the formation of H_2O_2 and hampering ornithine decarboxylase (ODC) induction (Wei et al. 2003).

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Table 1. Selected examples of soy food products.

Food item	Description
Soy flour	Soybeans ground finely enough to pass through a 100-mesh or smaller screen
Soymilk	A product of soybean extract made by crushing and filtering the soaking soy bean and boiling them for 30 minutes
Okara	A product with abundant of isoflavones. It is produced by the residue of crushed soybeans
Tofu	A product made by adding a coagulant notably calcium sulfate ($CaSO_4$) to soymilk, followed by heating the milk to make it coagulate. Upon filtration, tofu (also called soy curd) is generated
Yuba	A product which is also known as tofu skin
Soy nuts	A dried product produced by draining, roasting and baking soybeans which are soaked in water in advance
Miso	A traditional seasoning produced by fermenting soybeans with salt and koji (i.e., *Aspergillus oryzae* and *Asperigillus soyae*)
Soy sauce	A liquid condiment of Chinese origin. It is made from a fermented paste of soybeans and brine in the presence of *Aspergillus oryzae* or *Aspergillus sojae* molds
Tempeh	A product made by fermenting dehulled boiled soybeans with *Rhizopus oligoporus,* followed by binding the soybeans into a cake form. It sometimes serves as a meat substitute for vegans
Natto	A traditional Japanese food made by soaking, steaming, bagging and treating soybeans with *Bacillus subtilis var natto.* for 16-18 hours at 48–50 °C
Sufu	A product generated by fungal solid-state fermentation of tofu, followed by aging in brine containing alcohol

In fact, genistein and other isoflavones also show an affinity for peroxisome proliferation activated receptors, PPARα/γ (Chacko et al. 2005; Kim et al. 2004). A previous study has reported that genistein can inhibit monocyte adhesion to cytokine (TNF-α)-stimulated human vascular endothelial cells, and this process is dependent on the activation of PPARγ (Chacko et al. 2005). Compared genistein, daidzein is a less potent agonist on PPARγ; however, its agonist activity can be modulated by structural modification (Chacko et al. 2007; Chacko et al. 2005). Other than the aforementioned, isoflavones are able to modulate enzymatic activities during steroid synthesis and metabolism (Makela et al. 1995; Brooks and Thompson 2005). As far as thyroid hormone synthesis is concerned, thyroid peroxidase is one of the important targets associated with the conversion of tri-iodothyronine (T3) to thyroxine (T4). Isoflavones can inhibit such thyroid peroxidase (TPO)-catalyzed reactions during thyroid hormone synthesis (Divi, Chang, and Doerge 1997).

In addition to the activities presented above, isoflavones have been reported to exhibit anti-tumor effects by a previous study, which has introduced the green fluorescent protein (GFP)-expressing human breast cancer cells into mice (Vantyghem et al. 2005) and has examined the effects of the genistein-enriched diet on tumor growth. Results have shown that, although genistein has displayed no effect on the tumor size, it has effectively inhibited the proliferation of tumor cells (Vantyghem et al. 2005). Other than breast cancer, genistein has been reported to hinder metastasis in prostate cancer (Lakshman et al. 2008; Chambers 2009). More recently, genistein has been found to play a synergistic effect with the tumor protective calcitriol to inhibit the progression of osteosarcoma (Engel et al. 2017). All these studies have suggested the possible role played by isoflavones in combating cancers. Despite this, at this moment *in vivo* models used by most of these studies have adopted human cells for the establishment of murine cancer models, in which the immune system of mice has to be first disabled before the introduction of human cancer cells. The disruption of the immune system in this case may become a confounding factor. More studies are, therefore, required to verify the anti-tumor properties of isoflavones before isoflavones can be marketed for use in cancer prevention.

Finally, isoflavones (e.g., genistein) have been shown to stimulate collagen production by increasing collagen (COL1A2) gene expression (Greenwel et al. 1995) and by stimulating collagen synthesis in human dermal fibroblasts (HDF) (Sudel et al. 2005). These render them promising to be further exploited as nutrient supplements for postmenopausal women who have thinner dermis and are deficient in collagen (Thornfeldt 2006). Isoflavones can also increase the levels of GAG and hyaluronic acid (HA) in aging skin (Ghersetich et al. 1994; Schachtschabel and Freudenstein 1994), and can facilitate tissue repair and skin hydration maintenance (Sudel et al. 2005). These activities, together with other reported health benefits of isoflavones as presented above, constitutes the prevalent fundamental justifications supporting the use of soybean isoflavones in functional food development.

Safety concerns about isoflavone consumption

While isoflavones have been reported to show health benefits as mentioned above, concerns about possible adverse effects caused by isoflavones have also been raised. For example, genistein has been suggested to be a poison to topoisomerases, which are key to the regulation of the topological state of DNA (Nitiss 2009). If pregnant women consume foods containing topoisomerase poisons, the risk of leukemia in their infants will be ten-fold higher (Ross 2000; Ross et al. 1996). Although diets rich in genistein have been found to cause only mild topoisomerase toxicity (Baechler et al. 2016), the potential of genistein as a topoisomerase poison to cause chromosomal translocation and leukemia still should not be ignored (Gomez-Herreros et al.

Table 2. Major health effects brought about by isoflavones.

Effect	Action	Underlying mechanism	Reference(s)
Antioxidation	Combat oxidative stress by functioning as an antioxidant	Genistein exerts antioxidant effects by neutralizing free radicals.	Record, Dreosti, and Mcinerney 1995
		Dietary flavonoids and genistein can reduce oxidative DNA damage by preventing free radical damage to DNA. The antioxidant effect of genistein may explain the anticancer activity of isoflavones.	Cai, Rahn, and Zhang 1997
		Genistein is the most potent antioxidant among different soy isoflavones. The second best is daidzein.	Ruiz-Larrea et al. 1997
		Genistein increases superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) levels, and attenuates the decrease in these antioxidants during oxidative stress.	Zhang et al. 2013
		Genistein is an estrogen receptor (ER)-selective binding phytoestrogen, with a greater affinity to ERβ.	Lee et al. 2004
		Genistein works as an antioxidant by inhibiting tyrosine kinases and DNA topoisomerases I and II	Kuriyama and Yoshihiro 2016
Prevention of osteoporosis	Increase the bone mass density, thereby decreasing the risk of bone fractures and osteoporosis	Daily consumption of soybeans and related products reduces the risk of osteoporosis as well as bone loss	Yamaguchi and Gao-Balch 2013
		Genistein can activate the ER, and stimulates the proliferation and differentiation of osteoblasts through p38MAPK-Runx2 and NO/cGMP pathways.	Pan et al. 2005; Dai et al. 2013
		Genistein inhibits osteoclast formation and bone resorption by inducing osteoclastogenic inhibitor osteoprotegerin (OPG) and blocking NF-kB signaling	Ming, Chen, and Xian 2013
Epigenetic modification	Inhibit and modulate epigenetic changes	Genistein inhibits DNA methylation and increases the expression of tumor suppressor genes in breast cancer cells	Xie et al. 2014
		Soy phytoestrogens act on chromatins, and can modify transcription through demethylation and acetylation of histones in breast cancer cell lines	Dagdemir et al. 2013
Cancer prevention and inhibition	Reduce the occurrence and morbidity of cancer	The intake of soy products was linked with the reduction of the occurrence and / or mortality of breast cancer	He and Chen 2013; Applegate et al. 2018; Valachovicova, Slivova, and Sliva 2004
		Soy foods and their isoflavones (genistein and daidzein) have been found by a meta-analysis to possibly reduce the risk of prostate cancer.	Applegate et al. 2018
		Genistein upregulates tumor suppressor genes in prostate cancer cells and suppresses prostate carcinogenesis in an ER wild-type mouse model	Chiyomaru et al. 2013; Slusarz et al. 2012
		Genistein exerts anticancer effects in pancreatic cancer cells through induction of ROS-mediated mitochondrial apoptosis, cell cycle arrest and STAT3 regulation.	Bi et al. 2017
		Soy isoflavones show anti-tumor effects via their roles in antioxidation, DNA repair, inhibition of angiogenesis and metastasis, potentiation of radio- and chemotherapeutic agents, and antagonism of estrogen- and androgen-mediated signaling pathways.	Bilir et al. 2017; Mahmoud, Yang, and Bosland 2014
		Genistein and daidzein can reverse DNA methylation though ER agonism of estrogens.	Bilir et al. 2017; Adjakly et al. 2014
Cardiovascular protection	Improve lipid homeostasis and reduce cardiovascular risks	Consumption of soybeans can improve lipid homeostasis and reduce the cardiovascular risk by increasing the expression of cytochrome P450, which is involved in fatty acid and sterol metabolism.	Ronis et al. 2006
		Genistein has been suggested to prevent cardiovascular diseases via its vasodilator properties	Li, Wang, and Qu 2004
		Genisten may improve the contractility of the re-perfused heart by reducing the Ca^{2+} overload induced during ischemia/reperfusion (I/R) that results in diastolic contracture	Colareda, Ragone, and Consolini 2016
Metabolism modulation	Regulate lipid metabolism to reduce the risk of obesity, and provide relief from menopausal discomforts	Isofalvone aglycone is effective in reducing vasomotor symptoms with minimal side effects	Singhal and Shullai 2016; Lambert et al. 2017
		S-equol has been shown to have an excellent safety and pharmacokinetic profile in men and women, who have been administered with a dose up to 320 mg/day	Jackson, Greiwe, and Schwen 2013
		Soy Isoflavones can regulate lipid metabolism (e.g., suppressing lipogenesis and adipogenesis, and enhancing lipolysis and β-oxidation through the AKT/mTORC1 pathway)	Huang et al. 2016
Enhancement of cognitive functions	Bind to ERs, enhance cognitive functions, and even ameliorate symptoms of neurodegenerative diseases	Genistein ameliorates amyloid-β-induced impairment of short-term spatial memory in rats through an estrogenic pathway and induces attenuation of oxidative stress.	Bagheri et al. 2011; Habibi et al. 2017
		Genistein can lower the level of amyloid-β in the brain, reduce the number and the area of amyloid plaques (confirmed in vivo by positron emission tomography), and decrease microglial reactivity.	Bonet-Costa et al. 2016; Santos-Galduroz et al. 2010

Table 3. Examples of food products and dietary supplements in which soy components are marketed for health benefits.

Product type	Product name	Country/Region	Manufacturer	Health claims made by the manufacturer or distributor
Drink	Black bean tea	Japan	OSK Co., Ltd	• "Contain isoflavones that are beneficial to women. Women enjoying this tea can have their health protected at the same time."
	Black bean matcha	Taiwan	MAYUSHAN Foods Co.., Ltd	• "Contains black beans, whose isoflavones are able to give you youthful vigor."
	Vitasoy	China	Vitasoy International Holdings Limited	• "Vitasoy, being more than ordinary soft drinks." • "Vitasoy makes you taller, stronger, and slimmer."
Snack	Bean milk wafer	Japan	Bourbon Corporation Japan	• "Each package contains 5 mg of soy isoflavones, which can regulate hormonal secretion of women, reduce fat, and serve as calcium supplement"
Dietary supplement	Beauty foods - SMOOTH CLEAR AC	Japan	FANCL Co., Ltd	• "This dietary supplement is made from highly purified soybean isoflavones and the *Vitex negundo L.* extract. It can relieve acne caused by endocrine disorders during menstruation, and promote skin smoothness. In addition, it can control oil secretion by skin."
	Bone countermeasures of middle-aged and old women	Japan	FANCL Co., Ltd	• "This product includes soy isoflavones which have been proven to maintain the function of bone components. This product is a healthy food suitable for women, especially for bone care after menopause."
	Breast Support & Care	New Zealand	CTOMI Co., Limited	• "Being rich in phytoestrogens. This supplement is formulated to support healthy breast tissues."
	MENO herbal support	UK	The Natural Health Practice Co., Limited	• "A special organic supplement formulated for woman during and after the menopause" • "It is able to resist aging and regulate menstruation."
	Soybean isoflavone vitamin C tablets	China	Beijing Tongren Tang (Group) Co., Limited	• "This product contains vitamin C and soy isoflavones. It can enhance immunity and is especially designed for adult women with low immunity"
	Soy Extract Chewbles	Malaysia	Yang Ling Natural Hygiene Sdn Bhd	• "Isoflavones enhance the production of collagen and elastin for smoother and firmer skin and protect the skin from the aging and darkening effects of UV rays." • "Isoflavones tackle hair loss by blocking the effects of DHT (dihydrotestosterone), one of the causes of greasy scalp and hair loss in both men and women." • "Isoflavones help to ease menopausal symptoms" • "Isoflavones help to balance the hormones to alleviate premenstrual symptoms (PMS). improve cholesterol levels. and reduce cardiovascular risk."
	Peri – Menopause Support	USA	Elevate Recovery Supplement, LLC	• "This product soothes the body, preparing you for an easier transition into menopause while regulating hormones"

2017; Negrini et al. 1993). So far, concerns on health risks imposed by isoflavones lie mainly in four areas: induction of tumor growth, reproductive toxicity, immune retardation, and inhibition of infant development.

Induction of tumor growth

Genistein and estradiol, which is an estrogen steroid hormone that can promote cell proliferation (Brody and Wiqvist 1961; Liu et al. 2002; Quarmby and Korach 1984), and have highly similar structures. Previous studies have associated estrogens with the development of reproductive system tumors (Bardin et al. 2004; Yue et al. 2005; Baumann and Castiglione-Gertsch 2007; Colditz 2001; Davis and Bradlow 1995; Hiatt et al. 1984; Germain 2011), causing safety concerns about the intake of estrogen analogs such as genistein in long term (Murata et al. 2004). In fact, in mammals that have normal ovarian functions, genistein can prevent the occurrence of breast cancer via the antagonistic antagonism of genistein to the estrogen receptor (Peterson and Barnes 1996); however, genistein-induced inhibition of the proliferation of estrogen receptor positive cells occurs only when the amount of genistein is similar to that of estrogen in a body (Hsieh et al. 1998). Otherwise, genistein binds to the estrogen receptor to promote the proliferation of estrogen receptor positive cells (Hsieh et al. 1998). In addition, genistein induces noncompetitive inhibition of the activity of estrogen, and can increase the level of free 17β-estradiol in plasma (Poschner et al. 2017). This may increase the risk of breast cancer.

The carcinogenic effects of isoflavones have already been verified in preclinical trials. For example, genistein has been shown to participate in some synergistic processes (e.g., inducting the growth of ERβ/ERBB-2 cells (Yang et al. 2010), promoting the resistance to tamoxifen (Yang et al. 2010), and cooperating with 7,12-dimethylbenz-[a]-anthracene to stimulate ROS production and to promote breast cancer cell proliferation (Wei et al. 2015)) to enhance tumorigenesis and tumor progression. A long-term intake of low-dose genistein has also been found to stimulate MCF-7 tumor growth (Andrade et al. 2015). Apart from adults, the effect of the maternal intake of isoflavones on the offspring's risk of cancer should be noted. Effects of estrogens on intrauterine babies have been widely reported, particularly on the association between diethylstilbestrol and vaginal cancer

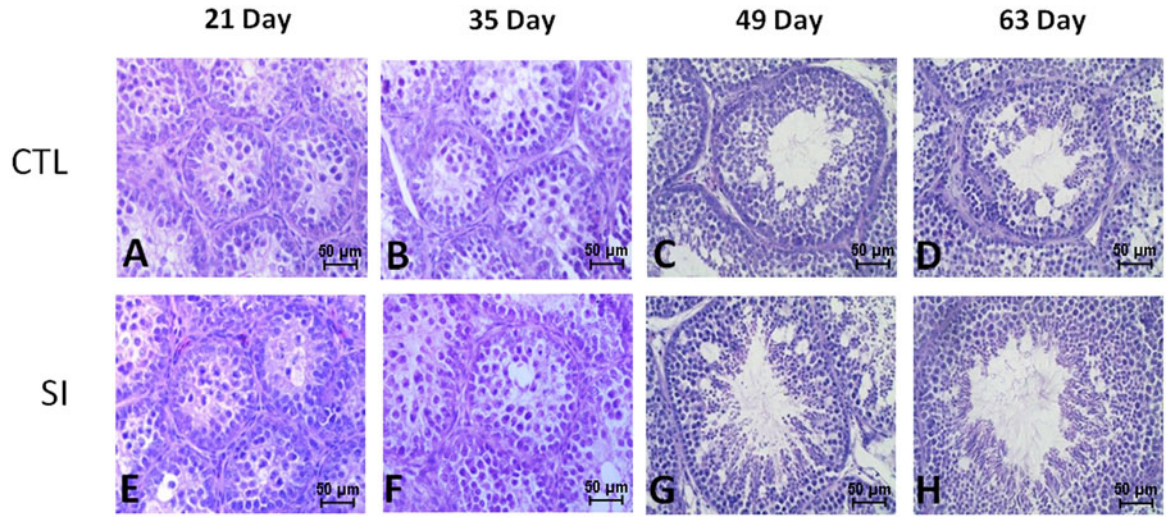

Figure 1. Morphology of chicken testis without receiving any treatment with isoflavones (A–D), and those treated with isoflavones in a dose of 5 mg/kg (E–H). On day 21, no significant difference in testis morphology was found (A, E). On day 35, seven to nine layers of germ cells were observed in those treated roosters (F), while only five to seven layers were found in the control group (B). On day 49 and 63, the diameters of the seminiferous tubes were significantly larger in the treatment group (G, H) than in the control groups (C, D). On day 63, the rate of spermatogenesis in the treatment group was also found to be higher (D, H). (Reproduced from Heng et al. 2017 with permission from Elsevier).

(Bongiovanni 1972). Apart from isoflavones, other soybean components may exert carcinogenic activities. This has been documented in a recent randomized clinical trial, which has shown that, after treatment with soy protein supplements for 7–30 days, the expression of multiple cell proliferation genes in breast cancer patients has been stimulated (Shike et al. 2014).

Reproductive toxicity

Soy isoflavones have been found to promote testicular growth in young roosters by increasing the secretion of reproductive hormones (Figure 1) (Heng et al. 2017); however, similar effects have not been observed in mammals, whose embryonic development has been found to be adversely affected by phytoestrogens in soy products instead. As revealed by earlier studies, isoflavones can weaken Leydig cell steroidogenesis and lower the plasma testosterone level (Opalka etal. 2004; Opalka et al. 2006). They can also reduce the rate of meiotic DNA synthesis in preleptotene spermatocytes (Svechnikov et al. 2005), lower the density of epididymal sperms (Delclos et al. 2001), impair sperm quality and male fecundity (Glover and Assinder 2006), and induce apoptotic cell death in male germ cells (Kumi-Diaka, Nguyen, and Butler 1999; Assinder et al. 2007). Similar observation has also been reported by Zhu et al. (2016), who have treated the testicles of newborn mice with daidzein for 72 hours. Testosterone secretion has been found to be inhibited when the concentration of daidzein reached 30 μmol L^{-1}. Further analysis has revealed that a series of key players [including the steroidogenic acute regulatory protein (StAR), the cholesterol side-chain cleavage enzyme (P450scc) and 3β-hydroxysteroid dehydrogenase (3β-HSD)] in androgen biosynthesis has been suppressed in the treated testis (Zhu et al. 2016). Apart from direct treatment with isoflavones, reproductive disturbance has happened in sheep and rabbits which have been fed with a large amount of soybean plants. This has also been attributed to the effects of the estrogenic-like substances present on the development of reproductive organs (Cassidy, Bingham, and Setchell 1994).

Physiologically, genistein can pass through the tubal epithelium and can affect the luminal secretion of amino acids in fallopian tubes (Simintiras and Sturmey 2017). The exposure to a high dose of genistein in the intrauterine and lactation periods can also lead to morphological changes in the mammary glands of the male offspring (You et al. 2002). It has adverse effects on the fertility (and the integrity of organ development) of the male offspring, and leads to the deterioration of the testicular structure (Meena et al. 2017). In rats, these effects have been manifested as the arrest of spermatogenesis with few spermatogonia, the enlargement of the intertubular space, the rupture of the epithelium and lumen, and a reduction in the number of sperm tails (Meena et al. 2017) (Figure 2). This may be due to the high sensitivity of the intrauterine offspring to environmental estrogen. In addition, the intrauterine exposure to genistein may change the expression of genes related to morphogenesis to result in abnormal development (El Sheikh Saad et al. 2013). Daily exposure of dams to genistein during late pregnancy and early lactation has been found to cause the male offspring to exhibit signs of anxiety and aggressive behavior after reaching adulthood, accompanied by changes in the neural nitric oxide synthase (nNOS) system in the basolatera amygdale (Rodriguez-Gomez et al. 2014). This suggests that genistein penetrates the placental barrier and affects the development of the central nervous system. Taking all these findings into account, the safety of isoflavone-based functional foods should be properly evaluated, especially when those foods are to be consumed by pregnant women.

Apart from the aforementioned, genistein can alter steroid-producing enzymes, affect the expression of steroid hormones, and inhibit the growth of antral follicles by

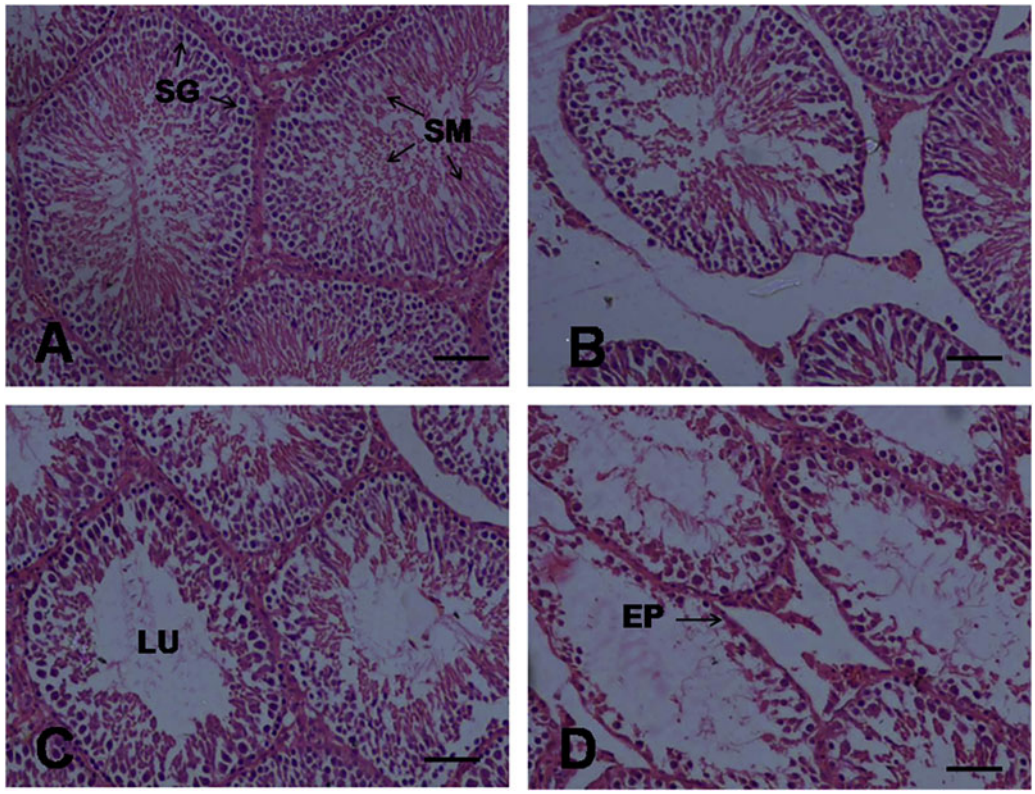

Figure 2. Morphology of rat testis without receiving any genistein exposure (A) and those exposed to 2, 20, or 100 mg of genistein respectively (B, C and D). The transverse section of testes from rats exposed to genistein showed symptoms of spermatogenesis arrest (e.g., fewer spermatogonia, enlarged intertubular space, and ruptured epithelium) (B, C and D). On the other hand, the transverse section of testes from the control rats showed the presence of normal tubular structures with spermatogenic cells at different stages of development. (Reproduced from Meena et al. 2017 with permission from Elsevier).

inhibiting the cell cycle (Patel et al. 2016), thereby subjecting women to an increased risk of infertility (Patel et al. 2016). *In vivo* studies have shown that the ovarian function, the uterine microenvironment, and the estrous cycle can be affected by exposure to genistein, and the severity is positively related to the length of exposure time (Jefferson, Padilla-Banks, and Newbold 2007; Chinigarzadeh et al. 2017). Because of this, the possible effect of genistein on the female offspring, especially in terms of premature sexual maturity (Guerrero-Bosagna et al. 2008), early puberty (Takashima-Sasaki et al. 2006) and the irregularity of the estrus (Romero, Cruz, and Pereira 2008), should not be ignored. More attention is, therefore, needed to be paid in future research to determine the risk associated with intrauterine exposure to isoflavones before isoflavones are extensively supplemented into food products.

Effects on growth and development

Hormones play an important role in regulating the growth and development of infants and adolescents. Studies have revealed that even low-dose exposure to genistein in childhood may lead to health problems in adulthood (Eustache et al. 2009; Wang et al. 2006). Children consume too much soy products may suffer from a higher risk of precocious puberty (Li et al. 2014; D'Aloisio et al. 2013; Guan, Huang, and Chen 2008). For example, in utero or lactational exposure to genistein may lead to mammary epithelial proliferation and even male mammary hyperplasia (Wang, Se, and You 2006). Exposure of infant mice to genistein also reduces the age of vaginal opening, increases the length of estrous, and accelerates the development of mammary glands in puberty (Li et al. 2014). The effect of genistein in inducing developmental dysplasia of reproductive organs is mediated by the competitive and noncompetitive antagonism of estrogen receptors, leading to a reduction in the secretion of autologous hormones (Poschner et al. 2017; Peterson and Barnes 1996; Hsieh et al. 1998).

In addition, early exposure to genistein in soybean formula has been found to induce genetic changes in reproductive tract tissues (Harlid et al. 2017; Hewitt et al. 2012) or even disrupt the integrity of neural circuitry development (Dinsdale and Ward 2010; Ponti et al. 2017). For example, the extent of DNA methylation in specific gene loci in the vaginal tissue has been found to be higher in girls fed with soy formula from birth than those fed with milk formula (Harlid et al. 2017; Hewitt et al. 2012). More recently, the association between neonatal exposure to genistein and glucocorticoid responses in adults has been studied (Whirledge et al. 2018). Results showed that neonatal exposure to genistein can alter the uterine transcriptome of mice, leading to disruption of the glucocorticoid signaling and inducing the occurrence of sterility in adulthood (Whirledge et al. 2018). Because soymilk is widely used as a substitute to infants who are allergic to milk, the possible effects of isoflavones on the integrity of organ development should be

more extensively studied so that the tolerable intake level of functional foods developed from soybeans can be properly determined for infants and adolescents (Caserta et al. 2007; Choi et al. 2008).

Impacts on immune functioning

Soybean isoflavones can serve as an immunomodulator to regulate the Th1/Th2 balance and play a positive role in the treatment of allergic diseases and autoimmune diseases (Fu et al. 2015; Zhang et al. 2008). The coin, however, has two sides. An earlier study has attempted to use genistein to improve the lung function or clinical outcome of adults and children with poorly controlled asthma, but has found that isoflavone supplementation fails to give any improvement to the disease condition (Smith et al. 2015). In fact, the effects of isoflavones on the immune system are multifaceted, so isoflavones have to be used with caution in the development of functional foods for immunomodulation. This has been documented in an earlier study (Yellayi et al. 2002), which has found that subcutaneous injection of genistein at a dose of 20 mg/kg (a dose close to the intake in infants who consume soy milk powders as a milk substitute (Setchell et al. 1997; Setchell et al. 1998)) leads to thymus atrophy in adult ovariectomized mice, and the effect is in a dose-dependent manner. Thymus atrophy has still occurred when genistein has been co-administered with an estrogen receptor antagonist (viz., ICI 182780) (Yellayi et al. 2002). This suggested that the thymic effects of genistein can also be mediated by mechanisms other than the estrogen receptor-mediated one (Yellayi et al. 2002).

In addition to causing thymus damage, genistein can act as an estrogen antagonist. The competitive binding of genistein and estrogen to estrogen receptors may influence the functioning of the immune system, for example by lowering the levels of IL-4 and IFN-γ in a dose-dependent manner (Kogiso et al. 2006). More recently, incubation with genistein has also been shown to reduce the survival rate of mouse macrophage RAW264.7 cells to 70% - 80% (after 24 hours) and 50–60% (after 48 hours) by inducing the G2/M arrest (Cui, Wienhoefer, and Bilitewski 2014). Because macrophages are not only key players in nonspecific immunity, but can also activate lymphocytes to initiate specific immunity. If genistein can cause dysfunction of macrophages, this implies that it may damage immune surveillance and lead to a higher risk of infectious diseases (Pollard 2009; Wynn, Chawla, and Pollard 2013). The adverse immunomodulating activity of isoflavones is especially damaging to those whose immune systems are immature. This is evidenced by the observation that prenatal and intrauterine exposure to dietary isoflavones can lead to an immune-suppressive effect in the offspring (Gaffer et al. 2018) and can cause an increase in the sensitization of the postnatal respiratory tract (Guo and Meng 2016), respectively, thereby resulting in a higher risk of infection and an increased sensitivity to allergens. Follow-up studies to decipher the mechanisms of action of genistein and other soybean isoflavones are, therefore, in dire need.

Implications for functional food development

Along with the rise of industrialization and capitalism, the processing and production of foods become more professionally-oriented. Currencies are used to obtain foods from large-scale industrial production due to the social division of labor. Large-scale food production, however, involves centralized management of food products. The distance between food consumption and production become much more distant than the past. Consumers, therefore, have no choice but trust the agri-food safety supervision mechanism. If any food producers neglect consumers' health in order to maximize their profits, or exaggerate the health benefits of certain food components while ignoring potential hazards involved, the trust relationship between food producers and consumers will be broken. This also applies to isoflavone-fortified foods and soybean products. Despite the advantages (e.g., possible health benefits, the ease of availability, and the natural origin of isoflavones) of using isoflavones in functional food development, future efforts are needed to be paid to verify the effects and safety of the developed food products for consumption (Figure 3). Efforts to the following three areas are particularly needed: elucidation of biochemical effects; evaluation of individual responses; and quality control for food products.

Elucidation of biochemical effects

At the moment, the biological effects of isoflavones are highly contradictory. For instance, while some studies have documented the anti-oxidant activity of soybean isoflavones (Record, Dreosti, and Mcinerney 1995; Cai, Rahn, and Zhang 1997; Ruiz-Larrea et al. 1997; Zhang et al. 2013; Lee et al. 2004; Kuriyama and Yoshihiro 2016), a recent study has revealed that genistein may stimulate the formation of reactive oxygen species (ROS) and enhance the activity of glutathione redox system (Chen et al. 2014). Similar discrepancies in the effect of genistein have also been reported in the case of bone health. Genistein is usually used as an alternative to estrogen for osteoporosis treatment (Bitto et al. 2009; Wang et al. 2007). By examining the osteogenic differentiation of embryo osteoblast precursor cells or human bone marrow mesenchymal stem cells (BMSCs), the performance of the drug in bone repair can be evaluated (Luo et al. 2017; Ma et al. 2017). Previous studies have revealed that genistein can stimulate osteoblast differentiation, inhibit osteoclast absorption, and increase bone mass. It, therefore, has been thought to be useful in preventing the occurrence of osteoporosis and in stimulating bone repair (King et al. 2015; Yamaguchi and Gao-Balch 2013). Counter-evidence, however, has been reported by a recent study (Zhang et al. 2016), which has found that genistin increases the expression of PPARγ to induce the differentiation of BMSCs into adipocytes to enhance the risk of osteoporosis (Zhang et al. 2016). Such a contradictory effect may be attributed to the difference in dose adopted in different studies, but more investigations are required to fully elucidate the underlying cause.

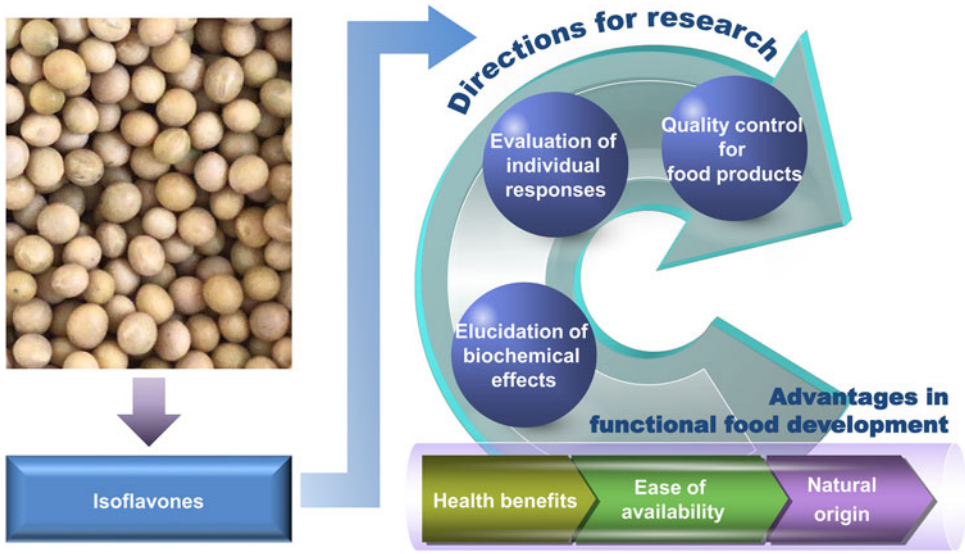

Figure 3. Advantages and future research directions for the use of isoflavones in functional food development.

Finally, the bidirectional estrogen-like effect exerted by genistein should not be overlooked when isoflavones are used in functional food development. Some people hold that the intake of maternal estrogen can prevent the occurrence of breast cancer in offspring (Song, Chen, and Hou 2009), others either believe that estrogen can promote cancer even at a low dose (Mehta et al. 2006) or think that the intake of genistein has not protective effect against the occurrence of cancer in offspring (Hilakivi-Clarke et al. 2002). In addition, the effects of the intake of soy protein or purified soy isoflavones on gene expression have been ill-defined till now (Liu et al. 2015). These effects should be fully elucidated when isoflavones or soybean extracts are adopted for functional food development.

Evaluation of individual responses

When isoflavone-fortified foods and soybean products are used as functional foods, it is worth noting that the health effects attained by different individuals may be different. Isoflavones can conjugate with mammalian estrogen receptors (ERs) (Martin et al. 1978), although their affinity is 100 times weaker than endogenous estrogens (Kuiper et al. 1997). As far as the conjugation of isoflavones to ERs is concerned, the impact of the receptor structure to the affinity with isoflavones has been ill-defined till now. In zebrafish, the differences in the amino acid composition between ERα and ERβ do not affect the affinities of the receptors to isoflavones (Sassi-Messai et al. 2009). Counter-evidence, however, is also available, showing that, although ERα and ERβ have highly homologous ligand-binding sites, their affinity to genistein can be different (Pike et al. 1999). This discrepancy may be explained by the differences in animal models, but further studies are required to clarify the actual mechanism behind.

Apart from this, the difference in metabolic activity on the health effects of isoflavones should not be overlooked when a functional food is developed. Upon food consumption, isoflavones inside the food product undergo

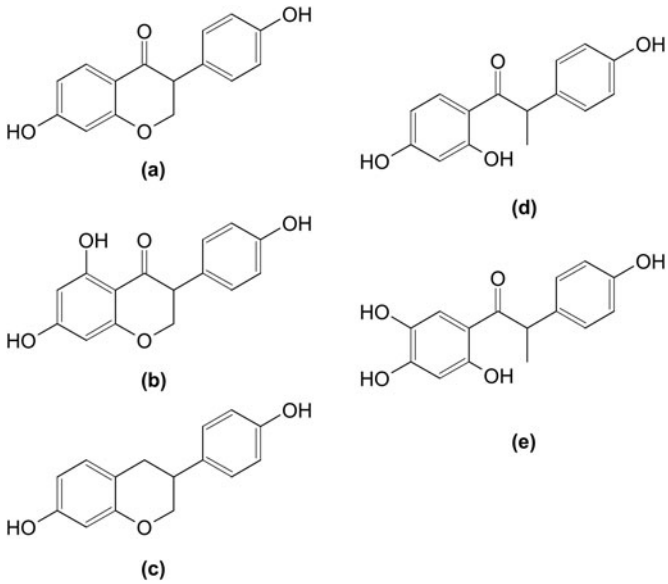

Figure 4. Structures of some bacterial metabolites of isoflavones: (a) dihydrodaidzein, (b) dihydrogenistein, (c) equol, (d) *O*-desmethylangolensin, and (e) 6-hydroxy-*O*-desmethylangolensin.

metabolism, which is mediated predominately by the bacterial action in the intestine. Structures of major bacterial metabolites of isoflavones are shown in Figure 4. By passive diffusion, the upper small intestine can absorb aglycones, which can reach the peak concentration in blood after around one hour after absorption (King, Broadbent, and Head 1996; Sfakianos et al. 1997). On the contrary, β-glucosides do not enter the blood circulation via passive diffusion, but can be hydrolyzed by either β-glucosidases, which derive from intestinal bacteria, or an enzyme in the intestinal mucosa (Day et al. 2000). Due to genetic variations among individuals, the hydrolysis of 6″-O-malonyl- or 6″-O-acetyl-7-O-β-glucosides, which are conducted by β-glucosidases in the small intestine, sometimes may not be able to occur effectively in some people. Under such circumstances, 6″-O-malonyl- or 6″-O-acetyl-7-O-β-glucosides transfer to the

Table 4. Isoflavone contents of soybeans and some of their products.

		Isoflavone content / per 100 g			
Processing	Food item	Genestein (mg)	Daidzein (mg)	Glycitein (mg)	Total isoflavones (mg)
Raw material	Soybeans, green	22.57	20.33	7.56	48.95
	Soybeans, mature seeds	81.35	62.90	15.28	15.28
	Soybean sprouts	18.76	12.86	2.87	34.39
Non-fermented product	Soy flour. full-fat, raw	98.77	72.91	16.11	178.10
	Soy flour, full-fat, roasted	85.11	89.45	16.40	165.04
	Soymilk curd, dried	42.45	40.85	0.00	83.30
	Tofu, fried	18.42	13.79	2.92	34.77
	Tofu, dried-frozen	51.03	29.58	3.44	83.20
	Yuba, raw	101.40	80.03	15.43	196.05
	Okara	4.46	3.61	1.30	9.39
	Soy nuts, mature seeds, dry roasted	75.77	62.13	13.32	148.49
Fermented product	Miso	23.23	16.42	3.00	41.44
	Soy sauce	0.39	0.78	0.14	1.18
	Tempeh	22.66	36.15	3.81	60.60
	Natto	37.66	33.22	10.54	82.28
	Sufu	5.46	7.50	0.78	13.75

Values adopted in this table are extracted from the USDA Food Composition Databases (USDA Nutrient Data Laboratory 2018).

large bowel where more microorganisms reside. This anaerobic environment provides a place not only for the occurrence of hydrolysis, but also for the production of dihydrodaidzein, O-desmethylangolensin and equol (7,4′-dihydroxyisoflavan) through reductive modification of the heterocyclic ring.

There is not much connection between puerarin (daidzein-8-C-glucoside) and O-glucosides. The presence of the C-C link between the glucose moiety and the isoflavone moiety may account for the resistance of puerarin to enzymatic hydrolysis. Physiologically, glucose transporters in the intestinal mucosal are responsible for the transportation of puerarin (Prasain et al. 2004). There is little metabolism happens before excretion of this compound (Prasain et al. 2004). Once aglucones enter intestinal cells, they are converted into two forms. In most cases, they are converted into β-glucuronides by UDP-glucuronyl transferases (Sfakianos et al. 1997) and in rare cases, into sulfate esters through the action of PAPS-sulfotransferases (Ronis et al. 2006). Liver is the site where glucuronidation and sulfation occur (Nakano et al. 2004). The metabolites get through the excretion process to the bile, and decouple in the lower bowel. This enables the metabolites to get reabsorbed, forming an enterohepatic circulation (Sfakianos et al. 1997). Peripheral tissues, especially mammary tumor-derived cells, are the sites for the formation of sulfate esters (Peterson et al. 1996; Peterson et al. 1998). Inflammatory cells, on the other hand, are key players in metabolism of isoflavones. In particular, the activated neutrophils can produce hypochlorous acid (HOCl), leading to chlorination of isoflavones (Boersma et al. 1999; Boersma et al. 2003). Those modified isoflavones can be glucuronidated and then enter into the bile for excretion. Activated macrophages, neutrophils and esinophils can also produce superoxides, which react with nitric oxide (NO) to form peroxynitrite that plays an important role in initiating 3'-nitration of isoflavones. All these metabolic activities are, at the end, governed by genetic factors. Because of this, the pharmacokinetics parameters (in terms of absorption, distribution, metabolism and excretion) after the consumption of isoflavones-fortified foods or soybean products can vary greatly among individuals due to genetic variations.

Quality control for food products

Soybeans are cultivated worldwide, with 90% of soybean production in the world occurred in tropical and semi-arid tropical regions, in which the temperature is generally high and the rainfall is low or erratic. Soybeans contain different major nutrients (including lipids, proteins, carbohydrates, and dietary fiber) and a range of bioactive components (Wallo, Nebus, and Leyden 2007), including phospholipids, proteases (e.g., soybean trypsin inhibitor (STI), and Bowman-Birk inhibitor (BBI) (Wei et al. 2001)), and isoflavones (Wei et al. 2001). The quantities of these bioactive components in soybeans are influenced by the cultivation method, farming conditions, and maturation status (Table 4). In another word, the nutrient values of soybeans grown in different locations and climatic conditions can be substantially different. This makes a proper selection of the origin of the crop vital when functional foods are produced from soybeans.

Apart from the origin of the crop, other factors may affect the quantity of active isoflavones in a food product. One important factor is the food processing method, which may cause changes in the structures of bioactive ingredients in soybeans. For example, fermentation may lead to the removal of glucosidic groups, resulting in the release of aglucone (Chun et al. 2007; Kuo et al. 2006). In addition, during the preparation of soymilk in boiling water, hydrolysis of the malonyl group may occur, leading to the generation of simple β-glucosides (Barnes, Kirk, and Coward 1994). This reaction also takes place when soy flour is extracted using hot alcoholic solutions. The malonyl group can be further decarboxylated to form 6″-O-acetyl-7-O-b-glucoside when soybeans are heated in a dry environment (such as extrusion of the soy protein concentrate or toasting of soybeans, soy flour or the hypocotyls) (Barnes, Kirk, and Coward 1994). These changes in chemical structures during food processing may change the biological effects of

bioactive components in soybeans, and should be taken into consideration when a functional food is developed.

Concluding remarks

Soybeans contain isoflavones, which are bioactive compounds of non-steroidal and phenolic nature. In this article, we have summarized the latest understanding of the biological activities of isoflavones. In fact, owing to their potential health benefits, isoflavones have attracted extensive interests in food industry and have emerged as a good candidate for functional food development. While reports on the health benefits of isoflavones are abundant in the literature, there are also studies raising concerns on the adverse health effects, particularly on the carcinogenic activity, reproductive toxicity, adverse effects on growth and development, and impacts on immune functioning. Future studies are required before a more balanced view of the benefits and adverse effects led by the intake of isoflavones can be reached. In addition, even though one day the health benefits of isoflavones are found to outweigh the adverse health effects, quality control of the functional food production, as well as proper management of food consumption, are needed. Finally, as the carcinogenic effect of isoflavones is associated, at least in part, with the level of estrogen in the body, the use of isoflavones in functional food development should be especially cautious when the foods are supposed to be consumed by women (e.g., postmenopausal women) whose ovarian function is comparatively weak (Hsieh et al. 1998).

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