Review

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An insight into the harmful effects of soy protein: A review

K. Sukalingam[1], K. Ganesan[2], S. Das[1], Zar Chi Thent[1]

[1]Department of Anatomy, 18th Floor, Pre-Clinical Block, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia. [2]Department of Biochemistry, Faculty of Medicine, International Medical School, Management and Science University, Shah Alam- 40100, Selangor, Malaysia

Abstract

Soy protein (SP) is a protein derived from soybean meal. SP is obtained from the removal of the outer shell of soybean and the fatty acid. The dietary supplementation of SP was was reported to have positive effects on human health. Therefore, the attention towards SP is increasing among the consumers, industrialist and researchers. However, the side effects and toxicity related to SP was not summarized, to date. This review summarized the toxic effects such as hormonal disturbances, carcinogenic and organotoxicity of SP based on the clinical and experimental studies. The review mainly focused on the effect of soy isoflavone-genistein on various organs. The main aim of the present review is to increase the public awareness on the harmful effect of SP on the various health aspects and draw the attention of the health care personnel and researchers. ***Clin Ter 2015; 166(3):131-139. doi: 10.7417/CT.2015.1843***

Key words: *carcinogenic, hormonal disturbances, soy isoflavone-genistein, Soy protein, toxicity*

Introduction

Seed legumes provide one-fifth of all plant proteins consumed by humans (1,2). Soy protein (SP) is a classic edible protein isolated from soy bean (*Glycine max)*, a leguminous plant related to peas, clover, and alfalfa (3). The nutritional content of SP is twofold that of meat, 4 folds that of eggs, and 12 folds that of milk (4). Since from past decades, SP was used as healthy, rich product for foods in many countries (5). At present, about 1.6 million tonnes of soybeans are cultivated Worldwide. Major production is from USA (38%), followed by Brazil (25%), Argentina (19%), China (7%), India (3%), Canada (2%), and Paraguay (2%). Worldwide statistic showed that approximately 64 million tonnes of SP are used for consumption (6).

In Malaysia, SP is mainly used in the meat industries as food binder and filler. The main purpose is to reduce the cost of production as SP is the substitute of the meat (7, 8). SP is produced from raw whole soybeans by removing the lipid and indigestible components to obtain the concentrated protein. Following the particular steps used in the process such as cooking, grinding, extracting, fermenting, and sprouting, isolated soy protein (ISP), soy protein concentrate, or soy flour was yielded as the form of final product (9).

Uses of Soy Protein

SP is utilized by consumers in the form of fermented and/or non fermented such as tofu, shoyu (soy sauce), tempeh and miso. It is easily available in the form of nuts, milk, yogurt and cheese. SP is present in variable amount in the products based on specific technology applied during the preparation (10). Soy contains approximately 70% of the total soy protein. SP is a "complete food" as it provides all nutrition and 9 essential amino acids (11). The digestibility of SP depends on the range of processed soy named as steamed soybeans 65%, tofu 93%, soy milk 93%, and SP isolate 95% (12). In human, both concentrated and isolated SP are easily digested and gives an equal nutritional value of the protein. In addition, SP does not possess any cholesterol and lactose. Digestibility standards for SP isolates and SP concentrates in humans is 93.5%, compared to the cow's milk and other proteins (13). The compounds in SP provide moisture, flavor retention and emulsification. It also increases the texture of many foods such as meat, peanut butter, and cheese.

Preparation of Soy Protein

SP is originated from "white flakes," prepared by dehulling, flaking and defatting the soy beans. Then, it is powdered into defatted flours and extracted with ethyl alcohol to remove flavour and sugars, followed by alkali extraction. Subsequently, the unwanted fibres were removed by the centrifugation, re-precipitation and drying. From the soy isolates, full-fat products, a variety of dried soy foods (soy milk and tofu) are produced. SP constituents are used for the absorption of water and fat, emulsification, aeration and heat setting (14).

Correspondence: Dr. Zar Chi Thent. Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia, 18th Floor, Pre-Clinical block, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia. Tel.: 006-03-91458634, Fax: 006-03-91458607.
E-mail: zarrchii@gmail.com

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Soy isolates are mainly used to develop the quality of meat products, enhance the moisture retention and protein content. SP concentrate and isolates were prepared by the several procedures such as ultrafiltration membranes (15), swellable gel method (16), hydrophobic interaction chromatography (17), pH and temperature methods (18), alternative defatting and extraction procedures (19, 20), defatted soy flake suspensions at pH 4.5 and pH 3.0 in the presence of calcium chloride (21-23), alkaline medium (24), ultra filtration (25) and mechanical method (26).

Active Ingredients of Soy Protein

SP possesses a rich source of proteins and other isoflavones. It contains numerous products such as oil, isoflavones (genistein, daidzein and glycitein) (27,28) and many other compounds (β-conglycinin, Glycinin, Soybean agglutinin, Glyceollins, Lunasin, saponins, phytosterols, phospholipids, ferritins and Glysojanin) (Table 1) (29, 30).

Therapeutic Uses of Soy Protein

SP contains essential amino acids required for human growth and maintenance (31). Due to the presence of beneficial effects in SP, there is an increase in SP consumption in the general population. SP has a lesser quantity of methionine, but, having adequate lysine, which is poor in most cereal proteins. Therefore, SP amino acid profile is corresponding to cereal amino acid profile. The content of essential amino acids present in soy protein depends on the cultivar that has been used.

Table 1. Amino acid composition in soy protein, adapted from reference (36).

Amino acid	mg/g protein
Arginine	77.16
Alanine	40.23
Aspartic acid	68.86
Cysteine	25.00
Glutamic acid	190.16
Glycine	36.72
Histidine	34.38
4-hydroxy proline	1.40
Isoleucine	51.58
Leucine	81,69
Methionine	10.70
Phenylalanine	56.29
Proline	52.91
Serine	54.05
Threonine	41.94
Tryptophan	12.73
Tyrosine	41.55
Valine	41.55

SP has a protective effect on plasma cholesterol, diabetes mellitus, cancer and obesity (32). It improved the kidney and gastrointestinal disorders (33). SP is safe to combine with other protein such as meat, milk, and cereal grains (34, 35). The nutritional competence of SP was confirmed in infant formulas, where protein needs are most important (36). SP is used as an extraordinary formula in geriatric, hospital, and postoperative feeding by providing absolute nutrition, caloric content, and a balance between calories such as carbohydrate, protein, and fat (37). Previous studies showed that SP has hypocholesterolaemic, anti-atherogenic effect and cardioprotective effect (38-40). Intake of isolated SP reduced the risk of coronary heart diseases (41-43).

Since SP is nutritive, the intake of SP varies from country to country. In Japan and Malaysia, SP intake ranges between 6.0 g and 10.5g/day, In comparison to Japan, soy intake of Hong Kong and China is approximately 3.0- 5.0 g/day. In Korea, the intake ranges seem as that of Japan and Hong Kong. According to WHO, SP is considered to have same protein quality as that of animal proteins. Egg white has a protein digestibility-corrected amino acid score of 1.00, soy concentrate 0.99, beef 0.92, and isolated soy protein 0.92. The scores above 1.0 are considered to indicate the protein contains essential amino acids in excess of the human requirements.

Adverse Effects of Soy Protein

Although SP is believed to possess several positive effects on human health, the reports suggest that the consumption of SP above its recommended dose is detrimental to the human body. SP contains major quantities of bioactive compounds with toxic and/or adverse effects. It was reported that the body mechanism was altered following the consumption of raw soybean (44). In another experimental study, the disruption of the intestinal brush border membranes, atrophy of the microvilli and reduced viability of the epithelial cells was observed with the use of raw soybean (45-51). It may be possibly due to the reduction of the nutritional value of raw soybean as it composed of toxic compounds. Researchers have found out that trypsin inhibitors and lectin is the major proteins responsible for the low nutritional value of raw soybean meals (48-50). Lectin binds to the intestinal epithelium and cause the gastrointestinal disturbance by altering the body mechanism (51) (Fig. 1).

Toxicity Studies on Soy Protein

Researchers reported that the injection of aqueous extract SP to the rabbits (52) and dogs (53) was found lethal. The crude extract contains a high urease activity, a toxic agent, which cause death in the animals. In general, SP produces two urease isoenzymes (54). The embryo-specific urease is synthesized only in the developing embryo (55, 56), whereas the ubiquitous urease is found in all tissues, namely cultured cells, leaves, embryos, roots and seed coats (57, 58). The increased level of urease in SP was shown to have anti-nutritional effects in rats (59, 60). The injection of an albumin-like fraction derived from raw soy beans was toxic in guinea pigs. Moreover, two other toxic proteins; soya toxin

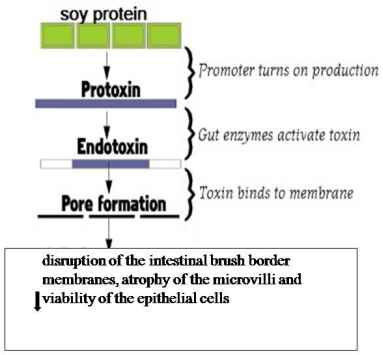

Fig. 1. Schematic diagram of mechanism of action of SP.

and soybean toxin were isolated from soybean seeds (61). It was revealed that SP induced toxic effect in the stomach of animals (62).

Effect of Soy Isoflavone- Genistein

Genistein, an active compound, in SP possesses oestrogenic effect that stimulate the multiplication of oestrogen receptor in the breast cancer (63, 64). Further investigation of in vitro and in vivo studies revealed that the dietary intake of genistein stimulates proliferation of implanted human breast cancer cells and influences the ovarian hormonal profile resulting in the formation of mutagenesis and carcinogenesis (65). Another study suggested that the increased risk of adenoma or adenocarcinoma was observed in genistein treated rats compared to the untreated genistein rats (65).

Table 2. In vivo studies on Soy isoflavones, soy products and their effects

No.	Soy Isoflavones/ soy products	Adverse effects	References
1.	Alpha and beta-conglycinin	Potential Allergenic effect	66-68
2.	Gly m 5 or Gly m 6	Potential Allergenic effect	68
3.	Glycinin	Potential Allergenic effect	69
4.	P34	Potential Allergenic effect	70
5.	Genestein	Hepatotoxicity & Genotoxicity	71
6.	Genistein and daidzein	Antithyroid activity	72-75
7.	Genestein	Goitrogenic and oestrogenic activity	76, 77
8.	Genestein	Hypothyroid activity	78
9.	Genestein	Postmenopausal breast cancer	79
10.	Genestein	Reproductive toxicity	80
11.	Genestein	Tumourogenic agents	81
12.	Genestein	Oestrogen-dependent (MCF-7) tumours	82 -84
13.	Daidzein	Oestrogenic activity	82
14	Genestin	Oestrogen-dependent breast cancer tumours	85, 86
15.	Glycitein	Oestrogenic activity	85
16.	Genestein	Oestrogen-dependent mammary tumours	86
17.	Genestein	Intestinal toxicity	87
18.	Genestein	Endocrine disruption	88,89
19.	Soy phytoestrogens	Reduce brain Neuroactivity	90
20.	Soy phytoestrogens	Infertility and hepatotoxicity	91, 92
21.	Soy tofu	Memory loss	93
22.	Soy tofu	Alzheimer's dementia	94
23.	Soy bean	Potential Allergenic effect	95, 96
24.	Genestein	Genotoxicity	97
25.	Genistein	Accelerates puberty	98, 99
26.	Genistein	Mammary gland Development	100
27.	Genistein	Growth toxicity, Myelotoxicity, Cytotoxicity	101-103
28.	Genistein and daidzein	Neurotoxicity	104

Effect of soy protein on endocrine glands

High quantity of SP contains a number of phytoestrogens such as zenistein, biochalin A and daidzein. Most of the phytoestrogens are potential endocrine disrupting chemicals that interrupt the normal functions of hormones as well as reproductive system (105). Various studies showed that phytoestrogens have significant effects on sexual development in terms of puberty time alteration, oestrogen cycle impairment, functions of ovary and alterations of pituitary as well as hypothalamus dysfunctions (103). Moreover, it was mentioned that increase consumption of SP produced harmful oestrogenic and goitrogenic activities (77).

A meta-analysis of 25 trials observed that soy phytoestrogens did not improve hot flashes or other menopausal symptoms (106). Intake of SP supplements for 12 weeks in the treatment of menopausal symptoms in patients with early breast cancer did not show any significant effect compared to placebo (107). Therefore, there is no concrete evidence to support any beneficial effect of SP intake on menopausal symptoms at this stage.

Effect of Soy Protein on Mammary Gland

The regular consumption of SP causes breast abnormalities due to the powerful oestrogenic effects (108). The chief isoflavone present in SP is genistein, which affects the development of the mammary gland. The chronic exposure to genistein caused ductal/alveolar hyperplasia in young pups (109). The low dose treatment of genistein in mice showed advanced maturation with enhanced duct elongation, where as high dose exhibited diminished lobular alveolar development (100).

The inhibitory action of genistein on topoisomerase II cause chromosomal damage with a threshold dose (110) and some experimental data suggested that genistein can stimulate breast cancer development via its oestrogenic activities (111). A previous case study showed that a high intake of SP produced the lower risk of breast cancer in adult (112). However, the recent Japanese cohort study reported that the consumption of SP and their products has no effect on breast cancer (113). In addition, another clinical study showed that isoflavones of SP stimulated epithelial cell proliferation in the breast of premenopausal women (114).

Effect of Soy Protein on Male Reproductive System

SP isoflavone, genistein is broadly consumed by humans. However, its oestrogenic action adversely causes the progression of the male reproductive system. In vivo study observed that sperm counts in epididymis and testes were decreased in the genistein-exposed group compared to the control group (115). Studies on genistein administration in animals caused hyperplasia of Leydig cells in the testis and severe damage to the epdidymis. Furthermore, the reduced sperm count and increased sperm motility in the stage of juvenile period was also observed (116, 117).

Effect of Soy Protein on Growth

Okano et al. (118) reported that the injection of SP albumin fraction showed toxicity and oral administration showed growth retardation. The protease inhibitors of SP consist of two groups, namely Kunitz inhibitor and Bowman-Birk inhibitor. Kunitz inhibitor is directed primarily against trypsin and Bowman-Birk inhibitor inhibits both chymotrypsin and trypsin at independent binding sites. Both proteinase inhibitors in SP are responsible for inhibition of growth hormones in rats (119), chicks (120) and mice (121). Moreover, these inhibitors decreased the digestibility of protein in the diet (122).

Effect of Soy Protein on Thyroid Gland

SP contains potent thyroid inhibitors, daidzein. SP acts as an anti-thyroid agent which decrease the absorption of iodine (123, 124). Hypothyroidism and goitre were reported in infants receiving soy-containing formula (125). Based on the thyroid function test, many researchers have highlighted that the high consumption of SP cause thyroid suppression and goitre (122) in iodine-deficient rodents and infants (126, 127). It was observed that extra iodine supplementation was required in the experimental group received SP compared to the control group. Hence, in the recent studies, consumption of soy formula milk is contraindicated in infants with congenital hypothyroidism (126, 127).

Additionally, SP stimulates the development of thyroid hyperplasia in iodine deficient rats (128, 129). The further study evidenced that consumption of SP may gradually reduce the functions of thyroid hormones and utilization of iodine (130). However, this problem is reversed by the dietary supplementation of adequate iodine.

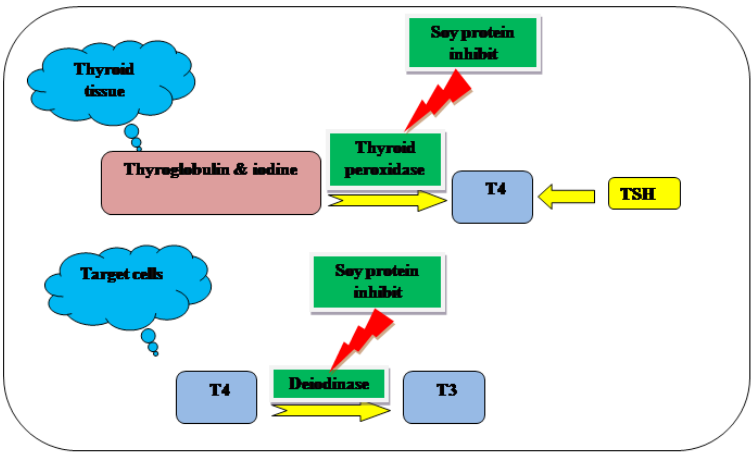

Figure 2. The schematic diagram illustrate the soy protein inhibit the catalytic activities of thyroid peroxidise (required for synthesis of T4 in thyroid tissues) and deiodinase (required for synthesis of biologically active T3 in target cells) resulting in the consequences of thyroid hyperplasia and more production of T4 by TSH.

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Carcinogenic property of soy protein

Pancreatic Cancer

Foods containing SP were proven to alter the biochemical and physiological mechanism of the pancreatic tissues in experimental animals. Increase consumption of raw soybean products enhanced the pancreatic carcinogenic process. Previous experimental studies observed that SP enhanced the growth of azaserine-induced pancreatic foci (131). Following 48 weeks of daily consumption of SP, it was observed that there were presence of hyperplastic and neoplastic nodules in the pancreas of the experimental rats (133). Feeding of SP stimulated the production of pancreatic carcinogens. The pancreatic growth induced by dietary soybean is due to the lectins and trypsin inhibitors (133).

Breast Cancer

In adults, the increase consumption of SP induces the risk of developing breast cancer. It was reported that SP stimulated the growth of mammary tumours in experimental animals (134, 135). SP supplements are contraindicated for female patients at higher risk of breast cancer (136). Further studies are advised to authenticate the underlying mechanisms of SP on the breast cancer.

Cancer

The relation between SP consumption and thyroid cancer has studied in Southeast Asian men and women. Population who consumed SP was shown to have two fold increase in the risk of developing thyroid cancer compared to the other populations (137). In a pivotal study, it was observed that thyroid carcinoma was induced in rats by feeding the iodine-deficient diet fortified with 30% SP. The mechanism by which SP induced cancer was discussed earlier (75). The elevated level of TSH resulted from the inhibition of thyroid peroxidase by the soy isoflavone lead to increase the follicular cell proliferation and developed the neoplastic changes (137).

Other toxic effects

Nephrotoxicity

SP inhibits protein tyrosine kinase and affects the kidney in abnormal immune conditions (138). It was observed that in the autoimmune disease, the consumption of SP (20%) and soybean oil (5%) has an adverse effect on kidney by elevating the proteinuria and serum creatinine level with decrease in creatinine clearance (139, 140). Further detailed investigations are mandatory to reveal the underlying mechanisms of nephrotoxic effect of SP.

Hepatotoxicity

Multiple studies have highlighted the hepatotoxic effect of SP and soy peptides (141, 142). Interestingly, SP extract is used as a "natural" hormone-replacement therapy pill. However, it is also important to mention that the active compounds present in SP alter the catabolism of oestrogen 4-hydroxyestrone (4OHE1), a putatively potent genotoxic oestrogen. Accumulation of 4OHE1 attributed to hepatotoxicity in long term treatment (143). Single-nucleotide polymorphism of lignans involved in the oestrogen pathway damaged the liver enzymes and the hepatic tissue architecture (144). Following consumption of SP, hepatotoxicity was observed in cheetahs (91).

Allergic Reaction

The incidence of SP is rising alarmingly. It is important to increase the public awareness about the SP as it is widely consumed by both vegetarian and non-vegetarian community. In the ranking of allergic food, the position of the soy derivatives is listed among as one of the most important allergic foods (135). According to a recent report by Jarmila et al. (145) one-third of the patients with atopic dermatitis had a history of consuming SP.

Conclusion

In summary, soy protein (SP) is isolated from soy bean, peas, and clover. Although SP has some therapeutic effects, high intake or prolong consumption of SP or raw soy bean is injurious to health. In the present review, it was observed that SP has adverse effects on the endocrine glands; carcinogenic effects on the pancreas, breast and thyroid gland; toxic effects on kidney and liver; and allergic reactions. This review also highlighted the effect of soy isoflavone- genistein on the multiple organs. It is believed that the present review will increase the public awareness of consumption of SP and will draw the attention of many researchers to specify the detailed analyses on SP and its compounds. Many of the studies stated the negative effects of the consumption of SP based on animal models and it is a questionable fact that the same result may be implied to humans. Nevertheless, the mechanism of action remains the same. It would be interesting to observe the effect soy protein consumption in humans in larger clinical trials to be conducted in the future.

Conflict of interest

The authors have declared that no competing interests exist.

References

1. Kendall HW, Beachy R, Eisner T, et al. World food supplies, in Bioengineering of Crops. Report of the Word Bank Panel on Transgenic Crops. ESDS Monograph Series: 23. Ed by World Bank, Washington, DC, 1997; 3-10
2. Pinstrup-Andersen P, Pandya-Lorch, Rosegrant MW. World food prospects: critical issues for the early 21st century, in Food Policy Report. Inter Food Policy Res, 1997
3. Liener IE, Possible adverse effects of soybean anticarcinogens. J Nutr 1995; 125:744S-50
4. Zhang HY, Yi JQ, Piao XS, Li PF, et al. The metabolizable energy value, standardized ileal digestibility of amino acids in soybean meal, soy protein concentrate and fermented soybean meal, and the application of these products in early-weaned piglets. Asian-Australas J Anim Sci 2013; 26(5):691-9
5. Singh A, Meena M, Kumar D, et al. Structural and Functional Analysis of Various Globulin Proteins from Soy Seed. Crit Rev Food Sci Nutr 2013 [Epub ahead of print]
6. Kristen SM, Soy Protein. J Perinat Educ 2003; 12(3):42-5
7. Babji AS, Mohd. Yusof S. The nutritional value of some processed meat products in Malaysia. Mal J Nutr 1995; 1: 83-94
8. Ramadhan K, Huda N, Ahmad R. Physicochemical characteristics and sensory properties of selected Malaysian commercial chicken burgers. Int Food Res J 2011; 18(4): 1349-57
9. Food and Drug Administration. Food labelling, health claims, soy protein, and coronary heart disease. Fed Reg 1999; 57:699-733
10. John W, Erdman Jr. Soy Protein and Cardiovascular Disease: A Statement for Healthcare Professionals From the Nutrition Committee of the AHA. Circulation 2000; 102: 2555-9
11. Riaz MN. Uses and benefits of soy fibre. Cereal Foods World 2001; 46(3): 98-100
12. Cui C, Zhao M, Yuan B, et al. Effect of pH and pepsin limited hydrolysis on the structure and functional properties of soybean protein hydrolysates. J Food Sci 2013;78(12): C1871-7
13. Rozenfeld P, Docena GH, Añón MC, et al. Detection and identification of a soy protein component that cross-reacts with caseins from cow's milk. Clin Exp Immunol 2002; 130 (1): 49-58
14. Lusas EW, Riaz MN. Soy Protein Products: Processing and use. J Nutr 1995; 125(3): 573S-80
15. Yanpeng Z, Wei Z, Ruijin Y, et al. Preparation and functional properties of protein from heat-denatured soybean meal assisted by steam flash-explosion with dilute acid soaking. J Food Engineering 2013; 119(1): 56-64
16. Wang H, Johnson LA, Wang T, Preparation of soy protein concentrate and isolate from extruded-expelled soybean meals. J Am Oil Chemist Soc 2004; 81(7): 713-7
17. Sewekow E, Kessler LC, Seidel-Morgenstern A, et al. Isolation Of Soyabean Protein P34 From Oil Bodies Using Hydrophobic Interaction Chromatography. BMC Biotechnology 2008; 8:27-32
18. Rickert DA, Meyer MA, Murphy PA. Effect of Extraction pH and Temperature on Isoflavone and Saponin Partitioning and Profile during Soy Protein Isolate Production. J Food Sci 2004; 69:C623-31
19. L'hocine L, Boye JI, Arcand Y. Composition and Functional Properties of Soy Protein Isolates Prepared Using Alternative Defatting and Extraction Procedures. J Food Sci 2006; 71:C137-45
20. L'Hocine L, Boye JI, Munyana C. Detection and quantification of soy allergens in food: study of two commercial enzyme-linked immunosorbent assays. J Food Sci 2007; 72(3): C145-53
21. Allaoua A, Youness Z, Joyce IB. Stability and Physical Properties of Emulsions Prepared with and without Soy Proteins. J Food Res 2012; 1(1): 254-67
22. He L, Jun L, Danshi Z, et al. Preparation of Soy Protein Isolate (SPI)-Pectin Complex Film Containing Cinnamon Oil and Its Effects on Microbial Growth of Dehydrated Soybean Curd (Dry Tofu). J Food Process Preserv 2014; 38(3): 1371-6
23. Russin TA, Arcand Y, Boye JI. Particle Size Effect on Soy Protein Isolate Extraction. J Food Process Preserv 2007; 31: 308-19
24. Vishwanathan KH, Govindaraju K, Singh V, et al. Production of okara and soy protein concentrates using membrane technology. J Food Sci 2011; 76(1): E158-64
25. Shallo HE, Rao A, Ericson AP, et al. Preparation of Soy Protein Concentrate by Ultrafiltration. J Food Sci 2001; 66:242-6
26. Na Cao, Yuhua Fu, Junhui He. Preparation and physical properties of soy protein isolate and gelatin composite films. Food Hydrocolloids 2007; 21(7): 1153-62
27. Messina M, Ho S, Alekel DL. Skeletal benefits of soy isoflavones: a review of the clinical trial and epidemiologic data. Curr Opin Clin Nutr Metab Care 2004; 7: 649-58
28. Messina M, McCaskill-Stevens W, Lampe JW. Addressing the soy and breast cancer relationship: review, commentary, and workshop proceedings. J Natl Cancer Inst 2006; 98(18): 1275-84
29. Ozawa Y, Sato H, Nakatani A, et al. Chemical Composition of Soybean Oil Extracted from Hypocotyle-Enriched Soybean Raw Material and Its Cholesterol Lowering Effects in Rats. J Oleo Sci 2001; 50:217-23
30. Law M. Plant sterol and stanol margarines and health. BMJ 2000; 320:861-4
31. Lönnerdal B. Nutritional aspects of soy formula. Acta Paediatr Suppl 1994; 402: 105-8
32. Azadbakht L, Shakerhosseini R, Atabak S, et al. Beneficiary effect of dietary soy protein on lowering plasma levels of lipid and improving kidney function in type II diabetes with nephropathy. Eur J Clin Nutr 2003; 57(10):1292-4
33. Broihier K. Fighting cancer with phytochemicals. Food Processing 1997; 58(11): 41-2
34. Friedman M, Brandon DL. Nutritional and health benefits of soy proteins. J Agric Food Chem 2001; 49(3):1069-86
35. Mark M, Virginia M. The Role of Soy in Vegetarian Diets. Nutrients 2010; 2(8): 855-8
36. Yetley EA, Park YK. Diet and heart disease: health claims. J Nutr 1995; 125:679S-85
37. Mateu-de Antonio J, Grau S, Luque S, et al. Albert I, Ribes E. Comparative effects of olive oil-based and soyabean oil-based emulsions on infection rate and leucocyte count in critically ill patients receiving parenteral nutrition. Br J Nutr 2008; 99(4):846-54
38. Carroll KK, Kurowska EM. Soy consumption and cholesterol reduction: review of animal and human studies. J Nutr 1995; 125: 594S-97
39. Sirtori CR, Evan R, Lovati MR. Soybean protein diet and plasma cholesterol: from therapy to molecular mechanisms. Ann NY Acad Sci 1993; 676:188-201
40. Tikkanen MJ, Adlecreutz H. Dietary soy-derived isoflavone phytoestrogens. Could they have a role in dietary heart disease prevention? Biochem Pharmacol 2000; 60:1-5

41. Potter SM, Bakhit RM, Esse-Sorlie DL, et al. Depression of plasma cholesterol in men by consumption of baked products containing soy protein. Am J Clin Nutr 1993; 58:501-6
42. Widhalm K, Brazda G, Schneider B, Effect of soy protein diet versus standard low fat, low cholesterol diet on lipid and lipoprotein levels in children with familial or polygenic hypercholesterolemia. J Pediatr 1993; 123:30-4
43. Bakhit RM, Klein BP, Essex-Sorlie DL, et al. Intake of 25 g of soybean protein with or without soybean fibre alters plasma lipids in men with elevated cholesterol concentration. J Nutr 1994; 124:213-22
44. Liener IE. Implications of antinutritional components in soybean foods. Crit Rev Food Sci Nutr 1994; 34: 31-67
45. Pusztai A, Ewen SWB, Carvalho AFFU, et al. Dietary lectins affect hormone balance of the body and modulate its general metabolism, in Gastrointestinal Tract and Endocrine System, Ed by Singer MV, Ziegler R and Rohr G. Kluwer Academic, Dordrecht 1994; 457-62
46. Pusztai A, Ewen SWB, Grant G, et al. Relationship between survival and binding of plants lectins during small intestinal passage and their effectiveness as growth factors. Digestion 1990; 46:308-16
47. Pusztai A, Grant G, Brown DS, et al. Lectins binding to the gut wall are growth factors for the pancreas: nutritional implications for transgenic plants, in Lectins—Biomedical Perspectives, Ed by Pusztai A and Bardocz S. Taylor and Francis, London, 1995; 141-54
48. Armour JC, Perera RLC, Buchan WC, et al. Protease inhibitors and lectins in soya beans and effects of aqueous heat-treatment. J Sci Food Agri 1998; 78:225-31
49. Anderson RL, Wolf WJ. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. J Nutr 1995; 125:581s-8
50. Rubio LA, Grant G, Daguid T, et al. Organs relative weight and plasma amino acid concentrations in rats fed diets based on whole legume (faba bean, lupin, chickpea, defatted soybean) seed meals or their fractions. J Sci Food Agric 1999; 79:187-94
51. Jindal S, Soni GL, Singh R. Biochemical and histopathological studies in albino rats fed on soybean lectin. Nutr Rep Int 1984; 29:95-106
52. Taliercio E, Loveless TM, Turano MJ, et al. Identification of epitopes of the â subunit of soybean â-conglycinin that are antigenic in pigs, dogs,rabbits and fish. J Sci Food Agric 2014; 94(11): 2289-94
53. Proot S, Biourge V, Teske E, et al. Soy protein isolate versus meat-based low-protein diet for dogs with congenital portosystemic shunts. J Vet Intern Med 2009; 23(4):794-800
54. Torisky RS, Griffin JD, Yenofsky RL, et al. A single gene (Eu4) encodes the tissue-ubiquitous urease of soybean. Mol Gen Genet 1994; 242(4): 404-14
55. Torisky RS, Polacco JC. Soybean Roots Retain the Seed Urease Isozyme Synthesized during Embryo Development. Plant Physiol 1990; 94(2): 681-9
56. Torisky RS, Griffin JD, Yenofsky RL, et al. A single gene (Eu4) encodes the tissue-ubiquitous urease of soybean. Mol Gen Genet 1994; 242:404-14
57. Werner AK, Medina-Escobar N, Zulawski M, et al. The ureide-degrading reactions of purine ring catabolism employ three amidohydrolases and one aminohydrolase in Arabidopsis, soybean, and rice. Plant Physiol 2013;163(2):672-81
58. Polacco JC, Winkler RG. Soybean leaf urease: a seed enzyme? Plant Physiol 1984; 74:800-3
59. Kumar S, Kayastha AM. Studies of histidine residues in soybean (Glycine max) urease. Protein Pept Lett 2012;19(6):657-62
60. Vasconcelos IM, Maia ABA, Siebra EA, et al. Nutritional study of two Brazilian soybean (Glycine max) cultivars differing in the content of antinutritional and toxic proteins. J Nutr Biochem 2001; 12:55-62
61. Vasconcelos IM, Trentin A, Guimar˜aes JA, et al. Purification and physicochemical characterization of soyatoxin, a novel toxic protein isolated from soybeans (Glycine max). Arch Biochem Biophys 1994; 312:357-66
62. Siebra EA. SBTX, uma nova toxina proteica das sementes de soja (Glycine max (L.) Merr) cv BR-10. Isolamento ecaracteriza¸c˜ao. MSc Thesis, Universidade Federal do Cear´a, Fortaleza, 1998
63. Messina M, Nagata C, Wu AH. Estimated Asian adult soy protein and isoflavone intakes. Nutr Cancer 2006; 55: 1-12
64. Taylor CK, Levy RM, Elliott JC, et al. The effect of genistein aglycone on cancer and cancer risk: a review of in vitro, preclinical, and clinical studies. Nutr Rev 2009; 67(7): 398-415
65. Delclos KB, Newbold R. NTP toxicity report of reproductive dose range-finding study of genistein (CAS No. 446-72-0) administered in feed to sprague-dawley rats. Toxic Rep Ser 2007; 79: 1-98
66. Adachi A, Horikawa T, Shimizu H, et al. Soybean beta-conglycinin as the main allergen in a patient with food-dependent exercise-induced anaphylaxis by tofu: food processing alters pepsin resistance. Clin Exp Allergy 2009; 39(1): 167-73
67. Krishnan HB, Kim WS, Jang S, et al. All three subunits of soybean beta-conglycinin are potential food allergens. J Agric Food Chem 2009; 57(3):938-43
68. Holzhauser T, Wackermann O, Ballmer-Weber BK, et al, Soybean (Glycine max) allergy in Europe: Gly m 5 (beta-conglycinin) and Gly m 6 (glycinin) are potential diagnostic markers for severe allergic reactions to soy. J Allergy Clin Immunol 2009; 123(2): 452-8
69. Liu X, Feng J, Xu ZR, et al. Oral allergy syndrome and anaphylactic reactions in BALB/c mice caused by soybean glycinin and beta-conglycinin. Clin Exp Allergy 2008; 38(2):350-6
70. Wilson S, Blaschek K, de Mejia E. Allergenic proteins in soybean: processing and reduction of P34 allergenicity. Nutr Rev 2005; 63(2):47-58
71. Singh P, Sharma S, Rath SK. Genistein induces deleterious effects during its acute exposure in Swiss mice. Biomed Res Int 2014; 20:615-7
72. Divi RL, Chang HC, Doerge DR. Anti-thyroid isoflavones from soybean: isolation, characterization, and mechanisms of action. Biochem Pharmacol 1997; 54(10):1087-96
73. Sosić-Jurjević B, Filipović B, Ajdzanović V, et al. Suppressive effects of genistein and daidzein on pituitary-thyroid axis in orchidectomized middle-aged rats. Exp Biol Med 2010; 235(5): 590-8
74. Šošić-Jurjević B, Filipović B, Wirth EK, et al. Soy isoflavones interfere with thyroid hormone homeostasis in orchidectomized middle-aged rats. Toxicol Appl Pharmacol 2014; 278(2):124-34
75. Doerge DR, Chang HC. Inactivation of thyroid peroxidase by soy isoflavones, in vitro and in vivo. J Chromatogr B Analyt Technol Biomed Life Sci 2002; 777(1-2): 269-79
76. Doerge DR, Sheehan DM. Goitrogenic and estrogenic activity of soy isoflavones. Environ Health Perspect 2002; 110(3):349-53

77. Ford JA, Clark, JR, Walters SG, et al. Estrogenic effects of genistein on reproductive tissues of ovariectomized gilts. J Animal Sci 2006; 84: 834-42
78. Chang HC, Doerge DR. Dietary genistein inactivates rat thyroid peroxidase in vivo without an apparent hypothyroid effect. Toxicol Appl Pharmacol 2000; 168(3): 244-52
79. Power KA, Thompson LU. Can the combination of flaxseed and its lignans with soy and its isoflavones reduce the growth stimulatory effect of soy and its isoflavones on established breast cancer? Mol Nutr Food Res 2007; 51(7):845-56
80. McCarver G, Bhatia J, Chambers C, et al. NTP-CERHR expert panel report on the developmental toxicity of soy infant formula. Birth Defects Res B Dev Reprod Toxicol. 2011; 92(5):421-68
81. Messina MJ, Loprinzi CL. Soy for breast cancer survivors: a critical review of the literature. J Nutr 2001; 131(11): 3095S-108
82. Messina M. A Close look at Soybeans. 4th Edition Nutritional Prospectives 2003; 176-7
83. Hsieh CY, Santell RC, Haslam SZ, Helferich WG. Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells in vitro and in vivo. Cancer Res 1998; 58(17): 3833-8
84. Allred CD, Ju YH, Allred KF, et al. Dietary genistin stimulates growth of estrogen-dependent breast cancer tumors similar to that observed with genistein. Carcinogenesis 2001; 22(10):1667-73
85. Trock BJ, Hilakivi-Clarke L, Clarke R. Meta-analysis of soy intake and breast cancer risk. J Natl Cancer Inst 2006; 98(7):459-71
86. Allred CD, Allred KF, Ju YH, et al. Dietary genistein results in larger MNU-induced, oestrogen-dependent mammary tumours following ovariectomy of Sprague-Dawley rats. Carcinogenesis 2004; 25(2):211-8
87. Yokooji T, Kawabe Y, Mori N, Muraka et al. mi T. Effect of genistein, a natural soy isoflavone, on the pharmacokinetics and intestinal toxicity of irinotecan hydrochloride in rats. J Pharm Pharmacol 2013; 65(2): 280-91
88. Verheus M, van Gils CH, Keinan-Boker L. Plasma phytoestrogens and subsequent breast cancer risk. J Clin Oncol 2007; 25(6):648-55
89. Zhang WZ, Wen-Ming CUI, Xin Z, et al. Subchronic Toxicity Study on Soy Isoflavones in Rats. Biomed Env Sci 2009; 22(3): 259-64
90. File SE, Hartley DE, Alom N, Rattray M. Soya phytoestrogens change cortical and hippocampal expression of BDNF mRNA in male rats. Neurosci Lett 2003; 338(2):135-8
91. Setchell KD, Gosselin SJ, Welsh MB, et al. Dietary estrogens-a probable cause of infertility and liver disease in captive cheetahs. Gastroenterol 1987; 93:225-33
92. Tachibana N, Matsumoto I, Fukui K, et al. et al. Intake of soy protein isolate alters hepatic gene expression in rats. J Agri Food Chem 2005; 53: 4253-7
93. Hogervorst E, Sadjimim T, Yesufu A, et al. High tofu intake is associated with worse memory in elderly Indonesian men and women. Dementia Geriatric Cogn Dis 2008; 26(1):50-7
94. Roccisano D, Henneberg M, Saniotis A. A possible cause of Alzheimer's dementia - industrial soy foods. Med Hypotheses 2014; 82(3): 250-4
95. Mittag D, Vieths S, Vogel L, et al. Soybean allergy in patients allergic to birch pollen: clinical investigation and molecular characterization of allergens. J Allergy Clin Immunol 2004; 113(1):148-54
96. L'Hocine L, Boye JI. Allergenicity of soybean: new developments in identification of allergenic proteins, cross-reactivities and hypoallergenization technologies. Crit Rev Food Sci Nutr 2007; 47(2):127-43
97. McClain RM, Wolz E, Davidovich A, et al. Genetic toxicity studies with genistein. Food Chem Toxicol 2006; 44: 42-55
98. Nikaido Y, Danbara N, Tsujita-Kyutoku M. Effects of prepubertal exposure to xenoestrogen on development of estrogen target organs in female CD-1 mice. In Vivo 2005; 19(3):487-94
99. Nikaido Y, Yoshizawa K, Danbara N. Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring, Reprod Toxicol 2004; 18(6):803-11
100. Padilla-Banks E, Jefferson WN, Newbold RR. Neonatal exposure to the phytoestrogen genistein alters mammary gland growth and developmental programming of hormone receptor levels. Endocrinol 2006; 147:4871-82
101. Choi EJ, Lee BH. Evidence for genistein mediated cytotoxicity and apoptosis in rat brain. Life Sci 2004; 75: 499-509
102. Ingham RR, Gesualdi DA, Toth CR, et al. Effects of genistein on growth and development of aquatic vertebrates. Bull Environ Contam Toxicol 2004; 72: 625-31
103. Guo TL, Germolec DR, Musgrove DL, et al.. Myelotoxicity in genistein-, nonylphenol-, methoxychlor-, vinclozolin- or ethinyl estradiol-exposed F1 generations of Sprague-Dawley rats following developmental and adult exposures. Toxicol 2005; 211: 207-19
104. Jin Y, Wu H, Cohen EM, Wei J, et al. Genistein and daidzein induce neurotoxicity at high concentrations in primary rat neuronal cultures. J Biomed Sci 2007; 14: 275-84
105. Kwack SJ, Kim KB, Kim HS, et al. Risk assessment of soybean-based phytoestrogens. J Toxicol Environ Health 2009; 72(21-22):1254-61
106. Krebs EE, Ensrud KE, MacDonald R, et al. Phytoestrogens for treatment of menopausal symptoms: a systematic review. Obstet Gynecol 2004; 104:824-36
107. MacGregor CA, Canney PA, Patterson G, et al. A randomized double-blind controlled trial of oral soy supplements versus placebo for treatment of menopausal symptoms in patients with early breast cancer. Eur J Cancer 2005; 41:708-14
108. Petrakis NL. Stimulatory influence of soy protein isolate on breast secretion in pre- and post-menopausal women. Cancer Epidemiol Biomarkers 1996; 5: 785-794
109. Delclos KB, Bucci TJ, Lomax LG, Latend et al. Effects of dietary genistein exposure during development on male and female CD (Sprague-Dawley) rats. Reprod Toxicol 2001; 15:647-663
110. Michael McClain R, Wolz E, Davidovich A, Bausch J. Genetic toxicity studies with genistein. Food Chem Toxicol 2006; 44(1):42-55
111. Ueda M, Niho N, Imai T, Shibutani M, et al. Lack of significant effects of genistein on the progression of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in ovariectomized Sprague-Dawley rats. Nutr Cancer 2003; 47(2): 141-7
112. Shu XO, Jin F, Dai Q, et al. et al. Soyfood intake during adolescence and subsequent risk of breast cancer among Chinese women. Cancer Epidemiol Biomarkers Prev 2001; 10:483-8
113. Nishio K, Niwa Y, Toyoshima H, et al. et al. Consumption of soy foods and the risk of breast cancer: findings from the Japan Collaborative Cohort (JACC) Study. Cancer Causes Control 2007; 18: 801-8

114. McMichael-Phillips DF, Harding C, et al. Morton M, Roberts SA, Howell A, Potten CS, et al. Effects of soy protein supplementation on epithelial proliferation in the histologically normal human breast. Am J Clin Nutr 1998; 68:1431S-5S
115. Lee BJ, Jung EY, Yun YW, et al. et al. Effects of exposure to genistein during pubertal development on the reproductive system of male mice. J Reprod Dev 2004; 50(4):399-409
116. Jung EY, Lee BJ, Yun YW, et al. et al. Effects of exposure to genistein and estradiol on reproductive development in immature male mice weaned from dams adapted to a soy-based commercial diet. J Vet Med Sci 2004; 66(11):1347-54
117. Ekor M, Emerole GO, Farombi EO. Phenolic extract of soybean (Glycine max) attenuates cisplatin-induced nephrotoxicity in rats. Food Chem Toxicol 2010;48(4):1005-12
118. Shireen KF Jr, Pace RD, Egnin M, Prakash C. Effects of dietary proteins and trypsin inhibitor on growth and lipid metabolism in hamsters. Malays J Nutr 2001;7(1):1-14
119. Nitsan Z. A comparative study of the nutritional and physiological significance of raw and heated soya beans in chicks and goslings. Br J Nutr. 1977; 37(1): 81-91
120. De La Barca AM, Wall A, López-Díaz JA. Allergenicity, trypsin inhibitor activity and nutritive quality of enzymatically modified soy proteins. Int J Food Sci Nutr. 2005; 56(3): 203-11
121. Gu C, Pan H, Sun Z, Qin G. Effect of soybean variety on anti-nutritional factors content, and growth performance and nutrients metabolism in rat. Int J Mol Sci. 2010 Mar 9;11(3):1048-56
122. Divi RI, Doerge DR. Anti-thyroid isoflavones from soybean: isolation, characterization and mechanisms of action. Biochem Pharmacol 1997; 54: 1087-96
123. Jeffrye SB, Jones DS. "Chapter 32 Clinical Approaches to Hormonal and Neuroendocrine Imbalances: Cellular Messaging, Part II- Tissue Sensitivity and Intracellular Response." Textbook of Functional Medicine. Ed. David S. Jones. Gig Harbor, WA.: Institute for Functional Medicine 2005; 590-609
124. Chao WX. Health Effects of Soy Protein and Isoflavones in Humans. J Nutr 2008; 138: 1244S-9S
125. Valentine Tom. 'If you eat soy, watch your thyroid function: new study', Quarterly newsletter of Carotec, Inc., Autumn 1997
126. Chorazy PA, Himelhoch S, Hopwood NJ, et al. Persistent hypothyroidism in an infant receiving a soy formula: case report and review of the literature. Pediatrics 1995; 96:148-50
127. Jabbar MA, Larrea J, Shaw RA. Abnormal thyroid function tests in infants with congenital hypothyroidism: the influence of soy-based formula. J Am Coll Nutr 1997; 16:280-2
128. Ikeda T, Nishikawa A, Imazawa T, et al. Dramatic synergism between excess soybean intake and iodine deficiency on the development of rat thyroid hyperplasia. Carcinogenesis 2000; 21: 707-713
129. Son HY, Nishikawa A, Ikeda T, et al. Lack of effect of soy isoflavone on thyroid hyperplasia in rats receiving an iodine-deficient diet. Jpn J Cancer Res 2001; 92:103-8
130. Xiao CW, L'Abbe´ MR, Gilani S, et al. Dietary soy protein isolate and isoflavones modulate hepatic thyroid hormone receptors in rats. J Nutr 2004; 134:743-9
131. Liener IE. Soybean protease inhibitors and pancreatic carcinogenesis. J Nutr. 1996;126(2):582-5
132. Nakai I, Deshmukh AR, Liener IE, et al. Effect of soybean flour on exocrine function in rat pancreas transplant with bladder drainage. Pancreas. 1992;7(3):334-8
133. Grant G, Dorward PM, Pusztai A. Pancreatic enlargement is evident in rats fed diets containing raw soybeans (Glycine max) or cowpeas (Vigna unguiculata) for 800 days but not in those fed diets based on kidney beans (Phaseolus vulgaris) or lupinseed (Lupinus angustifolius). J Nutr 1993; 123(12): 2207-15
134. Messina M, Wu AH. Perspectives on the soy-breast cancer relation. Am J Clin Nutr 2009; 89:1673-95
135. Wiwanitkit V. Soy milk toxicity: Additional concern. J Postgrad Med 2013; 59(4): 333
136. Parkin DM, Muir CS, Whelan SL, et al. Cancer incidence in five continents. IARC Scientific Publ. 1992. Lyon, France: IARC
137. Ralli M. Soy and the Thyroid: Can This Miracle Food Be Unsafe? Nutr Noteworthy 2003; 6(1): 1-5
138. Hong Y, Wang T, Huang C, et al. Soy isoflavones supplementation alleviates disease severity in autoimmune-prone MRL-lpr/lpr mice. Lupus 2008; 17(9):814-21
139. Zhao JH, Sun SJ, Horiguchi H, et al. A soy diet accelerates renal damage in autoimmune MRL/Mp-lpr/lpr mice. Int Immunopharmacol 2005; 5(11):1601-10
140. Jin X, Lok E, Caldwell D, et al. Dietary fats altered nephrotoxicity profile of methylmercury in rats. J Appl Toxicol 2009; 29(2):126-140
141. Wiwanitkit V. Excessive consumption of soybean milk and unexplained hepatitis. J Postgrad Med 2012; 58:226-7
142. Senthilkumaran S, Elangovan N, Menezes RG, Thirumalaikolundusubramanian P. Soy milk toxicity: Cause for concern. J Postgrad Med 2013; 59:249-50
143. Russo J, Russo IH. Genotoxicity of steroidal estrogens. Trends Endocrinol Metab 2004; 15:211-4
144. Usui T. Pharmaceutical prospects of phytoestrogens. Endocr J 2006; 53:7-20
145. Jarmila C, Kveçtuše E, Karel E, et al. Soy allergy in patients suffering from atopic dermatitis. Indian J Dermatol 2013; 58:325