Compositional Changes in Trypsin Inhibitors, Phytic Acid, Saponins and Isoflavones Related to Soybean Processing[1,2]

ROBERT L. ANDERSON AND WALTER J. WOLF

Biopolymer Research, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604

ABSTRACT Soybeans are high in protein but also contain a number of minor constituents traditionally considered to be antinutritional factors. These include trypsin inhibitors, phytic acid, saponins and isoflavones. These compounds are now thought to have beneficial biological effects in the diet, such as lowering blood cholesterol or preventing cancer. Soybean processing changes the content of these minor constituents in various ways. This review discusses the changes in content of trypsin inhibitors, phytic acid, saponins and isoflavones as soybeans are processed into the conventional protein ingredients, flours, concentrates and isolates, as well as some of the traditional Oriental soybean foods. J. Nutr. 125: 581S–588S, 1995.

INDEXING KEY WORDS:

- *soybean* • *isoflavone* • *phytic acid*
- *saponin* • *trypsin inhibitor*

Processing of soybeans, whether by traditional or Western methods, is necessary to produce animal feed ingredients, food for human consumption or functional protein ingredients intended for incorporation into a wide variety of human foods. During the course of soybean processing to change properties, the chemical composition may change as well. In this review we summarize the compositional changes that occur in trypsin inhibitors, phytate, saponins and isoflavones as a result of processing.

Proteins

The content and properties of oil and protein are the primary determinants of whole soybean value. Oil and protein are separated from each other early in most Western processing; in some traditional Oriental soy foods, oil and protein remain together whereas in others they are separated. Soybean oil is not included in this discussion.

The proteins of soy contribute to the nutritional value of foods and feeds and are responsible for a number of functional characteristics in a variety of foods. Heating is necessary to attain maximum nutritional value and to modify functional properties of soybean protein.

Kakade et al. (1973), using immobilized trypsin to remove trypsin inhibitors from a raw soy flour extract followed by rat feeding experiments, concluded that approximately 40% of the growth-depressing effect of the unheated extract was due to the trypsin inhibitors. The remainder of the growth depression was attributed to poor digestibility of the undenatured protein.

Trypsin inhibitors

Although soybean protein products require heat processing to achieve maximum nutritional value, partly through trypsin inhibitor denaturation, trypsin inhibitors also display anticarcinogenic properties (Messina and Barnes 1991). The predominant trypsin inhibitors in soybeans and derived materials are proteins and they are located, for the most part, with the main storage proteins in the protein bodies of the cotyledon (Horisberger et al. 1986, Horisberger and Tacchini-Vonlanthen 1983a, Horisberger and Tacchini-Vonlanthen 1983b). This being the case, trypsin inhibitors tend to fractionate with the milieu of storage proteins as soybeans are processed into ingredients and foods. There are exceptions to this generality that

[1] Presented at the First International Symposium on the Role of Soy in Preventing and Treating Chronic Disease, held in Mesa, AZ, February 20–23, 1994. The symposium was sponsored by Protein Technologies International, the soybean growers from Nebraska, Indiana and Iowa and the United Soybean Board. Guest editors for this symposium were Mark Messina, 1543 Lincoln Street, Port Townsend, WA 98368, and John W. Erdman, Jr., Division of Nutritional Sciences, University of Illinois, Urbana, IL 61801-3852.

[2] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

TABLE 1

Trypsin inhibitor content of soybeans and processed soy protein products

Type	Trypsin inhibitor activity[1]	
	mg/g sample	*mg/g protein*
Whole soybeans	16.7–27.2[2,3]	34.7–122.6[3,4]
Raw flour[5]	28–32	57.8
Toasted flour[5]	7.9–9.4	15.9
Concentrate[6]	5.4–7.3	8.4–11.2
Isolate[5,6]	1.2–30.0	1.4–29.4

[1] Measured by trypsin inhibition.
[2] Hafez (1983).
[3] Values, originally expressed as trypsin units inhibited, were converted to trypsin inhibitor units with the relationship that 1 μg of pure trypsin has an activity of 1.9 trypsin units.
[4] Kakade et al. (1972).
[5] Rackis et al. (1985).
[6] Peace et al. (1992).

will be discussed as they arise. Rackis et al. (1986) reviewed the literature through 1985 on protease inhibitor content of some plant foods.

Based on 19 varieties and strains, whole soybeans were reported to contain 17–27 mg of trypsin inhibitor/g (Hafez 1983) according to activity assays with the results expressed as though all activity resided in the main species, the Kunitz trypsin inhibitor (**Table 1**). Soybeans are approximately 40% protein and Kakade et al. (1972), after analyzing 108 strains and cultivars, reported they contain 35–123 mg trypsin inhibitor/g of protein. When soybeans are processed into raw defatted flour, a process in which the hulls and oil are removed, none of the trypsin inhibitors are removed but they become enriched in the flour to levels of 28–32 mg/g of flour, which equates to approximately 58 mg trypsin inhibitor/g of protein (Table 1).

Trypsin inhibitors, being proteins, are subject to denaturation and inactivation by heat. Heated or toasted flours are produced having a range of trypsin inhibitor activities depending upon their intended use. A fully toasted soy flour will have a trypsin inhibitor level of only 8–9 mg/g of flour or approximately 16 mg/g of protein. Soy protein concentrates are produced by washing defatted flours with either aqueous ethanol or acidified water. In these processes oligosaccharides and other constituents are removed but most of the protein is not; the protein content is therefore increased to ≥65%. The ranges of trypsin inhibitor activity are given in Table 1, 5–7 mg trypsin inhibitor/g of concentrate or 8–11 mg/g of concentrate protein. Soy protein isolates are processed to contain ≥90% protein. Depending upon the amount of washing of the precipitated curd as well as the trypsin inhibitor content of the starting soybeans, isolates contain trypsin inhibitor levels in the range of 1–30 mg/g or, because nearly all of this product is protein, the same range per gram of protein (Table 1).

Oriental and other soybean foods are generally low in trypsin inhibitor (**Table 2**). Soy sauce, produced by enzymic or acidic hydrolysis of a mixture of soybeans and wheat, is low in trypsin inhibitor content at 0.3 mg/g of sample or 3.3 mg/g of protein equivalent. It is unlikely that these values represent activity from the protein inhibitors but the origin of the measured activity is uncertain. Miso is a product made by fermentation of soaked and steamed soybeans. Table 2 shows a value of 4 mg/g of sample or 23 mg/g of protein for miso. However, when lipids are removed from miso by extraction, the trypsin inhibitor level falls to 4 mg/g of protein and it will decrease further if the miso is heated.

Tofu, which is made from heated soybean milk by coagulation of the proteins with a salt such as calcium sulfate followed by cooling, has a trypsin inhibitor content (9 mg/g of protein, Table 2) near to that associated with a fully toasted soy flour (16 mg/g of protein, Table 1). Soy infant formula is made from soy protein isolate with the addition of other nutrients. The trypsin inhibitor level in these products falls in the range of 0.3–3 mg/g of sample or 2–16 mg/g of protein (Table 2).

DiPietro and Liener (1989) differentiated between the Kunitz and Bowman-Birk inhibitor content of soybean flours, concentrates, isolates and a variety of foods (**Table 3**). The Kunitz soybean trypsin inhibitor present was quantitated by rocket immunoelectrophoresis and the Bowman-Birk inhibitor was measured by chymotrypsin inhibition. Soy flours were found to contain from 1.1 to 19.6 mg/g and from <0.2 to 4.9 mg/g of Kunitz and Bowman-Birk inhibitors, respectively. Concentrate samples contained a range of <0.5–6.1 mg/g of Kunitz and <0.2–1.0 mg/g of Bowman-Birk inhibitors whereas soy protein isolate inhibitor contents ranged from <0.5 to 3.6 mg/g and from 0.3 to 2.0 mg/g, respectively, for Kunitz and Bowman-Birk inhibitors. A dehydrated soymilk was found to

TABLE 2

Trypsin inhibitor content of Oriental and other soy foods

Type	Trypsin inhibitor activity[1]	
	mg/g sample	*mg/g protein*
Soy sauce[2]	0.3	3.3
Miso (unextracted)[2]	4.1	22.9
Miso (defatted)[2]	—	4.4
Tofu[2]	0.6	9.2
Soy infant formula[3]	0.3–2.7	2.2–15.5

[1] Measured by trypsin inhibition.
[2] Doell et al. (1981).
[3] Peace et al. (1992).

TABLE 3

***Kunitz and Bowman-Birk trypsin inhibitors in soy protein products and some foods*[1]**

Sample	Kunitz inhibitor	Bowman-Birk inhibitor
	mg/g sample	
Flours	1.1–19.6	<0.2–4.9
Concentrates	<0.5–6.1	<0.2–1.0
Isolates	<0.5–3.6	0.3–2.0
Dehydrated soymilk	11.3	1.9
Wheat-soy pancake mix	1.0	0.4

[1] Kunitz inhibitor measured by rocket immunoelectrophoresis; Bowman-Birk inhibitor measured by chymotrypsin inhibition. Data from DiPietro and Liener (1989).

contain 11.3 mg Kunitz inhibitor/g and 1.9 mg/g Bowman-Birk inhibitor whereas a wheat-soy pancake mix had 1.0 mg/g of Kunitz and 0.4 mg/g of Bowman-Birk inhibitors. A variety of other soy containing foods were found to have no more than approximately 1% of the quantity of these inhibitors present in raw soy flours. The ratio of Kunitz inhibitor to Bowman-Birk inhibitor ranged between 0.5 and 8.1.

Phytic acid

Phytic acid has long been recognized to interfere with the absorption of minerals, especially zinc. Recent work suggests that phytic acid may have anticarcinogenic properties as well (Messina and Barnes 1991); consequently, there is renewed interest in this compound. Phytic acid content of soybeans can vary considerably (**Table 4**). Field type cultivars, which are the usual items of commerce, fall into the range of 1.0–2.3%.

Table 4 also lists the various fractions and protein products that are derived from soybeans. The hulls contain only 0.1–0.5% phytic acid whereas the hypocotyl contains 0.9%. However, the hulls (8%) plus the hypocotyl (2%) represent only approximately 10% of the seed; thus the bulk of the phytic acid occurs in the cotyledons. The cotyledons contain 1.6% phytic acid, which is localized in the protein bodies and appears to be distributed between soluble protein-phytate salts and insoluble globoid inclusions (Prattley and Stanley 1982). Full-fat flour, which is essentially the cotyledon fraction, contains 1.5–1.8% phytic acid. Values for defatted flours, concentrates and isolates likewise are in the range of 1–2% phytic acid despite considerable additional processing as compared with full-fat flours. Texturized flours and concentrates are also in this range; phytic acid is stable to cooking (Davies and Reid 1979) and probably is not degraded during texturization by extrusion.

TABLE 4

Phytic acid content of soybeans and processed soy protein products

Product	Phytic acid content	Reference
	g/100 g dry matter	
Soybeans[1]	1.00–1.47	Lolas et al. (1976)
Soybeans[2]	1.39–2.30	Raboy et al. (1984)
Hulls	0.12	Lehrfeld (1989)
Hulls	0.50	Sutardi and Buckle (1985)
Hypocotyl	0.88	Thompson and Erdman (1982)
Cotyledons	1.58	Beleia et al. (1993)
Full-fat flour	1.51–1.81	Ranhotra et al. (1974)
Defatted flour	1.62–1.85	Ranhotra et al. (1974)
Defatted flour	1.30–1.63	Schuster and Bodwell (1980)
Textured flour	1.10–2.02	Davies and Reid (1979)
Textured vegetable protein[3]	0.95–1.63	Harland and Oberleas (1977)
Concentrate	1.25–2.17	Ranhotra et al. (1974)
Textured concentrate	1.48–1.50	Harland and Oberleas (1977)
Isolate[4]	0.97–1.69	Schuster and Bodwell (1980)
Isolate	1.61–2.00	Honig et al. (1984)
Spun isolate fiber	1.48	O'Neill et al. (1980)

[1] Fifteen field-type varieties.
[2] Thirty-eight field-type varieties.
[3] Authors did not specify whether products were derived from flours, concentrates or isolates.
[4] Fifteen samples.

Data on Oriental soybean foods are given in **Table 5**. The phytic acid values fall in the range of 1–3% except for okara and tempeh, which contain only 0.5–1.2% phytic acid. The lower values for tempeh are attributed to partial hydrolysis by phytase elaborated by *Rhizopus oligosporus*, the organism used in fer-

TABLE 5

Phytic acid content of Oriental soy foods

Product	Phytic acid content	Reference
	g/100 g dry matter	
Soymilk	1.68	Omosaiye and Cheryan (1979)
Soymilk	1.83	Beleia et al. (1990)
Tofu	1.5–2.5	van der Riet et al. (1989)
Tofu	1.96–2.88	Schaefer and Love (1992)
Okara (residue from soymilk)	0.5–1.2	van der Riet et al. (1989)
Tempeh	0.69–0.73	Sutardi and Buckle (1985)
Tempeh	0.96	Sudarmadji and Markakis (1977)

menting cooked soybeans to produce tempeh (Sudarmadji and Markakis 1977; Sutardi and Buckle 1985).

Saponins

Most listings of soybean antinutritional factors in the past included saponins, although with little or no justification. Toxicity was attributed to them simply by analogy with saponins from other sources that, indeed, are toxic. However, most reviewers have ignored studies reported 25 years ago showing that feeding soybean saponins to chicks, rats and mice failed to inhibit growth even when fed at levels three to five times those of a normal soybean meal diet (Ishaaya et al. 1969). More recently, hypocholesterolemic and anticarcinogenic effects have been attributed to soybean saponins (Messina and Barnes 1991). Consequently, there is justification for removing saponins from the list of antinutritional factors in soybeans (Liener 1981).

Soybean saponins are complex compounds consisting of nonpolar triterpenoid alcohol aglycones linked to one or more polar oligosaccharides resulting in molecules with amphiphilic properties similar to detergents. Early studies resulted in confusion about the number and structures of the aglycones but it is now believed that only three aglycones, soyasapogenol A, B and E, occur in intact saponins. Other soyasapogenols reported earlier evidently were artifacts of hydrolysis conditions (Price et al. 1987). Five major saponins have been isolated and structurally characterized (Kitagawa et al. 1985a, Kitagawa et al. 1985b, Kitagawa et al. 1988b). The main complexity arises from the structures of the attached oligosaccharides, which may be free or acetylated (Kitagawa et al. 1988a). Additional complexity has been reported recently in the form of 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one attached to the C-22 hydroxyl group of soyasapogenol B; this group is removed with the formation of maltol when the saponins are heated (Kudou et al. 1993).

This diversity of structure and composition has also made it difficult to analyze for soybean saponins. A variety of methods have been reported but at present high performance liquid chromatography techniques are available for analysis of the intact saponins (Kudou et al. 1993) as well as the aglycones obtained upon acid hydrolysis of the saponins (Ireland et al. 1986). The latter method assumes that the ratio of aglycone to sugars is 1:1 and this is not always justified (Price et al. 1986); hence, results may not always agree with analysis of intact saponins. Attention is also called to the fact that some of the early results obtained by thin layer chromatography (Fenwick and Oakenfull 1981, Fenwick and Oakenfull 1983) gave values that were almost 10-fold too high. These high values apparently resulted from impurities and use of a nonspecific detection reagent (Curl et al. 1985). Structural studies and problems of analysis are reviewed by Price et al. (1987).

TABLE 6

Saponin content of soybeans and processed soy protein products

Product	Saponin content
	g/100 g dry matter
Soybeans[1]	0.22–0.33
Soybeans[2]	0.53
Soybeans[3]	0.09–0.32
Cotyledons[4]	0.21–0.27
Hypocotyl-radicle[4]	1.67–1.98
Seed coat (hulls)[4]	0
Full-fat flour, unheated[2]	0.47
Full-fat flour, heated[2]	0.53
Protein concentrate[2]	0
Protein isolate[2]	0.81

[1] Kitagawa et al. (1984).
[2] Ireland et al. (1986).
[3] Shiraiwa et al. (1991).
[4] Taniyama et al. (1988).

Table 6 shows saponin contents for soybeans, seed parts and derived protein products. Soybeans contain 0.1–0.5% saponins; the high end of the range depends on a value determined by analysis of the sapogenols. The cotyledons contain 0.2–0.3% saponins whereas the level in the hypocotyl approaches 2%. However, the hypocotyl represents only approximately 2% of the seed; hence, it has little effect on the composition of flours and related products that may contain this fraction. The seed coat (hull) contains no saponins. Unheated and heated full-fat flours contain approximately 0.5% saponins; the results indicate that the saponins are heat stable (except for such changes as reported by Kudou et al. 1993). No saponins were detected in a protein concentrate prepared by aqueous alcohol extraction as would be expected based on the solubility properties of the saponins. Isolates have been reported to contain 0.8% saponins. Although water soluble, the saponins apparently complex with the proteins and hence are retained with the proteins during the isolation process.

Saponin content of several Oriental foods (**Table 7**) such as soymilk, yuba, tofu and natto is in the range of 0.3–0.4%, about the same as in soybeans. Okara (the insoluble residue remaining after preparation of soymilk), miso (fermented soybean paste) and natto (fermented, cooked soybeans) tend to be somewhat lower in saponin content at 0.1–0.3%.

With the exception of alcohol-extracted concentrates, the saponins tend to remain with the protein products derived from soybeans. Fermentation, as in the preparation of miso, results in some degradation of the saponins and thus a lower saponin content as compared with soybeans.

TABLE 7

Saponin content of Oriental soybean foods

Product	Saponin content
	g/100 g dry matter
Soymilk[1]	0.26–0.31
Soymilk[2]	0.39
Yuba (dried soymilk film)[2]	0.41
Okara (residue from soymilk)[2]	0.10
Tofu[2]	0.30–0.33
Miso[2]	0.15
Natto[2]	0.25

[1] Ireland et al. (1986).
[2] Kitagawa et al. (1984).

Isoflavones

Although long considered to be antinutritional factors, because of their estrogenic properties soybean isoflavones are now of great interest because of their anticancer activities (see references in Coward et al. 1993). Soybeans contain two major isoflavones, genistein and daidzein, and a minor one, glycetein. In the seed, the isoflavones are present primarily as β-glucosides and a portion of the glucosides also is substituted on the C-6 hydroxyl of the glucose by a malonyl group, especially in the hypocotyl (Kudou et al. 1991), which represents only approximately 2% of the seed. Acetyl derivatives also have been reported (Farmakalidis and Murphy 1985; Wang and Murphy 1994a, Wang and Murphy 1994b) but these may be degradation products resulting from decarboxylation of the malonylisoflavones during extraction and workup of the extracts (Horowitz and Asen 1989).

Table 8 shows low and high values for isoflavone contents of soybean varieties reported by Wang and Murphy (1994b); these values range from 1200 to 4200 μg/g. For additional information on variations of isoflavones with soybean varieties, crop year and location of growth, see Eldridge and Kwolek (1983) and Wang and Murphy (1994b). The glucoside forms of the isoflavone predominate. In the variety Pioneer 9111, for example, 6″-*O*-malonylgenistin accounts for 42% of the total followed by genistin (21%) and 6″-*O*-malonyldaidzin (16%). In contrast, the aglycones (genistein, daidzein and glycetein) make up only 2% of the total isoflavones.

Soy flour, made by defatting dehulled flakes and grinding, has an isoflavone profile approximating that of soybeans. Granules and textured vegetable protein products contain appreciable quantities of the 6″-*O*-acetyl derivatives of genistin and daidzin, presumably because of heat treatment during processing, which decarboxylates the malonyl glucosides.

Protein isolates contain reduced levels of isoflavones (600–1000 μg/g) as compared with soybeans and flours as a result of the aqueous processing used during manufacture. A concentrate made by aqueous alcohol extraction is very low in isoflavones (73 μg/g) because the isoflavones are soluble in aqueous alcohol and are thus largely removed during processing. Additional data on flours, concentrates and isolates were reported by Eldridge (1982), Coward et al. (1993) and Franke et al. (1994), although these workers did not report values for the acetylated or malonylated derivatives.

Isoflavone contents of traditional Oriental and Western soybean foods are summarized in **Table 9**. Roasted soybeans are low in the malonyl derivatives but higher in the acetyl forms as a result of the heat treatment. Beverage products approximate the composition for soybeans except for reduced levels of the malonyl derivatives. Tofu is greatly reduced in isoflavones because of the aqueous processing during manufacture. Tempeh is somewhat lower in isoflavone

TABLE 8

Isoflavone content of soybeans and processed protein products[1,2]

	Glucoside			Malonyl			Acetyl			Aglycone			
Product	Din	Gin	Glin	Din	Gin	Glin	Din	Gin	Glin	Dein	Gein	Glein	Total
		μg/g			μg/g			μg/g			μg/g		
Vinton 81 soybeans	234	326	66	121	290	58	tr	5	25	10	19	22	1176
Pioneer 9111 soybeans	637	888	60	690	1756	72	tr	2	33	28	30	19	4216
Soy flour	147	407	41	261	1023	57	tr	1	32	4	22	19	2014
Soy granule	727	870	132	106	193	60	72	135	48	12	27	22	2404
TVP A	507	634	146	93	192	60	187	320	90	12	29	25	2295
Protein isolate A	tr	137	34	20	100	39	6	nd	33	63	136	53	621
Protein isolate B	88	301	49	18	88	36	74	215	46	11	36	25	987
Protein concentrate	tr	18	31	nd	tr	nd	tr	1	nd	nd	nd	23	73

[1] Data from Wang and Murphy (1994a, 1994b).
[2] Din = daidzin; Gin = genistin; Glin = glycetin; Dein = daidzein; Gein = genistein; Glein = glycetein; tr = trace; nd = not detected.

TABLE 9

***Isoflavone content of traditional Oriental and Western soybean foods*[1,2]**

Product	Glucoside			Malonyl			Acetyl			Aglycone			Total
	Din	Gin	Glin	Din	Gin	Glin	Din	Gin	Glin	Dein	Gein	Glein	
		μg/g			μg/g			μg/g			μg/g		
Roasted soybeans	460	551	68	45	63	72	397	743	102	39	69	52	2661
Instant beverage A	444	775	76	39	144	40	5	24	33	18	44	20	1662
Instant beverage D	525	745	75	98	259	44	12	26	33	30	50	21	1918
Tofu	25	84	8	159	108	nd	8	1	29	46	52	12	531
Tempeh	2	65	14	255	164	nd	11	nd	nd	137	193	24	865
Bean paste (miso)	nd	96	21	nd	nd	19	1	2	nd	271	183	54	647
Honzukuri miso	72	123	18	nd	nd	22	1	11	nd	34	93	15	389
Fermented bean curd	nd	tr	nd	nd	nd	nd	nd	nd	nd	143	223	23	389
Soy hot dog	35	67	15	12	42	15	tr	4	14	8	16	8	236
Soy bacon	tr	27	14	tr	5	12	tr	3	nd	26	48	9	144
Tempeh burger	36	158	18	25	nd	nd	nd	1	nd	34	96	18	386
Tofu yogurt	42	80	12	61	79	nd	nd	tr	nd	tr	3	5	282
Soy Parmesan	tr	tr	nd	26	tr	nd	tr	tr	36	tr	6	20	88
Cheddar cheese A	tr	nd	12	nd	tr	nd	tr	tr	19	tr	4	8	43
Cheddar cheese B	16	46	17	67	7	nd	nd	tr	27	tr	9	8	197
Mozzarella cheese	7	33	15	17	6	nd	nd	9	19	tr	8	9	123
Flat noodle	tr	6	nd	15	37	37	nd	tr	nd	tr	13	19	127

[1] Data from Wang and Murphy (1994a).
[2] Din = daidzin; Gin = genistin; Glin = glycetin; Dein = daidzein; Gein = genistein; Glein = glycetein; tr = trace; nd = not detected.

content than tofu and contains elevated levels of the aglycones formed by enzymatic hydrolysis during fermentation. Similar trends are noted for bean paste (miso) and the other fermented soy foods.

Content of isoflavones in Western-style soy foods falls into the range of 43 μg/g for cheddar cheese–like product to 386 μg/g for tempeh burger. The values for these foods are considerably lower than those of soybeans because soy is only one of several ingredients used, including fat and water. Coward et al. (1993) and Dwyer et al. (1994) also report analyses for Oriental and Western soy foods.

It is apparent that processing generally does not remove the isoflavones from soybean protein products and foods, except for concentrates prepared by alcohol extraction. The form may be altered by heat treatment and by enzyme reactions during fermentation but the isoflavones remain. Lowering of isoflavone contents of some soybean foods also results from dilution because of the addition of other ingredients, e.g., salt in miso.

Conclusions

As is the case with many of the foods we consume today, soybean processing is useful and necessary to destroy or remove undesirable constituents, make nutrients more accessible or to improve palatability. However, processing toward these ends also leads to changes in the composition of the various soybean materials compared with whole soybeans. These changes may be intentional, as in the case of heating to diminish trypsin inhibitor activity; the result of differential solubilities, as when isoflavones and saponins are extracted into ethanol during the washing required to produce some concentrates; or they may be effected as a result of binding to the protein components throughout processing, as appears to occur with saponins and phytic acid. With the exception of the trypsin inhibitors, the compounds reviewed here generally are stable to processing of soybeans as practiced today. It should not therefore be difficult to maintain approximately the naturally occurring levels in the seed in many processed products if future studies dictate that these compounds are desirable in our diet.

LITERATURE CITED

Beleia, A., Ida, E. I. & Lethi, T. T. (1990) Phosphorus and phytic acid distribution during soy milk processing. Arq. Biol. Tecnol. 33: 623–629.

Beleia, A., Thu Thao, L. T. & Ida, E. I. (1993) Lowering phytic phosphorus by hydration of soybeans. J. Food Sci. 58: 375–377, 388.

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

Curl, C. L., Price, K. R. & Fenwick, G. R. (1985) The quantitative estimation of saponin in pea (*Pisum sativum* L.) and soya (*Glycine max*). Food Chem. 18: 241–250.

Davies, N. T. & Reid, H. (1979) An evaluation of the phytate, zinc, copper, iron and manganese contents of, and Zn availability from, soya-based textured-vegetable-protein meat-substitutes or meat-extenders. Br. J. Nutr. 41: 579–589.

DiPietro, C. M. & Liener, I. E. (1989) Soybean protease inhibitors in foods. J. Food Sci. 54: 606–609, 617.

Doell, B. H., Ebden, C. J. & Smith, C. A. (1981) Trypsin inhibitor activity of conventional foods which are part of the British diet and some soya products. Qual. Plant. Plant Foods Hum. Nutr. 31: 139–150.

Dwyer, J. T., Goldin, B. R., Saul, N., Gualtieri, L., Barakat, S. & Adlercreutz, H. (1994) Tofu and soy drinks contain phytoestrogens. J. Am. Diet. Assoc. 94: 739–743.

Eldridge, A. C. (1982) Determination of isoflavones in soybean flours, protein concentrates, and isolates. J. Agric. Food Chem. 30: 353–355.

Eldridge, A. C. & Kwolek, W. F. (1983) Soybean isoflavones: effect of environment and variety on composition. J. Agric. Food Chem. 31: 394–396.

Farmakalidis, E. & Murphy, P. A. (1985) Isolation of 6″-*O*-acetylgenistin and 6″-*O*-acetyldaidzin from toasted defatted soyflakes. J. Agric. Food Chem. 33: 385–389.

Fenwick, D. E. & Oakenfull, D. (1981) Saponin content of soya beans and some commercial soya bean products. J. Sci. Food Agric. 32: 273–278.

Fenwick, D. E. & Oakenfull, D. (1983) Saponin content of food plants and some prepared foods. J. Sci. Food Agric. 34: 186–191.

Franke, A. A., Custer, L. J., Cerna, C. M. & Narala, K. K. (1994) Quantitation of phytoestrogens in legumes by HPLC. J. Agric. Food Chem. 42: 1905–1913.

Hafez, Y. S. (1983) Nutrient composition of different varieties and strains of soybean. Nutr. Rep. Int. 28: 1197–1206.

Harland, B. F. & Oberleas, D. (1977) A modified method for phytate analysis using an ion-exchange procedure: application to textured vegetable proteins. Cereal Chem. 54: 827–832.

Honig, D. H., Wolf, W. J. & Rackis, J. J. (1984) Phytic acid and phosphorus content of various soybean protein fractions. Cereal Chem. 61: 523–526.

Horisberger, M., Clerc, M.-F. & Pahud, J.-J. (1986) Ultrastructural localization of glycinin and β-conglycinin in *Glycine max* (soybean) cv. Maple Arrow by the immunogold method. Histochemistry 85: 291–294.

Horisberger, M. & Tacchini-Vonlanthen, M. (1983a) Ultrastructural localization of Kunitz inhibitor on thin sections of *Glycine max* (soybean) cv. Maple Arrow by the gold method. Histochemistry 77: 37–50.

Horisberger, M. & Tacchini-Vonlanthen, M. (1983b) Ultrastructural localization of Bowman-Birk inhibitor on thin sections of *Glycine max* (soybean) cv. Maple Arrow by the gold method. Histochemistry 77: 313–321.

Horowitz, R. M. & Asen, S. (1989) Decarboxylation and exchange reactions in flavonoid glycoside malonates. Phytochemistry (Oxf) 28: 2531–2532.

Ireland, P. A., Dziedzic, S. Z. & Kearsley, M. W. (1986) Saponin content of soya and some commercial soya products by means of high-performance liquid chromatography of the sapogenins. J. Sci. Food Agric. 37: 694–698.

Ishaaya, I., Birk, Y., Bondi, A. & Tencer, Y. (1969) Soybean saponins IX. Studies of their effect on birds, mammals and cold-blooded organisms. J. Sci. Food Agric. 20: 433–436.

Kakade, M. L., Hoffa, D. E. & Liener, I. E. (1973) Contribution of trypsin inhibitors to the deleterious effects of unheated soybeans fed to rats. J. Nutr. 103: 1772–1778.

Kakade, M. L., Simons, N. R., Liener, I. E., & Lambert, J. W. (1972) Biochemical and nutritional assessment of different varieties of soybeans. J. Agric. Food Chem. 20: 87–90.

Kitagawa, I., Saito, M., Taniyama, T. & Yoshikawa, M. (1985a) Saponin and sapogenol. XXXVIII. Structure of soyasaponin A_2, a bisdesmoside of soyasapogenol A, from soybean, the seeds of *Glycine max* Merrill. Chem. Pharm. Bull. 33: 598–608.

Kitagawa, I. Saito, M., Taniyama, T. & Yoshikawa, M. (1985b) Saponin and sapogenol. XXXIX. Structure of soyasaponin A_1, a bisdesmoside of soyasapogenol A, from soybean, the seeds of *Glycine max* Merrill. Chem. Pharm. Bull. (Tokyo) 33: 1069–1076.

Kitagawa, I., Taniyama, T., Nagahama, Y., Okubo, K., Yamauchi, F. & Yoshikawa, M. (1988a) Saponin and sapogenol. XLII. Structures of acetyl-soyasponins A_1, A_2, and A_3, astringent partially acetylated bisdesmosides of soyasapogenol A, from American soybean, the seeds of *Glycine max* Merrill. Chem. Pharm. Bull. (Tokyo) 36: 2819–2828.

Kitagawa, I., Wang, H. K., Taniyama, T. & Yoshikawa, M. (1988b) Saponin and sapogenol. XLI. Reinvestigation of the structures of soyasapogenols A, B, and E, oleanene-sapogenols from soybean, structures of soyasponins I, II, and III. Chem. Pharm. Bull. (Tokyo) 36: 153–161.

Kitagawa, I., Yoshikawa, M., Hayashi, T. & Taniyama, T. (1984) Quantitative determination of soyasaponins in soybeans of various origins and soybean products by means of high performance liquid chromatography. Yakugaku Zasshi 104: 275–279.

Kudou, S., Fleury, Y., Welti, D., Magnolato, D., Uchida, T., Kitamura, K. & Okubo, K. (1991) Malonyl isoflavone glycosides in soybean seeds (*Glycine max* Merrill). Agric. Biol. Chem. 55: 2227–2233.

Kudou, S., Tonomura, M, Tsukamoto, C., Uchida, T. Sakabe, T., Tamur, N. & Okubo, K. (1993) Isolation and structural elucidation of DDMP-conjugated soyasaponins as genuine saponins from soybean seeds. Biosci. Biotechnol. Biochem. 57: 546–550.

Lehrfeld, J. (1989) High-performance liquid chromatography analysis of phytic acid on a pH-stable, macroporous polymer column. Cereal Chem. 66: 510–515.

Liener, I. E. (1981) Factors affecting the nutritional quality of soya products. J. Am. Oil Chem. Soc. 58: 406–415.

Lolas, G. M., Palamidis, N. & Markakis, P. (1976) The phytic acid-total phosphorus relationship in barley, oats, soybeans, and wheat. Cereal Chem. 53: 867–871.

Messina, M. & Barnes, S. (1991) The role of soy products in reducing risk of cancer. J. Natl. Cancer Inst. 83: 541–546.

Omosaiye, O. & Cheryan, M. (1979) Low-phytate, full-fat soy protein product by ultrafiltration of aqueous extracts of whole soybeans. Cereal Chem. 56: 58–62.

O'Neill, I.K., Sargent, M. & Trimble, M. L. (1980) Determination of phytate in foods by phosphorus-31 Fourier transform nuclear magnetic resonance spectrometry. Anal. Chem. 52: 1288–1291.

Peace, R. W., Sarwar, G. & Touchburn, S. P. (1992) Trypsin inhibitor levels in soy-based infant formulas and commercial soy protein isolates and concentrates. Food Res. Int. 25: 137–141.

Prattley, C. A. & Stanley, D. W. (1982) Protein-phytate interactions in soybeans. I. Localization of phytate in protein bodies and globoids. J. Food Biochem. 6: 243–253.

Price, K. R., Curl, C. L. & Fenwick, G. R. (1986) The saponin content and sapogenol composition of the seed of 13 varieties of legume. J. Sci Food Agric. 37: 1185–1191.

Price, K. R., Johnson, I. T. & Fenwick, G. R. (1987) The chemistry and biological significance of saponins in foods and feedingstuffs. Crit. Rev. Food Sci. Nutr. 26: 27–135.

Raboy, V, Dickinson, D. B. & Below, F. E. (1984) Variation in seed total phosphorus, phytic acid, zinc, calcium, magnesium, and protein among lines of *Glycine max* and *G. soja*. Crop Sci. 24: 431–434.

Rackis, J. J., Gumbmann, M. R. & Liener, I. E. (1985) The USDA trypsin inhibitor study. I. Background, objectives, and procedural details. Qual. Plant. Plant Foods Hum. Nutr. 35: 213–242.

Rackis, J. J., Wolf, W. J. & Baker, E. C. (1986) Protease inhibitors in plant foods: content and inactivation. In: Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods (Friedman, M., ed.), pp. 299–347. Plenum Press, New York, NY.

Ranhotra, G. S., Loewe, R. J. & Puyat, L. V. (1974) Phytic acid in soy and its hydrolysis during breadmaking. J. Food Sci. 39: 1023–1025.
Schaefer, M. J. & Love, J. (1992) Relationships between soybean components and tofu texture. J. Food Qual. 15: 53–66.
Schuster, E. M. & Bodwell, C. E. (1980) Phytic acid content of thirty-two commercial soy products. Fed. Proc. 39: 659 (abs.).
Shiraiwa, M., Harada, K. & Okubo, K. (1991) Composition and content of saponins in soybean seed according to variety, cultivation year and maturity. Agric. Biol. Chem. 55: 323–331.
Sudarmadji, S. & Markakis, P. (1977) The phytate and phytase of soybean tempeh. J. Sci. Food Agric. 28: 381–383.
Sutardi & Buckle, K. A. (1985) Phytic acid changes in soybeans fermented by traditional inoculum and six strains of *Rhizopus oligosporus*. J. Appl. Bacteriol. 58: 539–543.
Taniyama, T., Yoshikawa, M. & Kitagawa, I. (1988) Saponin and sapogenol. XLIV. Soyasaponin composition in soybeans of various origins and soyasaponin content in various organs of soybean. Structure of soyasaponin V from soybean hypocotyl. Yakugaku Zasshi 108: 562–571.
Thompson, D. B. & Erdman, J. W., Jr. (1982) Phytic acid determination in soybeans. J. Food Sci. 47: 513–517.
van der Riet, W. B., Wight, A. W., Cilliers, J. J. L. & Datel, J. M. (1989) Food chemical investigation of tofu and its byproduct okara. Food Chem. 34: 193–202.
Wang, H. & Murphy, P. A. (1994a) Isoflavone content in commercial soybean foods. J. Agric. Food Chem. 42: 1666–1673.
Wang, H. & Murphy, P. A. (1994b) Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year, and location. J. Agric. Food Chem. 42: 1674–1677.