Oxalate Content of Soybean Seeds (*Glycine max*: Leguminosae), Soyfoods, and Other Edible Legumes

Linda K. Massey,*,† Reid G. Palmer,‡ and Harry T. Horner§

Department of Food Science and Human Nutrition, Washington State University, Spokane, Washington 99201; and CICGR, ARS, USDA, Departments of Agronomy and Zoology/Genetics, and Department of Botany, Iowa State University, Ames, Iowa 50011

Consumption of soybeans and food products made from them is increasing because of their desirable nutritional value. However, the oxalate content of seeds from 11 cultivars of soybean showed relatively high levels of total oxalate from 0.67 to 3.5 g/100 g of dry weight. Oxalate primarily was found as calcium oxalate crystals. Thirteen tested commercial soyfoods contained between 16 and 638 mg of total oxalate per serving. These values compare to those of three other legume foods, peanut butter, refried beans, and lentils, which contained 197, 193, and 100 mg of total oxalate per serving, respectively. After oxalate has been absorbed from the diet, it cannot be metabolized and is excreted by the kidney into urine, where it binds to calcium forming an insoluble salt that may precipitate to form kidney stones. The amounts of total oxalate in soybean seeds, soy foods, and other common legume foods exceed current recommendations for oxalate consumption by individuals who have a history of calcium oxalate kidney/urinary stones. This study serves as the basis to find soybean cultivars lower in oxalate, which will have lower risk for kidney stone formation after human consumption.

Keywords: *Breakfast links; calcium; crystals; Glycine; Leguminosae; lentils; magnesium; oxalate; peanut butter; phosphorus; potassium; refried beans; soy burger patty; soynuts; soybean; textured vegetable protein; tofu; tempeh; soy yogurt; soy cheese*

INTRODUCTION

Human consumption of soybeans and products made from them is increasing, due to the reported health benefits of eating them in various prepared forms (*1*). Soybeans are regarded as a highly nutritious food source because of their excellent oil (61% polyunsaturated fat and 24% monounsaturated fat) and protein (eight essential amino acids) contents (*2*). The U.S. Food and Drug Administration has recently approved a label health claim that foods containing at least 6.25 g of soy protein per serving reduce the risk of cardiovascular disease (*3*). Besides these nutritional benefits, soybean seeds are rich in isoflavones (*4*), compounds that are being studied for their potential of reducing bone loss and breast cancer (*1*). Even though there are many studies dealing with the phytate content in seeds and its binding with calcium (*5*, *6*), the nutritional consequences of the oxalate (Ox) content of soybeans and its binding with calcium have been overlooked. This is likely due to the paucity of information regarding Ox content of soybeans and other legumes commonly consumed by humans, in contrast to spinach and rhubarb, other high-Ox-containing plants (*7*).

Zindler-Frank (*8*) reported that the majority of species in the Leguminosae family contain Ox in one or more of their tissues. Ilarslan et al. (*9*, *10*) studied the changing concentrations of Ox and calcium oxalate (CaOx) in developing soybean seeds (cv. Harosoy) and found the Ox level to be relatively high in the mature seeds. However, there have been no studies, to date, on the Ox content of soyfoods or Ox absorption and urinary excretion after the consumption of soybeans or food products produced from them. Absorbed Ox cannot be metabolized by humans and is excreted in the urine (*11*). In humans, high urinary Ox increases the risk of CaOx kidney stones because CaOx is poorly soluble in the urine. Saturated Ox in solution binds to Ca to form crystals that aggregate (*12*), often becoming large enough to block the urinary stream. Kidney stones not only are very painful but also caused an estimated $1.83 billion in direct medical costs in 1995 (*13*), not including the value of time lost from work.

In higher plants, Ox is derived from several different metabolic pathways (*14*, *15*), and different functions (*16*) have been suggested for both soluble Ox and CaOx, depending upon the plant species and plant organ. The Ox content of some plants is known to vary by genotype (*7*) and possibly within cultivars under different growth conditions. For example, the Ox content of star fruit (carambola) varied 10-fold among 15 commercial varieties (*17*), and 78 rhubarb cultivars (*18*) varied up to 72% among them. In contrast, the level of Ox in 10 commercial potato cultivars varied only 2-fold (*19*). We do not know of any studies that relate soil Ca content with soybean seed Ox content.

The purpose of this study was to assay selected cultivars of commercial soybean seeds (referred to hereafter as soybeans), a number of commercial soy

* Author to whom correspondence should be addressed [telephone (509) 358-7621; fax (509) 358-7627; e-mail massey@wsu.edu].

† Washington State University.

‡ CICGR, USDA, ARS, and Departments of Agronomy and Zoology/Genetics Iowa State University.

§ Department of Botany, Iowa State University.

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Table 1. Oxalate (Ox), Calcium (Ca), Phosphorus (P), Potassium (K), and Magnesium (Mg) in Mature Soybean Seeds of 14 Samples from 11 Different Cultivars (Milligrams per Gram of Dry Weight)

line/sample name		Ox			Ca	P	K	Mg
		insoluble	soluble	total				
Clark	1	12.7	2.1	14.8	2.54	6.95	29.5	2.76
Verede	2	4.8	1.9	6.7	1.85	7.55	32.4	2.85
Williams	3	9.9	1.6	11.5	2.13	7.37	29.7	2.81
Minsoy	4	9.0	1.4	10.4	2.34	7.86	29.1	2.96
Cutler 71	5	13.6	2.4	16.0	2.85	6.32	28.5	2.62
BSR 101	6	10.5	2.7	13.2	2.27	6.62	28.7	3.16
Hark	7	8.3	1.5	9.8	2.12	7.69	29.1	3.43
Harosoy	8	9.6	1.7	11.3	2.25	6.66	27.5	3.27
L95-1573 (1998)	9	16.5	3.0	19.5	2.25	7.31	30.6	2.96
L95-1573 (1999)	10	18.2	2.1	20.3	3.90	6.52	27.1	3.47
L95-1409 (1998)	11	19.7	2.8	22.5	2.59	7.56	30.0	2.96
L95-1409 (1999)	12	18.4	2.0	20.4	3.45	6.11	26.8	3.24
L95-1116 (1998)	13	32.2	2.8	35.0	3.89	8.28	30.4	3.44
L95-1116 (1999)	14	17.9	1.6	19.5	3.69	7.72	28.6	3.60

foods, and other edible legumes to ascertain their Ox and associated mineral contents for their potential to increase the risk of CaOx kidney stone formation in humans.

MATERIALS AND METHODS

Sources of Soybeans, Soy Foods, and Other Legume Products. Eleven different cultivars of *Glycine max* (L.) Merr. were analyzed for both Ox and mineral contents. Eight of the cultivars (cv. Clark, Verede, Williams, Minsoy, Cutler 71, BSR 101, Hark, and Harosoy) (see Table 1) were obtained from the soybean collection at Iowa State University, Ames, IA (ISU). Three additional cultivars, each collected over two growing seasons [L95-1573 (1998 and 1999), L95-1409 (1998 and 1999), and L95-1116 (1998 and 1999)] (see Table 1), were obtained from the soybean collection at the University of Illinois, Champaign−Urbana, IL. Thirteen soy food products and three other legume foods were purchased locally at groceries in Spokane, WA (see Table 2 for brand names).

Identification of CaOx in Situ. Developing young seeds and cotyledons from mature seeds of cv. Harosoy were cleared (cell cytoplasm removed leaving cell walls and CaOx crystals) according to procedures of Ilarslan et al. (*9*). Individual seed crystals were also isolated and analyzed by X-ray diffraction according to the procedure of Ilarslan et al. (*9*). The preparations were viewed with a compound light microscope between crossed polarizers, photographed, and scanned for visualization.

Mineral Analyses of Seeds. Three minerals, Ca, K, and Mg, were chosen for analysis because of their presumed association with Ox, and P was also included as an estimate of phytate. The seeds were dry-ashed at 490 °C, and then the ash was digested with aqua-regia (mixture of one part of nitric acid and three parts of hydrochloric acid). The mineral concentrations in the digests were assayed using inductive coupled argon plasma emission techniques (*20*, *21*) with a Thermo Jarrell-Ash ICP/IRIS with a charged injection device (*22*). The analyses were carried out in the Department of Horticulture Analytical Laboratory, ISU (Henry G. Taber, Professor-in-Charge).

Calcium Analyses of Soy Food Products. The Ca concentrations of soy foods were determined by blending 5 g of the soybeans or soy foods in 100 mL of 3 N HCl. The supernatant was analyzed using atomic absorption spectrophotometry (Perkin-Elmer model 2380) in lanthanum chloride (*23*). The soy food analyses were carried out at Washington State University (WSU).

Ox Analyses of Seeds. To measure total Ox, 20 seeds from each cultivar were dried in a 37 °C oven. Each sample of 20 seeds was divided into two equal lots, and each lot was ground separately in a Krup coffee grinder until the particles had reached a size that could no longer be homogenized. Each lot was weighed, and to determine total Ox, a 0.1 g sample was placed into a sterile 15 mL polypropylene centrifuge tube. Using a kit (Urinalysis Diagnostics Kit by Sigma, St. Louis, MO; kit 591), 4 mL of diluent was added to each weighed sample and vortexed for 30 s. Fifty microliters of this mixture was added to 1 mL of kit reagent A and 100 μL of kit reagent B (oxalate oxidase), and then it was incubated for 5 min at room temperature. One milliliter of the supernatant was measured against the sample blank using a spectrophotometer. An Ox standard and two urine controls were run to ensure kit quality and validity.

To determine insoluble Ox of each soybean line, 0.1 g of the dried, ground seeds was placed into a 15 mL centrifuge tube. Four milliliters of 0.5% bleach ($HClO_4$) was added to the tube, vortexed for ~30 s, and then incubated overnight. During the incubation time, disintegration of the cytoplasm occurred; however, the cell walls and the CaOx crystals were retained and later identified microscopically between crossed polarizers. After incubation, the samples were vortexed again and cen-

Table 2. Oxalate and Calcium Contents of Soy Foods and Other Legumes

	brand	serving size (g)	Ox (mg/g)	Ox (mg/serving)	Ca (mg/g)	Ca (mg/serving)
soynuts	Genisoy	28	14.0	392	2.00	56
soy beverage	Soy Dream	240	1.4	336	0.30	122 (40)[c]
tofu prepared with Ca	Azumaya	85	2.8	235	2.90	247 (100)[c]
tofu prepared with Ca	Small Planet	85	1.36	116	nd[d]	nd
tofu prepared with Mg	Small Planet	85	1.10	94	0.70	60
tofu prepared with Mg	Freda's	85	0.50	43	nd	nd
textured vegetable (soy) protein (1998) (hydrated)	Bob's Red Mill	85 (24 g dry)	5.84	496	2.40	204 (80)[c]
textured vegetable (soy) protein (1995) (hydrated)	Bob's Red Mill	85	7.50	638	nd	nd
breakfast links	MorningStar	45	1.54	69	0.31[a]	14
soy burger patty	Gardenburger	67	8.7	58	0.72[a]	48
soy yogurt	Brown Cow Farm	240	0.47	113	nd	nd (300)[c]
soy cheese, Cheddar flavor	TofuRella	28	0.56	16	nd	nd
tempeh	Soy Deli	83	0.28	23	0.93[b]	77 (60)[c]
peanut butter	Skippy	32	7.05	225	0.34[a]	10
lentils, cooked	Western Family	85	1.18	100	0.9[a]	16
refried beans	Rosarita	85	2.27	193	0.32[a]	27

[a] Data from Hands (*34*). [b] Data from *Soyfoods Guide*: soyfoods.com/Soyfoodsguide.pdf (accessed March 23, 2001). [c] Value given on food label of food package. [d] Not determined.

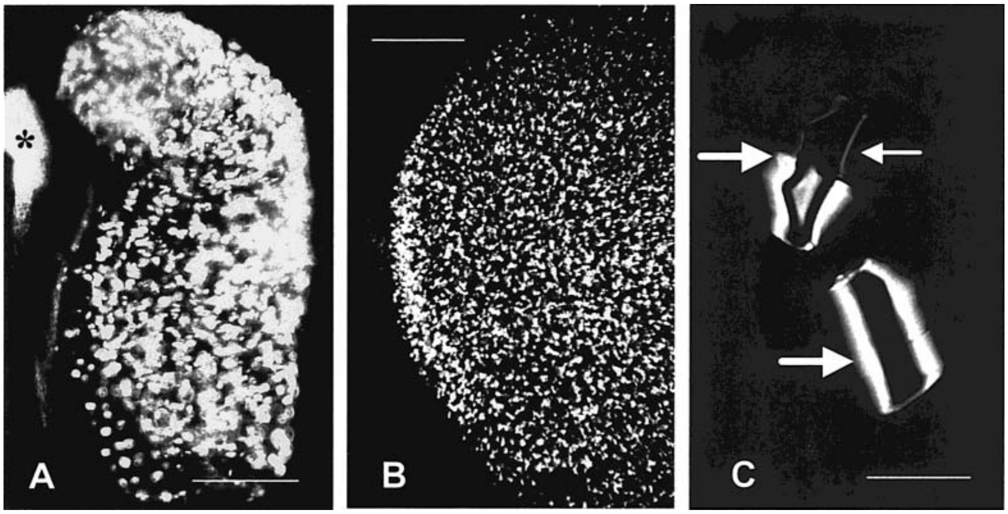

Figure 1. CaOx crystals in soybean seeds of cv. Harosoy and in isolation; all were observed between crossed polarizers with light microscopy: (A) cleared developing seed showing a large number of CaOx crystals throughout the integuments [asterisk (*) identifies hilum region; bar = 1 mm]; (B) portion of an isolated, cleared cotyledon from a mature seed showing a large number of CaOx crystals (bar = 200 μm); (C) two isolated CaOx−monohydrate prismatic, twin crystals [one straight (lower crystal) and one kinked (upper crystal)] displaying twin planes (larger arrows) and less birefringence of half of partially extinguished, kinked crystal (smaller arrow) (bar = 25 μm).

trifuged at ~2800 rpm. The supernatant was discarded, and the remaining sample was dried at 60 °C for 48 h. Samples were assayed for insoluble Ox. Soluble Ox was determined by subtracting the insoluble Ox from the total Ox. The analyses were carried out in the Department of Botany, ISU.

Ox Analyses of Soy Foods and Other Legume Products. Total Ox was extracted by three sequential extractions from each sample with 5% HCl acid according to the method of Ohkawa (*24*). Ox in the pooled supernatants was then determined by high-pressure liquid chromatography (HPLC) (*25*). Intra-assay variability of the Ox assay was 4.7%. Purified NaOx (Sigma) was the standard.

RESULTS

Whole cleared soybean cv. Harosoy seeds in mid-development displayed many CaOx crystals in their integuments (Figure 1A), and mature, isolated cotyledons (Figure 1B) displayed many CaOx crystals in their tissues. In developing seeds, the crystals occurred in both the integument tissues and young cotyledons. As the embryo and its cotyledons enlarged and began to fill the seed, the integuments became crushed and the crystals disappeared (*10*). Near or at maturity when the cotyledons make up most of the seed volume, the crystals appeared throughout the cotyledons. All crystals, regardless of location, were twin prismatics that were either straight (completely bright) or kinked (half partially extinguished) (Figure 1C), as shown between crossed polarizers. These crystals were determined to consist of only CaOx monohydrate in a previous study (*9*).

Total Ox, soluble Ox, insoluble Ox, and Ca, P, K, and Mg contents of 14 samples from 11 cultivars of soybean are shown in Table 1. Total Ox of the 14 samples ranged from 6.7 to 35.0 mg/g of dry weight, a ~5-fold difference. Soluble Ox was less variable, ranging from 1.4 to 3.0 mg/g, only a 2-fold difference. Soluble Ox ranged from 28% of total Ox in cv. Verede to 8% in cv. L95-1116. The analyses of the four minerals showed ranges of 1.85−3.90 mg/g for Ca, 6.11−8.28 mg/g for P, 26.8−32.4 mg/g for K, and 2.62−3.47 mg/g for Mg. Table 1 shows that cv. Verede had the lowest insoluble and total oxalate contents of any of the cultivars tested, and it also had the lowest Ca content. In contrast, cv. L95-1116 (1998) had the highest insoluble and total Ox contents and the highest Ca content. The other cultivars were intermediate for these values as well as for the other three minerals analyzed.

A comparison (Table 1) of the three cultivars grown over two consecutive years shows that (1) on the basis of the two-year average they have a greater total Ox content than the other eight cultivars and (2) total Ox varied from 4% for cv. L95-1573 to 44% for cv. L95-1116 between the two years. These results indicate that, among genetically different cultivars grown in the same general region under the same environmental conditions, the variation in total oxalate was substantial.

Ox and Ca contents of soy foods, lentils, beans, and peanut butter are shown in Table 2. The data clearly show that Ox is retained during the production of commercial soy foods. Soy nuts, basically an intact soybean (consisting mostly of cotyledons), have 14.0 mg of oxalate/g, similar to the 11 lines analyzed. Two batches of textured vegetable (soy) protein from the same processor, but purchased three years apart, were similar in Ox content at 7.50 mg/g (1995) and 5.84 mg/g (1998). The two Mg-precipitated tofu samples had less than half the Ox of the two Ca-precipitated tofu samples. It is unknown whether the salt used or the Ox content of the soybean seed line, or both, caused the difference. Soy cheese had the lowest Ox per serving at 16 mg, whereas tempeh had the lowest Ox content per gram, only 0.28 mg. It is unknown whether the fermentation of tempeh reduces the Ox content. Peanut butter, lentils, and refried beans had similar levels of Ox as soy foods, 225, 100, and 193 mg per serving, respectively.

DISCUSSION

The microscopic results and chemical tests for CaOx and Ox both clearly show that soybeans contain relatively high levels of Ox, primarily in the form of CaOx crystals in the seeds. The Ox content of the two highest Ox soybean cultivars was lower than that of spinach per gram, an average of 2.73 mg for L95-1116 and an average of 2.15 mg for L95-1409, compared to 6.57 mg (*26*) and 6.18 mg reported for spinach (*24*). Food products developed from soybeans, as well as other selected legumes, also contain high levels of Ox. The Ox content of peanut butter was similar to that of spinach with 6.20 mg/g but was 5 times higher than the 1.16 mg/100 g previously reported (*26*), suggesting that peanut cultivars may also have substantial variability in their Ox content. The Ox contents of lentils and refried beans were lower than that of peanut butter, with 1.04 and 2.27 mg/g, respectively. Soy beverage had 1.40 mg/g, whereas tofu prepared with Ca had only 1.36 mg/g. Breakfast links had 1.54 mg of oxalate/g.

There was a correlation between total Ox content and Ca content in the seeds of cv. Verede and cv. L95-1116 (1998), suggesting that as Ox increases, Ca binding increases. Some of the other cultivars showed much lower total Ox and Ca contents, suggesting that these, or other lower Ca- and Ox-containing cultivars, need to be studied for their suitability in making food products.

The critical factor in a food's effect on urinary Ox is not the total Ox but the amount of Ox that is absorbed from that food and ultimately excreted in the urine. It has generally been assumed that CaOx is not significantly absorbed in humans because it is virtually insoluble in aqueous solutions, only ∼1 mg/100 mL (*27*). In fact, diet therapy for CaOx kidney stones recommends the inclusion of high-Ca food in every meal to bind food Ox in order to reduce its absorption (*28*). However, Heaney and Weaver (*29*) reported that 10% of a load of CaOx was absorbed by healthy humans. Recently, Hanes et al. (*30*) reported passive uptake of CaOx as an intact molecule, at least in rats. Less than 2% of the load was absorbed via this pathway.

Even if CaOx is poorly absorbed, not all Ox in soy is Ca bound. Ox not bound to Ca is assumed to be bound to potassium and/or sodium and is referred to as soluble Ox, as these salts have solubilities in the range of 2.5−16.7 g/100 mL (*24*). Absorption of Ox from NaOx solutions has been reported to vary from 2 to 20% in healthy humans without gastrointestinal disease (*31*). Soluble Ox in soybean is relatively constant in the lines analyzed, ranging only from 14.7 to 29.9 mg/100 g. In the soybean cultivars analyzed for this study, molar amounts of Ox exceeded amounts of Ca by 8−28%. Oxalate also forms insoluble salts with magnesium, iron, and copper; these salts have solubilities only from 3 to 22 mg/100 mL (*27*). Oxalate may be the second compound in legumes that reduces iron absorption, as phytate binding apparently only accounts for about half of the inhibitory effect of soy on iron absorption (*32*).

No soy foods, and only peanut from all other legumes, have been individually tested for actual absorption of Ox in human feeding studies. Brinkley et al. (*26*) reported that 100 g of peanuts, which contained 116 mg of Ox, increased urinary Ox by 4.4 mg in the 8 h after their consumption, a 3.8% absorption of the total oxalate fed. Additional studies are in progress to determine the absorption of Ox from these and other soybean cultivars used for food products and widely available commercial soy foods.

The nutritional problem with the Ox content of soybean comes with using cultivars that are high in both soluble and insoluble Ox. Verede is a soybean cultivar developed for the fresh vegetable market, and the three L95 cultivars were bred for natto production (a cooked, whole soybean product fermented with *Bacillus natto*). Additional studies need to be conducted to determine the range of variation in Ox among all available cultivars of soybean. Then those cultivars with the lowest Ox content could be used for human feeding studies to determine whether the increases in urine Ox levels remain below an acceptable level. The absorptions of insoluble versus soluble Ox need to be compared as well as the effects of different processing procedures on Ox content and absorption.

Foods made from soybeans tested in this study varied widely in Ox levels, but all were high compared to the recommendations for patients with CaOx kidney stones. Currently, they are advised to limit their intake of foods containing >10 mg per serving, with a total intake not to exceed 50−60 mg per day (*33*). Under these guidelines, no soybean or soy food tested could be recommended for consumption by patients with a history of CaOx kidney stones.

There are no known low-oxalate cultivars at the present time. Soybean cultivars need to be studied for the several compounds of interest in human nutrition, including oxalate, phytate, and the isoflavones, to determine if there are cultivars that exist, or could be bred or genetically engineered, which could be nutritionally advantageous when compared to commonly used cultivars high in oxalate. Research is needed on how to control Ox synthesis without disrupting normal seed development and quality. With this knowledge, mutants might be found that have little or no Ox in them, leading to the possibility of their use in making suitable food products with lower Ox content. All of these issues are of great importance for soybeans to continue to be highly sought after for their nutritional value for human consumption.

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