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## **Oxalate and Phytate of Soy Foods**

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The consumption of foods made from soybeans is increasing because of their desirable nutritional value. However, some soy foods contain high concentrations of oxalate and/or phytate. Oxalate is a component of calcium oxalate kidney stones, whereas phytate is an inhibitor of calcium kidney stone formation. Thirty tested commercial soy foods exhibited ranges of 0.02-2.06 mg oxalate/g and 0.80-18.79 mg phytate/g. Commercial soy foods contained 2-58 mg of total oxalate per serving and 76–528 mg phytate per serving. Eighteen of 19 tofu brands and two soymilk brands contained less than 10 mg oxalate per serving, defined as a low oxalate food. Soy flour, textured vegetable soy protein, vegetable soybeans, soy nuts, tempeh, and soynut butter exhibited greater than 10 mg per serving. The correlation between oxalate and phytate in the soy foods was significant (r = 0.71, P < 0.001) indicating that oxalate-rich soy foods also contain higher concentrations of phytate. There also was a significant correlation, based on molar basis, between the divalent ion binding potential of oxalate plus phytate and calcium plus magnesium (r = 0.90, P < 0.001) in soy foods. Soy foods containing small concentrations of oxalate and moderate concentrations of phytate may be advantageous for kidney stone patients or persons with a high risk of kidney stones.

KEYWORDS: Calcium; kidney stones; oxalate; phytate; soy foods

#### INTRODUCTION

The human consumption of soy products is increasing, not only because of their high nutritional value but also because of their reported health benefits, such as reduction of cardiovascular disease, osteoporosis, and cancer risks (1-3). To the contrary, the recent finding that soybeans and soy foods have relatively high concentrations of oxalate (4) raises a concern regarding the risk of kidney stones for humans consuming soy foods. Oxalate binds with calcium in human urine to form a poorly soluble salt that is typically near saturation. Small crystal formation occurs when urinary calcium and oxalate concentrations reach supersaturation. A kidney stone forms when the calcium oxalate crystals aggregate or deposit on a "seed" crystal such as uric acid. In human urine, calcium oxalate supersaturation is influenced more by oxalate concentration than calcium concentration (5). About 2-8% of the oxalate in ingested soy foods is absorbed and excreted in the urine by healthy humans ( $\delta$ ). Kidney stone formers exhibit a higher rate of oxalate absorption than nonstone formers (7), and their increase in urinary oxalate after consumption of soy foods may be large enough to increase the risk of calcium oxalate precipitation, which potentially increases their risk of kidney stones.

Soybeans also contain high concentrations of myoinositol hexakisphosphate (phytate, 8). Phytate has long been considered as an antinutrient because it reduces the bioavailability of minerals in humans. However, studies suggest that phytate exhibits effective anticarcinogenic action against many types of cancers (9). In addition to its anticarcinogenic activity, phytate is also a potential inhibitor of calcium kidney stone formation, related to both its antioxidant activity (10) and its ability to inhibit crystal formation (11, 12). Although humans synthesize phytate, most urinary phytate originates from the diet and not from endogenous synthesis (13, 14).

Published research on oxalate content of soy foods is limited to Massey et al. (4). In this study, the concentration of oxalate in the 13 soy foods assayed exceeded the 10 mg limit that the American Dietetic Association recommends for oxalate consumption by individuals with a history of calcium oxalate kidney stones. The soy food list in that study is not that extensive, and there are many other soy foods now in the market. In addition

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Table 1. Total Oxalate, Phytate, Calcium (Ca), Magnesium (Mg), and Sodium (Na) Content in Tofus<sup>a</sup>

product	brand	serving size (g)	oxalate (mg/g)	oxalate (mg/svg)	phytate (mg/g)	phytate (mg/svg)	Ca (mg/g)	Mg (mg/g)	Na (mg/g)
tofu prepared with CaSO <sub>4</sub>	Pete's	85	0.02	1.7	0.89	76	0.26	0.17	0.01
tofu prepared with CaSO <sub>4</sub> <sup>b</sup>	Pete's	85	0.04	3.6	1.0	85	0.26	0.17	0.01
extra firm tofu prepared with CaCl <sub>2</sub>	Mori-Nu	85	0.03	2.6	1.85	157	0.35	0.26	0.51
soft tofu prepared with CaCl <sub>2</sub>	Mori-Nu	85	0.03	2.6	1.74	148	0.26	0.33	0.02
soft tofu prepared with CaCl <sub>2</sub> <sup>b</sup>	Mori-Nu	85	0.03	2.7	2.1	178.5	0.26	0.33	0.02
extra firm tofu prepared with MgCl <sub>2</sub>	Nasoya	85	0.03	2.6	1.77	150	0.32	0.67	0.02
firm tofu prepared with CaCl <sub>2</sub>	Mori-Nu	85	0.03	2.6	1.88	160	0.3	0.31	0.27
firm tofu prepared with CaSO <sub>4</sub>	Hinoichi	85	0.04	3.4	6.21	528	1.46	0.51	0.11
Japanese traditional tofu prepared with CaSO <sub>4</sub>	PacSoy	85	0.05	4.3	4.62	393	2.84	0.32	0.02
Japanese traditional tofu prepared with CaSO <sub>4</sub> <sup>b</sup>	PacSoy	85	0.09	8.1	2.1	178.5	2.84	0.32	0.02
Kinugoshi soft tofu prepared with CaSO <sub>4</sub>	PacSoy	85	0.05	4.3	1.57	133	1.08	0.3	0.02
fried tofu prepared with MgCl <sub>2</sub>	Soy Deli	85	0.05	4.3	5.96	507	0.83	1.22	0.03
tofu prepared with MgCl <sub>2</sub>	White Wave	85	0.06	5.1	4.09	348	1.88	0.79	0.03
silken tofu prepared with CaSO <sub>4</sub> + MgCl <sub>2</sub>	Azumaya	85	0.06	5.1	ND	ND	0.04	0.02	0.00
firm tofu prepared with CaCl <sub>2</sub>	Mori-Nu Lite	85	0.07	6	ND	ND	0.02	0.01	0.03
silken tofu prepared with CaSO <sub>4</sub> + MgCl <sub>2</sub>	Nasoya	85	0.07	6	ND	ND	0.06	0.02	0.00
firm tofu prepared with MgCl <sub>2</sub>	Small Planet	85	0.09	7.7	3.57	303	0.62	1.48	0.10
firm tofu prepared with CaSO <sub>4</sub> + MgCl <sub>2</sub>	Azumaya	85	0.09	7.7	ND	ND	0.11	0.02	0.00
firm tofu prepared with CaSO <sub>4</sub> + MgCl <sub>2</sub>	Nasoya	85	0.09	7.7	ND	ND	0.05	0.02	0.00
soft tofu prepared with CaSO <sub>4</sub> + MgCl <sub>2</sub>	Nasoya	85	0.1	8.5	ND	ND	0.12	0.02	0.00
extra firm tofu prepared with CaSO <sub>4</sub> + MgCl <sub>2</sub>	Azumaya	85	0.1	8.5	ND	ND	0.06	0.03	0.00
firm tofu prepared with MgCl <sub>2</sub>	Soy Deli	85	0.13	11.1	ND	ND	0.04	0.07	0.00

<sup>a</sup> On a wet weight or as purchased basis. ND, not determined. <sup>b</sup> The second sample was purchased 3 months after the first.

to the limited numbers of published oxalate concentrations of selected foods, the fact that many oxalate-rich foods contain high concentrations of phytate makes it difficult for dietitians to recommend consumption of specific foods to persons at risk for kidney stones. Anderson and Wolf (8) published a review on the changes in phytate concentration related to the processing of soybeans.

Analyses of both oxalate and phytate concentrations are necessary to determine the availability of soy foods that are nutritionally advantageous for kidney stone patients or persons at risk for kidney stone formation. The present study was undertaken to determine the concentrations of oxalate and phytate in selected commercial soy foods and to investigate the correlation between oxalate and phytate in soy foods. Moreover, calcium and magnesium were assayed to identify any association with oxalate and phytate in processed soy foods.

#### MATERIALS AND METHODS

**Sources of Soy Foods.** Forty commercial soy foods were purchased locally in Spokane, WA. Among these, three tofu brands from two different lots were purchased 3 months apart. Only foods with soy as the sole source of protein were purchased.

Oxalate Analysis of Soy Foods. A representative sample of 5-40 g from each soy food was homogenized with a suitable volume of 2 N HCl in a beaker using a homogenizer (PowerGen 125, Fisher Scientific, Pittsburgh, PA). Duplicate samples were analyzed as follows: 20 g of the homogenate was transferred to a 50 mL polypropylene centrifuge tube and centrifuged for 5 min at 5000 rpm. The supernatant was transferred to a 100 mL graduated cylinder. The residual was extracted twice more, and the supernatants were combined in the graduated cylinder. The final volume of the supernatant was made to 100 mL using distilled water. The oxalate concentration in each sample was determined using an oxalate oxidase commercial kit (Urinalysis Diagnostics Kit by Sigma, kit 591, St. Louis, MO). One milliliter of diluent was added to 1 mL of supernatant in a culture tube and vortexed for 30 s. The pH was adjusted to 6 using 10 N NaOH. The mixture was transferred to a 15 mL centrifuge tube containing 300 mg of charcoal and vortexed for 5 min followed by centrifugation for 5 min at 5000 rpm. To remove charcoal traces, the resultant supernatant was transferred to a 2 mL centrifuge vial and centrifuged for 5 min at 10000 rpm. Ten microliters of the final supernatant was

added to 200  $\mu$ L of kit reagent A and 20  $\mu$ L of kit reagent B (oxalate oxidase) in a microplate well, and the mixture was held for 5 min at room temperature. The absorbance of the sample was determined at 590 nm against the sample blank and the oxalate standard using a microplate reader (Tecan Sunrise, Phoenix Research Products, Hayword, CA). The oxalate analyses were carried out at Washington State University.

**Phytate Analysis of Soy Foods.** Phytate analysis of the soy foods was carried out at Iowa State University according to the method of Harland and Oberleas (*15*). Briefly, soy foods were extracted with 2.4% HCl for 3 h at room temperature followed by centrifugation. The supernatant was filtered and run through an anion exchange column. Phytic acid was eluted with 7 mol/L NaCl and subjected to Kjeldhal digestion with concentrated H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub>. The inorganic P concentration was measured using a colorimetric method utilizing ammonium molybdate and sulfonic acid reagents. The phytic acid concentration was determined based on its P content of 28.2%. Red wheat bran (3 ± 0.3%) obtained from AOAC (Association of Official Analytical Chemists) was used as the control; the measured values were within the reference values.

**Mineral Analysis of Soy Foods.** Calcium, magnesium, and sodium were analyzed because of their presumed association with oxalate and phytate or utilization in soy food manufacture. The extracted supernatants prepared for oxalate analyses were used for mineral analyses. One-half milliliter of each supernatant was adjusted to 10 mL using 2 N HNO<sub>3</sub>. The mineral concentrations in each supernatant were determined using inductive-coupled argon plasma emission technique (Optima 2000 DV, PerkinElmer Optical Emission Spectrometer, Norwalk, CT).

**Statistical Analysis.** Linear correlations between oxalate and phytate and between the molar divalent ion binding potential of oxalate plus phytate and the molar concentration of calcium plus magnesium were calculated by using MINITAB Release 14 Statistical Software for Windows (Minitab Inc., State College, PA). Significant correlation coefficients were predetermined as  $P \le 0.05$ .

### RESULTS

Oxalate, calcium, magnesium, and sodium concentrations in 40 soy foods, on a wet weight or as purchased basis, are presented in **Tables 1** (tofu) and **2** (other soy foods).

**Oxalate.** The total oxalate concentration in 19 selected brands of tofu ranged from 0.02 to 0.13 mg/g. Eighteen of the 19 tofus

Table 2.	Total	Oxalate,	Phytate,	Ca,	Mg,	and	Na	Content	in	Soy	Food	ls
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product	brand	serving size (g)	oxalate (mg/g)	oxalate (mg/svg)	phytate (mg/g)	phytate (mg/svg)	Ca (mg/g)	Mg (mg/g)	Na (mg/g)
soymilk	Pacific Soy	240	0.02	5.86	0.8	192	0.11	0.25	0.01
soymilk	Westsoy	240	0.02	5.18	1.33	319	0.2	0.25	0.07
soya powder	Fearn	28	0.68	18.99	14.05	393	2.25	2.56	0.02
soy flour	Bulk	28	0.8	22.47	11.02	309	1.55	2.48	0.02
soy flour	Arrowhead Mills	28	2.06	57.77	13.33	373	2.61	2.46	0.02
soy protein	Bulk (Fred Meyer)	28	0.15	4.11	16.2	454	2.13	0.62	8.63
textured vegetable (soy) protein	Red Mill	28	1.5	42.04	18.79	526	3.53	3.63	0.02
edamame soybeans (vegetable)	Hearty	85	0.16	13.6	2.73	232	0.59	0.82	0.04
sweet soybeans (vegetable)	Safeway	85	0.2	17	2.89	246	0.65	0.77	0.05
soybeans (vegetable)	C & W	85	0.23	19.6	2.34	199	0.59	0.78	0.01
shelled edamame (vegetable)	Safeway	85	0.32	27.2	2.53	215	0.53	0.57	0.07
edamame (vegetable)	Safeway Select	85	0.44	37.4	2.43	207	0.69	0.7	0.14
soy beans	Red Mill	28	0.54	15.1	13.06	366	1.83	2.12	0.16
roasted soynuts	Good Sense	28	0.84	23.5	9.4	263	1.72	2.28	0.02
soy nuts	GenSoy	28	0.87	24.4	8.32	233	2.19	2.55	0.08
tempeh	White Wave	85	0.47	40	6.17	524	1.08	0.99	0.03
tempeh	Turtle Islan	85	0.65	55.3	4.31	366	1.27	0.9	0.20
soynut butter "original creamy"	I. M. Healthy	28	0.38	10.6	4.67	131	0.83	0.85	3.54
soynut butter "low carb"	I. M. Healthy	28	0.63	17.6	7.64	214	1.78	1.58	2.38
soy protein	Liquid Aminos	28	0.09	2.5	ND	ND	0.01	0.03	30.40
soy sauce	Kikkoman	28	0.11	3.1	ND	ND	0.01	0.04	32.40

contained less than 10 mg oxalate per serving. Two selected brands of soymilks exhibited similar oxalate concentrations of 0.02 mg/g. Oxalate concentrations in five brands of vegetable soybeans (edamame) ranged from 0.16 to 0.44 mg/g. Soy nuts, soy flour, tempeh, and soynut butter exhibited relatively high oxalate concentrations, with more than 10 mg oxalate per serving.

Phytate. The 11 tofu samples analyzed from the 19 overall exhibited phytate concentrations ranging from 0.89 to 6.21 mg/ g. Soy flour, soy protein, and soy nuts contained high concentrations of phytate as compared with the other soy foods. The textured vegetable (soy) protein contained the highest phytate concentration (18.79 mg/g) among the 30 soy foods. Soy milks contained from 0.8 to 1.33 mg/g. The samples from five brands of vegetable soybeans were similar to each other in their phytate concentrations with a mean of 2.58 mg/g. The samples from two tempeh brands exhibited intermediate phytate concentrations. A comparison (Table 1) of three tofu brands purchased 3 months apart showed differences in oxalate and phytate concentrations that exceeded technical variation. Oxalate and phytate differences in the Pete's and Mori-Nu tofus would not be clinically significant, but the oxalate content of the PacSoy tofu was double and the phytate value was one-half in the second sample.

**Cations.** The analysis of the three minerals showed ranges of 0.01-3.53 mg/g for calcium, 0.01-3.63 mg/g for magnesium, and 0.00-8.63 mg/g for sodium. There were significant correlations between oxalate and phytate concentrations (r = 0.71, P < 0.001) and between the molar divalent ion binding potential of oxalate plus phytate and molar concentration of calcium plus magnesium (r = 0.90, P < 0.001) among the 30 soy foods (**Figures 1** and **2**). Sodium values per serving were 241 mg for the bulk soy protein from Fred Meyer, 66 and 99 mg for the two soynut butters, and 840 and 907 mg, respectively, for the liquid soy protein and soy sauce.

#### DISCUSSION

The data presented in **Tables 1** and **2** clearly show a wide range of oxalate values in commercial soy foods. Foods containing greater than 10 mg of oxalate per serving are considered high oxalate foods by the American Dietetic As-



**Figure 1.** Correlation between oxalate and phytate in 30 soy foods (r = 0.71; P < 0.001).



**Figure 2.** Correlation between phytate plus oxalate with calcium plus magnesium on molar basis in 30 soy foods (r = 0.90; P < 0.001).

sociation (16). The oxalate content of the 40 soy foods tested in this study ranged from 2 to 58 mg oxalate per serving, indicating that some of these soy products are considered high oxalate foods. Massey et al. (4) reported that the oxalate content of 13 commercial soy foods ranged from 16 mg/g for soy cheese to 638 mg/g for textured vegetable soy protein. All of the soy foods tested in that study were high oxalate foods. In our present study, 18 of 19 tofus contained less than 10 mg of oxalate per serving. Soymilk, soy protein, and soy sauce also were low in oxalate. On the other hand, soy foods made with relatively minimal processing, such as soy flour, textured vegetable protein, soy nuts, tempeh, and soy nut butter, contained concentrations of oxalate greater than the 10 mg/serving.

Different cooking methods have different effects on food oxalate. For example, 17% of total oxalate in spinach is reduced by blanching for 15 min (17), whereas baking oca tubers (New Zealand sweet potatoes) may increase oxalate by 79% (18). Unfortunately, there are no studies on the effects of cooking on the oxalate content of soybeans. Soluble oxalate, presumed to be the potassium salt, may be lost from soybeans during processing, explaining the presence of a positive correlation between the molar divalent ion binding potential of oxalate plus phytate and the molar concentration of calcium plus magnesium in soy foods. The amount of retained oxalate is dependent on both the oxalate content of the soybean cultivar used and the processing technique. Because our soy foods were produced from unknown soybean cultivars, it was not feasible to follow the mass balance of the oxalate during production.

Unlike oxalate, there are many studies dealing with phytate concentrations in soybeans and soy foods (8, 19). The bulk of phytate in soybeans occurs in the cotyledons, and the phytate is localized in the protein bodies (8). In general, our soy foods contained relatively high concentrations of phytate, suggesting that some of these foods may be advantageous for kidney stone patients or persons with a high risk for kidney stones. Phytate has been shown to be an inhibitor of calcium kidney stone formation in many studies and was used to treat patients at risk for kidney stones 40 years ago (20); however, no recommendations have been established for its therapeutic use.

The critical factor in the effect of a food on urinary oxalate and phytate is the amount of oxalate and phytate absorbed from the food and ultimately excreted in the urine. About one-half of urinary oxalate derives from endogenous synthesis, but on a typical diet, which contains 150-200 mg oxalate, the other half results from absorption of oxalate from the diet (21). Oxalate in soybeans is primarily in the form of calcium oxalate crystals (4). It has generally been assumed that calcium oxalate in the diet is not significantly absorbed in humans because calcium oxalate is virtually insoluble in aqueous solutions ( $\sim 1 \text{ mg/}$ 100 mL; 22). However, Hanes et al. (23) reported the passive uptake of calcium oxalate as an intact molecule in rats; about 2% of the calcium oxalate load was absorbed via this pathway. Massey et al. (6) examined human oxalate absorption following consumption of soybeans and soy foods by assaying urinary oxalate excretion. Eight healthy individuals with no history of kidney stones consumed eight oxalate loads comprised of two soybean cultivars, two tofus, a soy beverage, a textured vegetable protein, soynuts, and 8.3 mmol of sodium oxalate solution. After correction for preload baseline excretion, the absorption ranged from 2.1% from one high oxalate soybean cultivar to 5.4% from soynuts. Increases in urinary oxalate ranged from  $1.7 \pm 2.1$  to  $10.9 \pm 13.8$  mg for the two soybean cultivars and five soy foods during the 8 h after ingestion. Because normal urinary oxalate excretion is defined as 10-39 mg per day, Massey et al. (6) concluded that frequent consumption of soybeans and soy foods in the diet may increase urinary oxalate excretion to 40 mg or more per day; a concentration defined as hyperoxaluria, which is a risk factor for calcium oxalate kidney stone formation. However, all soy foods fed in this study contained greater than 10 mg oxalate per serving.

The concentration of phytate in selected rat tissues and organs is highly affected by dietary intake, indicating that the majority of the phytate observed in tissues and organs has a dietary origin and is not a consequence of endogenous synthesis (24). Urinary excretion of phytate by rats is dependent on dietary supply. Grases et al. (25) reported that urinary phytate increased as dietary phytate increased up to a maximum of 20.9 mg/kg body weight. At that intake, about 2% of dietary phytate was absorbed and excreted in the urine. When dietary phytate was not present, urinary phytate was very low, indicating that most of the urinary phytate originated from the diet and not from endogenous synthesis. Similar patterns of phytate excretion as seen in rats were observed in humans. Grases et al. (13) conducted a twophase study with phytate-poor diet and phytate normal diet, respectively, in which seven healthy human subjects consumed three selected commercial preparations containing doses of phytate (400 mg as calcium/magnesium salt, 3200 mg as calcium/magnesium salt, and 1400 mg as sodium salt). After 2 weeks of consuming a diet deprived of phytate, the urinary concentrations of phytate decreased to around 20-25% of normal concentrations typically observed in humans. The three different sources and doses of phytate increased the excreted amounts of phytate continuously during 8 h up to a maximum of 0.08 mg, an amount lower than that obtained by consuming normal diet (0.12 mg). The three sources and doses of phytate resulted in similar excretion profiles suggesting a maximum excretion concentration that cannot be exceeded by ingesting greater quantities of phytate regardless of the phytate salt.

In an observational study, Grases et al. (26) also reported that urinary excretion of phytate by stone formers was on the average half the concentration observed for nonstone formers. Healthy nonstone formers excreted significantly more phytate per day than stone formers,  $3.27 \pm 0.41$  vs  $2.01 \pm 0.34$  mg, respectively. When the total urine volume was considered, the concentration of phytate was  $2.94 \pm 0.20$  mg/L in healthy nonstone formers as compared to  $1.13 \pm 0.17$  mg/L in stone formers. In another study, Conte et al. (27) fed 120 mg phytate/day to 17 stone formers and determined its effect on lithogenicity, a risk index calculated from urine composition. Twelve of the 17 stone formers with a positive index before treatment exhibited a negative index after phytate treatment as compared to only one of the 19 control patients.

In the present study, soy foods contained phytate in the range of 76–528 mg/serving. Therefore, a diet with one serving of these soy foods daily would contain between 76 and 528 mg of phytate. Humans absorb and excrete less than 1% of phytate intake, so daily excretion would be less than 0.76–5.3 mg after eating these soy foods. This is similar range to the 2–4 mg per day reported by Grases et al. (26) as typical in his study of Spanish populations. Conte et al. (27) fed 120 mg of purified phytate to 17 stone formers and observed a significant benefit, so some soy foods are potentially a much greater influence on kidney stone risk.

Several soy foods tested in this study contained significant amounts of sodium, including one soy protein powder and the two soynut butters, from 66-241 mg/day. The liquid soy protein and soy sauce were very high at 840-907 mg/serving. The current recommendation for sodium intake is 1150 mg/day (50 mmol) so regular consumption of these soy foods could increase urinary calcium, which increases the risk of kidney stones (28).

This study investigated a variety of soy foods and provides basic information for future human feeding studies. Humans prone to form calcium oxalate kidney stones are recommended to limit their intake of soy foods containing greater than 10 mg of oxalate per serving. Consuming foods containing phytate may lower the risk of forming calcium oxalate kidney stones. There are soy food choices that fit these recommendations without the need to consume unusual amounts of soy foods. Further studies are needed to determine the bioavailability of phytate from soy foods and establish the amount of each food required for urinary concentrations of phytate to provide an effective inhibition of calcium oxalate kidney stone formation.

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