

Changes in Oxalate and Phytate Concentrations During Soymilk Processing from the Seeds of Korean Soybean Cultivars

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Abstract A high dietary oxalate intake may lead to calcium oxalate (CaOx) kidney stones in the gastrointestinal tract. Most soy foods contain high concentrations of oxalate and/or phytate. This study analyzed the changes in oxalate (Ox), phytate (InsP₆), and calcium (Ca) during soymilk processing from the seeds of Korean recommended soybean cultivars (cvs). The contents of Ox, InsP₆, and Ca in 21 cvs ranged from 14, 108, and 148 to 231, 279, and 246 mg/100 g of dry seed, respectively. Seven cultivars were selected from the 21 cvs by the distributions of Ox, InsP₆, and Ca. Then, each contents of soymilk prepared from the 7 cvs were determined. All contents were lower in soymilk than in seeds, but the Ox to InsP₆ ratios changed from varying ratios (0.1-0.8) to normal ratios (0.8-1.0) in all cvs except 'Paldalkong'. Consequently, during soymilk processing, the Ox content was decreased and the InsP₆ content was remained higher than the Ox content although the Ox was likely to be less reductive than the InsP₆. These results may provide better information for minimizing the risk of formation of CaOx kidney stones due to consumption of soy products.

Keywords: soybean cultivar, soymilk, oxalate, phytate, kidney stone

Introduction

The human consumption of soybeans and products made from them is increasing due to the reported health benefits of eating soybeans in various prepared forms (1). Soybeans are regarded as a highly nutritious food source because of their excellent content of oil and protein (8 essential amino acids). Soybean oil contains high concentration of essential fatty acids as well as tocopherols and, thus, is considered as one of the highly functional edible oils (2). And soybean proteins are composed of 2 major components, glycinin (11S globulin) and β -conglycinin (7S globulin). Glycinin plays an important role in the functional properties of soybean protein (3,4). In addition to these nutritional benefits, soybean seeds are rich in isoflavones that are known to prevent osteoporosis, cancer, and cardiovascular disease (5-7). However, the recent finding that soybeans and soy foods have relatively high concentrations of oxalate (Ox) is a cause of concern regarding the risk of kidney stones for humans consuming soy foods.

Ox forms water-soluble salts with Na⁺, K⁺, and NH₄⁺ ions, and also binds to Ca²⁺, Fe²⁺, and Mg²⁺, rendering these minerals unavailable (8). Ox binds to calcium (Ca) in human urine to form a poorly soluble salt that is typically near saturation. The formation of small crystals occurs when the urinary Ca and Ox concentrations reach supersaturation. A kidney stone forms when the calcium oxalate (CaOx) crystals aggregate or deposit on a 'seed' crystal, such as uric acid (9). About 2-8% of the Ox in ingested soy foods is absorbed and excreted in the urine by

healthy humans (10). Kidney stones formers exhibit a higher rate of Ox absorption than nonstone formers (11), and the increase in urinary Ox following the consumption of soy foods may be large enough to increase the risk of CaOx precipitation in the former group, potentially increasing their risk of kidney stones.

Soybeans also contain high concentrations of phytate (InsP₆). InsP₆ is a fully phosphorylated form of inositol (*myo*-inositol hexakisphosphate), a compound that also exists in seeds, and has both beneficial and detrimental effects on human nutrition and health (12,13). Similar to Ox, InsP₆ can be synthesized by the human body. However, like Ox, the main source of InsP₆ for humans is plant foods. InsP₆ has long been considered an antinutrient due to its ability to complex metal ions, including zinc (14), calcium (15), and iron (16-19), reducing their bioavailability. However, InsP₆ has recently been proposed to reduce kidney stone formation by inhibiting the formation of CaOx and Ca phosphate crystals (20).

Soymilk, a popular food in the traditional Asian diet, is obtained by squeezing the homogenate of water-soaked soybeans. During the processing of soymilk, soybeans are soaked and then heated to 100°C for a period of time (21,22). Al-Wahsh *et al.* (23) reported that soy foods containing low concentrations of oxalate and moderate concentrations of phytate may be advantageous for kidney stone patients or persons with a high risk of kidney stones. Therefore, an analysis of changes in the Ox and InsP₆ concentrations during the soymilk processing from soybean seeds would be useful to identify soy foods that are nutritionally advantageous for kidney stone patients or persons at risk for kidney stone formation. However, there have been very few reports of changes in the Ox and InsP₆ concentrations during the transition from soybean seeds to soymilk, although there have been reports of the

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concentrations of Ox and InsP₆ in selected commercial soy foods, soybeans, soymilks, and various vegetables. In this study, to present information for safe digestion of soybean cultivars (cvs) and soy food, we selected 7 soybean cvs from 21 Korean recommended soybean cvs based on their Ox, InsP₆, Ca, and Mg contents, prepared soymilk from seeds of the 7 cvs, and investigated the changes in the concentrations of Ox, InsP₆, and Ca during soymilk processing.

Materials and Methods

Materials Seeds of 21 recommended Korean soybean cvs [*Glycine max* (L.) Merr.], produced in 2005, were obtained from a local farm in Korea. The cvs were composed of 1) 'Paldalkong', 2) 'Dankyongkong', 3) 'Sinpaldalkong', 4) 'Kwangkyokong', 5) 'Changyupkong', 6) 'Hwangkeumkong', 7) 'Baekwoonkong', 8) 'Eunhakong', 9) 'Daewonkong', 10) 'Bukwangkong', 11) 'Changkyongkong', 12) 'Bangsakong', 13) 'Saealkong', 14) 'Duckyokong', 15) 'Danyeobkong', 16) 'Namhaekong', 17) 'Marikong', 18) 'Taekwangkong', 19) 'Muhankong', 20) 'Solokkong', and 21) 'Danbeakkong'. Hydrochloric acid, sulfuric acid solution, sulfosalicylic acid, and ferric chloride for oxalate analysis were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Oxalate analysis of soybean and soymilk One g samples of soybean seeds were prepared by grinding in a Philips grinder (HR-2870; Philips, China) and filtering with a 100-mesh sieve. Soymilk samples of 10 g were used. The Ox contents of soybeans and soymilk were analyzed according to the procedures of Savage *et al.* (8) and Kim *et al.* (24), with some modifications. For the total Ox, each sample was placed in a 50-mL beaker, 30 mL of 2 N HCl were added, and the samples were homogenized at 16,000 rpm for 1 min using a rolling chopper-type polytron homogenizer (Ultra Turrax T25; IKA Lab. Co., Kuala Lumpur, Malaysia). Then, the beakers were shaken in a water bath at 80°C for 15 min, and the extracts were allowed to cool and filtered with Whatman filter paper (8 µm, No. 2; Whatman, Maidstone, England). The filtrates were transferred quantitatively to 100-mL volumetric flasks and the volume brought to 100 mL with 2 N HCl. The samples were then diluted 10-fold with distilled water and filtered with 0.45-µm regenerated cellulose microfilters (Sartorius AG, Göttingen, Germany). The amounts of total Ox in the filtrates were measured using high performance liquid chromatography (HPLC; Thermo Separation Products, Waltham, MA, USA). An Aminex HPX-87H ion exclusion column (300×7.8 mm i.d.; Bio-Rad, Hercules, CA, USA) and a Micro-Guard Cation H cartridge (30×4.6 mm, Bio-Rad) were used as the solid phase. The mobile phase was 0.008 N H₂SO₄ solutions and the flow rate was 0.6 mL/min, and the signal at 215 nm was monitored using a ultraviolet (UV) detector (Spectra System UV1000; Thermo Separation Products) for the total Ox solution. Injection volumes of 20 µL were delivered using an autosampler (Spectra System AS1000; Thermo Separation Products), and quantitation was performed by electronic integration of the peak area using MultiChro™ Version 5.0 (Yullin Technology, Seoul, Korea). For the extraction of soluble Ox, distilled water was used as the extraction medium

instead of 2 N HCl. The insoluble Ox concentration was calculated by subtracting the soluble Ox from the total oxalate. All measurements were performed in triplicate.

Phytate analysis of soybean and soymilk Two g samples of soybean seeds were prepared by grinding in a Philips grinder and filtering with a 100-mesh sieve. Soymilk samples of 20 g were used. The amounts of InsP₆ in the soybeans and soymilk were measured according to the method of Haug and Lantzsch (25), with some modifications based on the method of Mameesh and Tomar (26). Briefly, each sample was transferred quantitatively to a 50-mL volumetric flask, and made up to 50 mL with 0.2 N HCl containing 5% Na₂SO₄ to solubilize the InsP₆. The mixture was filtered with Whatman filter paper (20-25 µm, No. 4, Whatman), and 10 mL of the filtrate was diluted to 40 mL. Ten mL of the diluted filtrate were mixed with 12 mL of ferric chloride solution (10 mM FeCl₃·6H₂O in 0.2 N HCl solution). The mixture was heated in a boiling water bath for 75 min, cooled in an ice bath for 15 min, and centrifuged (2,500×g, 30 min) to precipitate any ferric phytate formed during the reaction. The supernatant was filtered with Whatman filter paper (8 µm, No. 1, Whatman), and the filtrate was transferred to a 50-mL volumetric flask, and made up to 50 mL with distilled water. The InsP₆ in the diluted filtrate was determined by a colorimetric method using Wade's reagent [0.03%(w/v) FeCl₃·6H₂O and 0.3%(w/v) sulfosalicylic acid in distilled water]. One mL of Wade's reagent was added to 3 mL aliquots of each sample, and the samples were mixed on a vortex mixer for 5 sec. The absorbance of each sample was determined at 500 nm against a water blank using a spectrophotometer (UV-1650PC; Shimadzu, Kyoto, Japan). A standard curve was prepared using sodium phytate. All measurements were performed in triplicate.

Mineral analysis of soybean and soymilk The amounts of divalent minerals such as Ca and Mg were analyzed because of their presumed association with Ox and InsP₆ and their utilization in the manufacture of soy foods. The Ca and Mg contents of soybeans and soymilk were analyzed according to the procedures of Al-Wahsh *et al.* (23) and Lachas *et al.* (27), with some modifications. Two g of soybean cvs were grinded using a Philips grinder. The resulting powders and soymilk powders dried from 20 g of each soymilk solution using a drying oven were burned at 550°C until the samples became light ash in color. The burned soybean and soymilk ashes were adjusted to 10 mL with 0.5 N HNO₃ and then filtered using GF/C filter paper. The mineral concentrations in each filtrate were determined using an inductively coupled argon plasma spectrometer system (ICAP 61E; Thermo Jarrell Ash, Franklin, MA, USA). All measurements were performed in triplicate.

Soymilk preparation Soymilk was prepared from soybeans using the method of Toda *et al.* (28). Soybeans (120 g/batch) were washed 3 times with distilled water and soaked in distilled water at 25°C for 18 hr. The hydrated seeds were drained and ground into homogenates using a rolling chopper-type polytron homogenizer with distilled water equivalent to 10 times the weight of the dry seed. Raw soymilk was separated using filter cloth (100-mesh).

Soymilks were prepared by incubating raw soymilk in boiling water for 5 min followed by cooling in flowing water. The soymilks were incubated at 4°C until the measurement of Ox, InsP₆, Ca, and Mg.

Statistical analysis All treatments were replicated 3 times, and samples were analyzed in duplicate. The values presented are averages of triplicates. Significant differences between the sample means were determined at the $p < 0.05$ level by analysis of variance (ANOVA) using SAS version 8.2 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Oxalate, phytate, calcium, and magnesium contents of Korean soybean cultivars The relative distributions of the total Ox, InsP₆, and Ca contents in the seeds of 21 Korean recommended cvs were shown in Fig. 1. The total Ox contents of the seeds varied widely, from 13.8 to 231.2 mg/100 g of dry seed, whereas the Ca contents were distributed in a narrow range from 148 to 245 mg/100 g of dry seed. The total Ox contents were similar to the range of 82 to 285 mg/100 g reported by Horner *et al.* (29), whereas the Ca contents were somewhat lower than the range of 185 to 389 mg/100 g reported by Massey *et al.* (30). The total InsP₆ contents ranged from 0.11 to 0.28 g/100 g of dry seed, which is lower than the ranges in

soybean reported by Horner *et al.* (0.77-2.22 g/100 g of dry seed) (29) and Raboy *et al.* (1.39-2.3 g/100 g of dry seed) (31). There was no relationship between the total Ox, total InsP₆, and Ca concentrations in the cvs studied. On the basis of Ox concentrations and absorption, Grentz and Massey (32) reported that frequent consumption of soy products may increase the excretion of Ox to more than 40 mg/day, which is defined as hyperoxaluria and may lead to kidney stone formation. In addition, because Ox may also reduce mineral absorption (12), excess consumption raises the potential for mineral deficiencies. Therefore, we postulated that a lower Ox content and/or a higher InsP₆ content in soy foods would be beneficial for decreasing the potential risk of oxalate kidney stones and other disorders with the ingestion of soy foods. However, Massey *et al.* (30) and Al-Wahsh *et al.* (23) reported that the total Ox content in soy products is much lower than in commercial soybean seeds. These reports suggested that the total Ox content in soybean decreases during the manufacture of soy foods. Therefore, we wondered whether the potential risk of the consumption of Korean cvs would decrease with soy food processing.

Selection of 7 cultivars for soymilk preparation To investigate changes in the Ox, InsP₆, and Ca contents during the soymilk processing from soybean seeds, 7 cvs were selected from 21 Korean recommended cvs based on

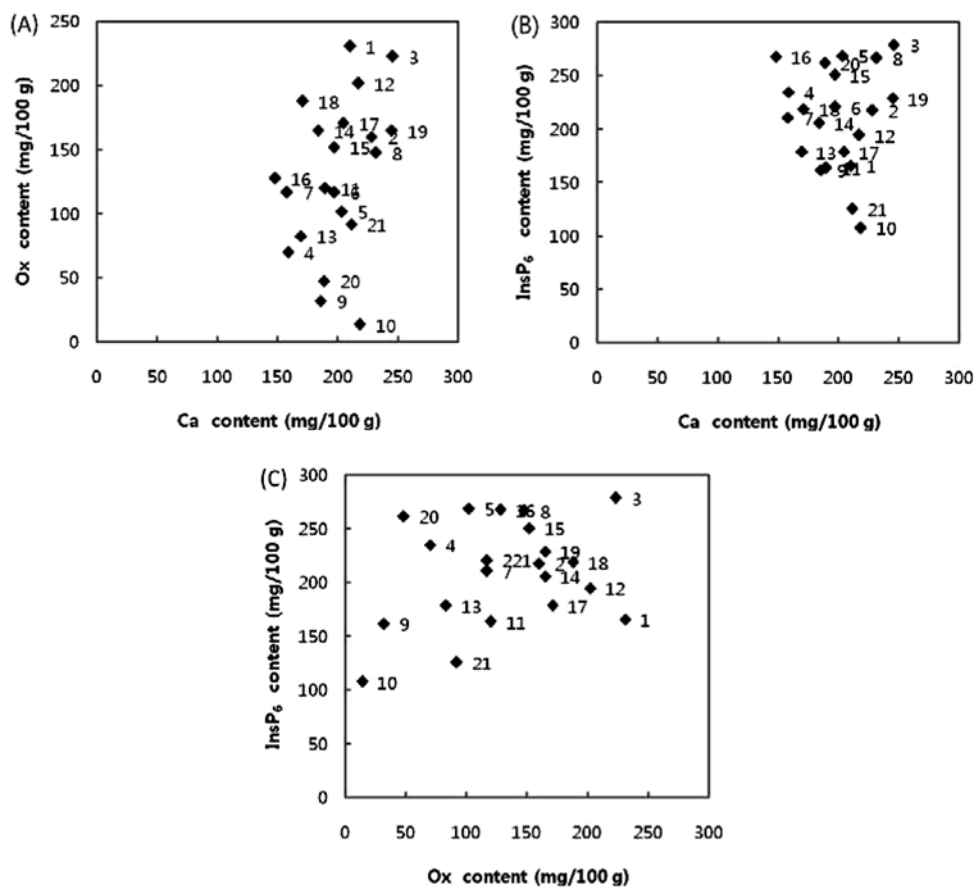


Fig. 1. Correlation between (A) Ox and Ca, (B) InsP₆ and Ca, and (C) InsP₆ and Ox in the seeds of 21 Korean recommended soybean cultivars.

Table 1. Total oxalate (Ox), insoluble Ox, soluble Ox, phytate (InsP₆), divalent mineral concentrations, and Ox/InsP₆ and Ox/Ca ratios in seeds of 7 soybean cultivars

Cultivar	Total Ox (mg/100 g)	Insol Ox (mg/100 g)	Sol Ox (mg/100 g)	InsP ₆ (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)	Ox:InsP ₆ ratio	Ox:Ca ratio
'Paldalkong'	231.2±18.5 ^{a1)}	217.0±18.8 ^a	14.2±3.2 ^a	166.4±11.5 ^c	210.2±12.5 ^{bc}	137.8±8.9 ^c	1.4±0.15	1.1±0.11
'Sinpaldalkong'	222.8±16.7 ^a	206.9±17.3 ^a	15.9±4.5 ^a	279.3±21.3 ^a	245.6±18.9 ^a	166.3±10.2 ^b	0.8±0.09	0.9±0.10
'Daewonkong'	31.8±8.9 ^e	19.3±9.4 ^e	12.5±3.1 ^a	162.4±8.5 ^c	185.7±15.4 ^d	143.0±7.9 ^c	0.2±0.06	0.2±0.05
'Bukwangkong'	13.8±4.5 ^f	0.4±5.2 ^f	13.3±2.6 ^a	107.7±10.7 ^d	218.4±21.0 ^b	180.7±11.9 ^a	0.1±0.04	0.1±0.02
'Danyeobkong'	152.4±13.5 ^b	138.6±14.1 ^b	13.8±4.1 ^a	250.5±21.5 ^b	196.9±18.5 ^{cd}	149.0±14.6 ^c	0.6±0.08	0.8±0.10
'Namhaekong'	128.5±14.3 ^c	114.9±14.5 ^c	13.6±2.4 ^a	268.1±22.6 ^{ab}	148.0±10.5 ^c	149.5±10.3 ^c	0.5±0.07	0.9±0.11
'Solokkong'	48.1±6.3 ^d	35.2±6.9 ^d	13.0±2.8 ^a	262.0±9.9 ^{ab}	188.7±14.2 ^d	142.2±9.8 ^c	0.2±0.03	0.3±0.04

¹⁾Means in the same column followed by the same letter are not significantly different ($p < 0.05$) as determined by Duncan's multiple range test.

Table 2. Oxalate (Ox), phytate (InsP₆), divalent mineral concentrations, and Ox/InsP₆ and Ox/Ca ratios in soymilks made from 7 soybean cultivars

Cultivar	Ox (mg/100 g)	InsP ₆ (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)	Ox:InsP ₆ ratio	Ox:Ca ratio
'Paldalkong'	30.7±5.6 ^{a1)}	27.8±6.1 ^a	71.4±8.6 ^a	34.5±5.4 ^b	1.1±0.31	0.4±0.09
'Sinpaldalkong'	29.1±5.9 ^a	32.8±5.2 ^a	44.4±6.4 ^{bc}	32.9±6.1 ^{bc}	0.9±0.23	0.7±0.16
'Daewonkong'	31.2±6.8 ^a	30.6±11.7 ^a	28.5±5.9 ^d	10.7±3.9 ^d	1.0±0.45	1.1±0.33
'Bukwangkong'	31.9±5.1 ^a	34.7±5.3 ^a	38.0±8.7 ^c	31.6±6.2 ^{bc}	0.9±0.20	0.8±0.23
'Danyeobkong'	32.6±8.2 ^a	34.8±12.7 ^a	53.7±10.0 ^b	63.7±10.2 ^a	0.9±0.42	0.6±0.19
'Namhaekong'	28.7±6.3 ^a	36.8±15.2 ^a	23.9±4.1 ^d	23.9±7.7 ^c	0.8±0.36	1.2±0.34
'Solokkong'	27.3±3.9 ^a	32.8±4.2 ^a	49.2±5.7 ^b	33.4±8.6 ^b	0.8±0.16	0.6±0.10

¹⁾Means in the same column followed by the same letter are not significantly different ($p < 0.05$) as determined by Duncan's multiple range test.

their Ox, InsP₆, and Ca contents. Table 1 showed the concentrations of total Ox, InsP₆, divalent minerals such as Ca and Mg, and the total Ox/InsP₆ and total Ox/Ca ratios in soybeans of the 7 selected cvs. The total Ox and insoluble Ox contents of seeds of the 7 cvs ranged from 13.8 and 0.4 to 231.2 and 217.0 mg/100 g of dry seed, respectively, whereas the soluble Ox contents ranged from 12.5 to 15.9 mg/100 g dry seed. The soluble Ox contents were similar to the range of 14.7 to 29.9 mg/100 g reported by Massey *et al.* (30). The total Ox contents in 'Paldalkong' and 'Sinpaldalkong' were higher than those in seeds of the other cvs, but the soluble Ox contents were lower. The percentages of soluble Ox contents in the soybean seeds were in the low range of 6.2 to 10.6%, except for 'Daewonkong' and 'Bukwangkong'. The InsP₆ contents ranged from 107.7 to 279.3 mg/100 g of dry seed, and the Ca and Mg concentrations ranged from 148.0 and 137.8 to 245.6 and 180.7 mg/100 g of dry seed, respectively. The InsP₆ and Ca contents in 'Sinpaldalkong' were the highest among the 21 cvs, and the InsP₆ content in 'Bukwangkong' and the Ca content in 'Namhaekong' were the lowest. There was no correlation between the total Ox and InsP₆ within or among the 7 cvs, and no significant relationships of Ca and Mg in any of the cvs. The ratio of Ox to InsP₆ in the 'Paldalkong' and 'Sinpaldalkong' cvs was higher than reported elsewhere (23,29), and the ratios of Ox to InsP₆ and Ox to Ca in 'Bukwangkong' and 'Solokkong' were much lower than those in other soybean seeds. These results showed that the potential risk of kidney stones from Korean soybean cvs such as 'Paldalkong' and 'Sinpaldalkong' were somewhat higher than that indicated by other reports (23,29). The recommended soybean cvs, including

'Bukwangkong', 'Solokkong', 'Paldalkong', and 'Sinpaldalkong', are commonly consumed in Korea. Therefore, the consumption of soybean seeds, especially of the 'Paldalkong' and 'Sinpaldalkong' cvs, requires processing into products such as soymilk and *tofu* in order to decrease the potential risk of kidney stones from these cvs.

Oxalate, phytate, calcium, and magnesium contents of soymilk The concentrations of total Ox, InsP₆, and divalent minerals including Ca and Mg, and the ratios of total Ox/InsP₆ and total Ox/Ca in soymilk are shown in Table 2. The total Ox and InsP₆ contents ranged from 27.3 and 27.8 to 32.6 and 36.8 mg/100 g of liquid soymilk, respectively, and the Ca and Mg contents ranged from 23.9 and 10.7 to 71.4 and 63.7 mg/100 g of liquid soymilk, respectively. The contents of all of these components of soybean seeds were reduced during soymilk processing. The widely varying distributions of the Ox and InsP₆ contents in soybean seeds were in contrast to the regular distributions in the resulting soymilks. These results are similar to the consistent distributions and low contents of Ox in commercial soy foods reported by Al-Wahsh *et al.* (23) and Massey *et al.* (30). The conversion into regular distributions of Ox contents during the transition from soybean seed to soymilk may be related to the extraction of soluble Ox during the heat treatment step of soymilk processing based on the other reports (23,30) and the regular distribution of soluble Ox in soybean seed.

The ratios of Ox to InsP₆ in soymilk prepared with seeds of most of the soybean cvs, except 'Paldalkong', increased into the regular range (0.8-1.0) from the more widely varying range (0.1-0.8) in soybean seeds, whereas the

ratios of Ox to Ca in soymilk remained in a large range from 0.4 to 1.2 (Table 2). These results suggested that the potential risk of oxalate kidney stones during soymilk processing is decreased because the Ox content was decreased, and the InsP_6 content related to the inhibition of CaOx was still higher than Ox content related to the formation of CaOx although the Ox content is less reductive than the InsP_6 content. However, the Ca content, related to the formation of CaOx, was transferred, to varying extents, into soymilk.

Even though CaOx is poorly absorbed, not all Ox in soy is bound to Ca. Ox that is 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 to 16.7 g/100 mL (33). The potential outbreak rate of oxalate kidney stones increased with increase of amounts and duration of Ox intake (10). However, controlling the intake period of soy products is difficult because these products are frequently ingested in Asian countries. Therefore, the use of low-Ox seeds and the control of the Ox and InsP_6 contents during soy processing may be suitable methods for reducing the potential risks of soybean seeds and soy products. Consumption of the recommended Korean soybean cvs such as 'Namhaekong' and 'Solokkong', especially in processed form, could reduce the potential risk, because the ratio of Ox to InsP_6 is low in both the seeds and soymilk of these cvs.

In conclusion, Ox and Ca, which were related to the formation of CaOx and, consequently, to oxalate kidney stones, were abundant in soybean seeds; additionally, InsP_6 , which was related to the inhibition of CaOx formation, was also abundant (23,29). Therefore, generally, the possibility of CaOx formation after the digestion of soybean seeds was very lower. However, the outbreak rate of oxalate kidney stones increased with increase of Ox intake and duration of Ox consumption. In soybean seeds used in this study, the Ox content was found to vary more than the InsP_6 content, but all were high compared to the recommendations for patients with oxalate kidney stones. Currently, they are advised to limit their intake of foods containing >10 mg/serving, with a total intake not to exceed 50-60 mg/day (30). Therefore, the long-term intake of soybean with a high Ox content could lead to a greater risk of oxalate kidney stones, more so than consuming soybean with a high InsP_6 content in patient and people with a history of oxalate kidney stone (10).

During the preparation of soymilk from soybean seeds, the Ox and InsP_6 contents were found to decrease into a regular range, and the ratios of Ox to InsP_6 in most of the soybean cvs, except 'Paldalkong', were increased because the Ox content was less reductive than the InsP_6 content, but the InsP_6 content was still higher than Ox content. In contrast, the Ca content, related to the formation of CaOx, was transferred into soymilk in a more varying manner. Therefore, the potential risk of oxalate kidney stones with the consumption of soymilk is likely to be lower than with soybean seeds, because of the increased possibility that the Ox content is decreased and the inhibition of CaOx is more active than the formation of CaOx. In this sense, the Korean recommended soybean cultivar such as 'Namhaekong' and 'Solokkong' were suitable for use in soy processing to reduce potential risks, as the ratio of Ox to InsP_6 in these

cvs is low in both the seeds and the soymilk. Consequently, we proposed it was effective method for the safe intake of soy food that soybean seeds with low Ox content used in the digestion of soybean and soy products.

This study demonstrated changes in the Ox, InsP_6 , and Ca contents that occur during the processing of seeds of Korean soybean cvs into soymilk. These results may be providing better information of how to breed and select optimal soybean cvs and useful as guidelines in the manufacture of soy products, with a view to minimizing the risk of oxalate kidney stones following the consumption of soy products.

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