Food Sci. Biotechnol. Vol. 16, No. 3, pp. 444 ~ 450 (2007)

Food Science and Biotechnology

Antioxidant Activity and Isoflavone Profile of *Rhynchosia nolubilis* Seeds Pickled in Vinegar (*Chokong*)

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Abstract The antioxidant activity and isoflavone content of *chokong*, *Rhynchosia nolubilis* seeds pickled in vinegar at 4°C for 2 weeks, were investigated. The polyphenol content and 2,2-diphenyl-1-pycrylhydrazyl (DPPH) radical-scavenging capacity were lower in *chokong* than in raw seeds. Based on isoflavone analysis, the aglycone (daidzein and genistein) content was high in *chokong* while the content of the corresponding glucosides (daidzin and genistin) was similar to that in raw seeds. Thermal processing, in which seeds were heated in vinegar at 121°C for 20 min, reduced the polyphenol content but did not affect the DPPH radical-scavenging capacity compared to the pickling process. The heated seeds had a 2.6 to 2.7 times higher glucoside content and 51 to 55% lower aglycone content than *chokong*, depending on the kind of vinegar used. During pickling and thermal processing, vinegars were more effective at eluting antioxidants and isoflavones from seeds than other solutions such as acetic acid, citric acid and HCl solutions, distilled water, and phosphate buffer (pH 7.0).

Keywords: *Rhynchosia nolubilis* seed, *chokong*, vinegar, antioxidant, isoflavone

Introduction

Legumes are of great interest with regard to the presence of various micronutrients and phytochemicals and their reported beneficial effects on health (1, 2). One such legume, *Rhynchosia nolubilis* seeds (RNS), also known as black soybean, *sumoktae,* or *yakkong*, has been traditionally used in Korea for curing and/or preventing various diseases including neuralgia, kidney disease, senile dementia, and postmenopausal osteoporosis. Recent studies showed that its potent effects may be closely related to its higher antioxidant and isoflavone contents (3-5) relative to other legumes (6, 7).

Antioxidants are compounds that inhibit biological oxidation and contribute to preventing or curing oxidation-associated diseases such cancer, cardiovascular diseases, cataracts, atherosclerosis, diabetes, asthma, hepatitis, liver injury, arthritis, immune deficiency diseases, and aging (8-10). Polyphenolic substances including flavonoids and phenolic acids are of particular interest and have been shown to be potent natural antioxidants with minimal side effects (11-14). The potential use of RNS as a functional food is related to its high polyphenol content (3-5). The anthocyanins responsible for the black color of RNS skins, for example, are polyphenols abundant in these seeds and have excellent antioxidant activity (5, 15).

Isoflavones are well-known beneficial compounds found in legumes as well. These flavonoids are reported to potentially prevent many prevalent chronic diseases, including hormone-related cancers, osteoporosis, and cardiovascular diseases, in addition to alleviating menopausal symptoms (1). The activity of isoflavones is presumed to be due to their ability to act as weak estrogen by binding to estrogen receptors due to their structural similarity to 17-β-estradiol and other synthetic estrogens (16-19), and in part due to their antioxidant activity (20). Beneficial effects of isoflavones on obesity have been suggested as well by recent studies in which genistein, in particular, appears to have a direct effect on lipid metabolism in liver and adipose tissue (21, 22).

Chokong, RNS pickled in vinegar, are of great interest as an anti-obesity food, however, apart from its anti-obesity effects, there are few studies addressing its basic chemical or physical properties (23, 24).

In this study, we have investigated the effectiveness of vinegar relative to other pickling solutions in the preparation of *chokong* with regard to antioxidant activity and isoflavone content in the seeds. Thermal processing of *chokong* was also investigated as an alternative to the pickling process.

Materials and Methods

Materials *Rhynchosia nolubilis* seeds (RNS), brown rice vinegar and brewing vinegar were purchased from a local market in Daegu, Korea. All solvents used in chromatographic analysis were of HPLC grade and obtained from Fisher Scientific (Pittsburgh, PA, USA). Folin-Ciocalteu phenol reagent, 2,2-diphenyl-1-pycrylhydrazyl (DPPH), isoflavone standards (daidzein, genistein, daidzin, and genistin) and fluorescein were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

Preparation of *chokong* and its extract *Chokong* was prepared by the pickling process, specifically, 20 g of RNS were soaked in 60 mL of vinegar (brown rice vinegar and brewing vinegar) for 2 weeks at 4°C. Other pickling solutions were also tested for comparison; acetic, citric,

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Received January 19, 2007; accepted February 22, 2007

and hydrochlolic acid solutions with pH values similar to that of vinegar, distilled water, and 50 mM phosphate buffer at pH 7.0. Thermal processing was used as an alternative to pickling in which *chokong* was prepared by autoclaving the seeds in the soaking solutions listed above for 20 min at 121°C.

After processing, the seeds were washed with a sufficient amount of distilled water to remove the residual soaking solution followed by freeze-drying and grinding of the seeds. The resulting powder was extracted with 80% aqueous methanol (1:10, w/v) with vortexing for 2 hr at room temperature and centrifuged for 10 min at 10,620×g. The final extract was filtered through a 0.22 μm PTFE filter unit (Millipore Co., Bedford, MA, USA). All soaking solutions remaining after pickling or thermal processing were also filtered for subsequent analysis.

Analysis of pH and soluble solid content After pickling or thermal processing, the pH of soaking solutions was measured with a pH meter (Suntex Co., Taipei, Taiwan). The soluble solid content of soaking solutions was obtained by freeze-drying (Labconco Co., Kansas City, MO, USA) each solution after processing.

Determination of total polyphenol content The total polyphenol contents of seed extracts and soaking solutions were estimated according to the method of Kim *et al.* (25). Samples were diluted 100 fold with water, and 1.0 mL of each sample was added to 1.0 mL of 0.2 N Folin-Ciocalteu phenol reagent and incubated for 3 min at room temperature followed by the addition of 1.0 mL of saturated sodium carbonate. After incubating for 1 hr at room temperature, the absorbance at 765 nm was measured and the results expressed as μg of gallic acid/mL.

Measurement of DPPH radical-scavenging capacity The method of Kim *et al.* (25) was used with some modification to determine the DPPH radical-scavenging activity of seed extracts and residual soaking solutions. One tenth mM methanolic DPPH solution was diluted with methanol until the absorbance at 525 nm was 0.95-0.99, and 3 mL of DPPH solution was added to 0.1 mL of each sample. The mixture was shaken well and left to stand at room temperature for 30 min, and the absorbance at 525 nm measured. The capacity to scavenge the DPPH radical was calculated according to the following equation:

$$\text{DPPH radical-scavenging capacity (\%)} = [1-(ABS_{sample}/ABS_{control})] \times 100$$

Analysis of isoflavone content The isoflavone contents of seeds and residual soaking solutions were analyzed by the method of Coward *et al.* (26) with some modification. Briefly, isoflavones in each sample were separated using a SUPELCO™ LC-18 column (5 μm, 250 cm × 4.6 mm i.d., Bellefonte, PA, USA) with a linear gradient mobile phase of 1 mL/min flow rate, where 1% aqueous acetic acid solution was replaced by acetonitrile for 20 min in the linear gradient (510 pump with gradient controller, Waters, Milford, MA, USA). After injection of 20 μL of sample (Rheodyne injector, Rheodyne LLC; Rohnert Park, CA, USA), absorbance at 254 nm was monitored with a UV/Vis detector (UV-2077; Jasco, Tokyo, Japan) for the quantification of daidzin, genistin, daidzein, and genistein. Fluorescein was used as an internal standard.

Statistical analysis All experiments were determined in triplicate and represented as mean standard deviations. Significance was verified at a level of $p<0.05$ by performing Duncan's multiple range tests using the SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA) software package.

Results and Discussion

Changes in the pH and soluble solid content of various soaking solutions During the pickling of RNS for *chokong* preparation, the pH of brown rice vinegar increased from 2.52 to 4.15, and from 2.61 to 4.01 for brewing vinegar (Table 1), similar to the results of Kim *et al.* (23). A similar increase was observed with other acidic soaking solutions, but not with water or phosphate buffer. The changes in pH are presumed to be due to the elution of some component from the seeds. Indeed, the soluble solid content of each pickling solution increased after completion of the pickling process, and the efficiency of elution from 1 g of seeds was higher with the use of vinegars than other solutions (Table 1). The increased elution during thermal processing, was probably due to disruption of the seed structure as a result of heating, with more elution occurring with vinegars than other solutions.

The higher elution of soluble solids into vinegar with both processes is thought to be due to the presence of more solutes in vinegar than other solutions. That is,

Table 1. pH and solid contents of soaking solutions after pickling and thermal processing

	Soaking solution[1)]	BRV	BV	A	C	H	W	P
pH	Initial	2.52	2.61	2.71	2.16	2.23	5.45	7.00
	After pickling	4.15	4.01	4.81	4.18	5.94	5.94	6.64
	After thermal processing	3.68	3.79	4.52	3.41	5.68	6.01	6.67
Solid (g/g)[2)]	After pickling	0.18	0.17	0.08	0.08	0.05	0.06	0.08
	After thermal processing	0.23	0.22	0.12	0.11	0.09	0.09	0.10

[1)]BRV, brown rice vinegar; BV, brewing vinegar; A, acetic acid solution; C, citric acid solution; H, hydrochloric acid solution; W, distilled water; P, phosphate buffer (50 mM, pH 7.0).
[2)]Content of soluble solids eluted from 1 g of seeds into soaking solution.

vinegar may be a more hypertonic solution causing greater osmotic disruption of intact seed structure resulting in the greater elution of soluble solids.

Polyphenol content and DPPH radical-scavenging capacity The polyphenol content of seeds and soaking solutions was quantified after the pickling process as an indication of antioxidant capacity. Following extraction with ten volumes of 80% methanol, 306 and 297 μg polyphenols were identified per 1 mL of methanolic extracts of *chokong* prepared using brown rice vinegar and brewing vinegar, respectively, while 645 μg/mL was obtained from raw seed extract (Fig. 1). These values corresponded to 3.06 and 2.97 mg per gram of dried *chokong*, and 6.45 mg per gram of raw seeds, revealing a decrease in the polyphenol content of seeds due to pickling. Seeds pickled in other solutions had a reduced polyphenol content as well, but more so than *chokong*. The reduction of polyphenyls in *chokong* was the result of elution as shown by the increased polyphenol content of the vinegars; 672 μg/mL in brown rice vinegar and 625 μg/mL in brewing vinegar which corresponds to the elution of 2.02 and 1.88 mg of polyphenyls per gram of seeds, respectively, by considering the seeds:vinegar ratio (1:3, w/v, that is, 20 g per 60 mL) in the pickling process. These values indicate that 66 and 63% of polyphenols remained in the seeds after the pickling process using brown rice vinegar and brewing vinegar, respectively. The efficiency of vinegars at eluting polyphenols surpassed that of the other solutions tested (214 to 428 μg/mL), resulting in a lower polyphenol content of *chokong* than other tested seeds.

The DPPH radical-scavenging capacity of *chokong* (16 to 17%) was much weaker than the raw seeds (87%) and its activity was even lower than that of seeds pickled in other solutions (Fig. 1). The analysis of solutions obtained after pickling, however, showed that the vinegars possessed stronger activity than the other solutions. The changes in DPPH radical-scavenging capacity of seeds and vinegars correlate well with the polyphenol contents after pickling (4). The phenolic acids and anthocyanins of the seed hull are most likely the important polyphenols contributing DPPH radical-scavenging activity to the vinegars because of their high water solubility (27) and antioxidant capacity (5, 15). Hydrochloric acid and phosphate buffer solutions had higher antioxidant capacities despite having a similar polyphenol content to the other pickling solutions, perhaps due to different sorts of eluents.

The polyphenol contents and antioxidant activities of seeds and soaking solutions were also investigated after thermal processing. The polyphenol content of all samples was reduced following thermal processing relative to pickling as shown in Fig. 2. For instance, decreases of 37, 20, and 15% were seen in raw seeds, and *chokong* prepared using brown rice vinegar and brewing vinegar, respectively. A similar effect on the polyphenol content of beans due to heating has been reported by Bressani *et al.* (28). The polyphenol content was lowered by 13 and 8% in the respective brown rice vinegar and brewing vinegar as well, but it was still higher in the vinegars than other solutions indicating a higher efficiency of polyphenol elution from seeds.

The seeds and soaking solutions had different DPPH radical-scavenging capacities after thermal processing (Fig. 2), with corresponding changes in polyphenol content. Specifically, all of the soaking solutions showed reduced radical-scavenging activity while the seeds had increased or unchanged activity relative to the soaking solutions. Although the reduced radical-scavenging activity in the soaking solutions can be explained by the decrease in polyphenol content due to heating, this does not explain why the seeds did not show a similar reduction. A plausible explanation might be that heating disrupted the seed structure and allowed the extraction of antioxidants other than polyphenols capable of scavenging the DPPH radical. Further investigation will be necessary for to determine the exact reason for this result.

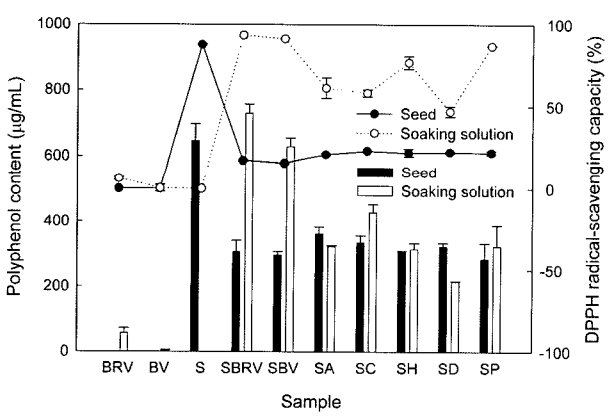

Fig. 1. Polyphenol content (□, ■) and DPPH radical-scavenging activity (○, ●) in seeds and soaking solutions after pickling. BRV, brown rice vinegar; BV, brewing vinegar; S, raw seeds; SBRV, SBV, SA, SC, SH, SD, and SP: seeds and soaking solutions after pickling RNS in brown rice vinegar, brewing vinegar, acetic acid solution, citric acid solution, hydrochloric acid solution, distilled water, and phosphate buffer (50 mM, pH 7.0); respectively.

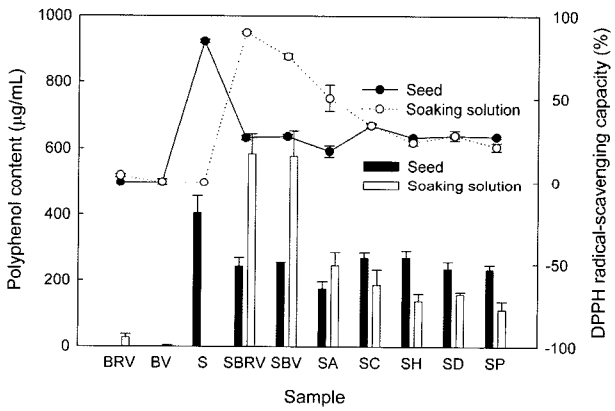

Fig. 2. Polyphenol content (□, ■) and DPPH radical-scavenging activity (○, ●) of seeds and soaking solutions obtained after thermal processing. BRV, brown rice vinegar; BV, brewing vinegar; S, raw seeds; SBRV, SBV, SA, SC, SH, SD, and SP: seeds and soaking solutions after pickling RNS in brown rice vinegar, brewing vinegar, acetic acid solution, citric acid solution, hydrochloric acid solution, distilled water, and phosphate buffer (50 mM, pH 7.0); respectively.

In summary, vinegars were more efficient at eluting polyphenols and antioxidants from seeds than other soaking solutions in both pickling and thermal processing, for the same reasons stated for the soluble solids. The pickling process was superior to thermal processing in terms of polyphenol content and antioxidant activity.

Isoflavone content To date, 3 types of isoflavone have been identified in each of 4 chemical groups from legumes and their processed products: the aglycones daidzein, genistein, and glycitein; the χ-glucosides daidzin, genistin, and glycitin; the acetyl-β-glucosides 6"-O-acetyl-β-daidzin, 6"-O-acetyl-β-genistin, 6"-O-acetyl-β-glycitin and the malonyl-β-glucosides 6"-O-maloyl-β-daidzin, 6"-O-malonyl-β-glycitin and 6"-O-malonyl-β-glycitin (29). Most isoflavones in soy are found in the esterified forms (97.98%) (30), with the malonyl conjugates of daidzin and genistin found in their respective glycoside forms while glycitin and its malonyl conjugate account for a very small portion. The amount of acetyl conjugates and aglycones were reported to be negligible (6, 29, 30). Kim *et al.* (6) reported the isoflavone content of RNS to be as high as 2.49 mg/g with the 6"-O-malonyl conjugate of daidzein and genistein comprising 95%, and the glucoside form 5%.

Isoflavone profiles have been known to change not only due to food processing methods such as soaking, heating, filtration, cooking, extraction, and fermentation (31-33) but even during storage (34, 35). This change is of particular interest because the structural alteration of isoflavones affects their rate of absorption, and possibly further metabolism and clinical effectiveness (36), although there is some controversy in these areas (37, 38). In light of this, changes in levels of the major isoflavones during *chokong* preparation were measured, specifically glucoside daidzin, genistin, and their corresponding aglycone daidzein and genistein derivatives. Each isoflavone showed a respective retention time of 8.53, 9.66, 11.29, and 12.57 min on HPLC separation (data not shown).

As shown in Table 2 and Fig 3, the amount of aglycones increased in *chokong* prepared using brown rice vinegar (383 μg/g) and brewing vinegar (449 μg/g) while the amount of aglycones in the raw seeds decreased. An increase was also identified in seeds pickled in other solutions, with the most drastic increase seen for seeds pickled in acetic acid and less so for seeds soaked in hydrochloric acid, water, and phosphate buffer.

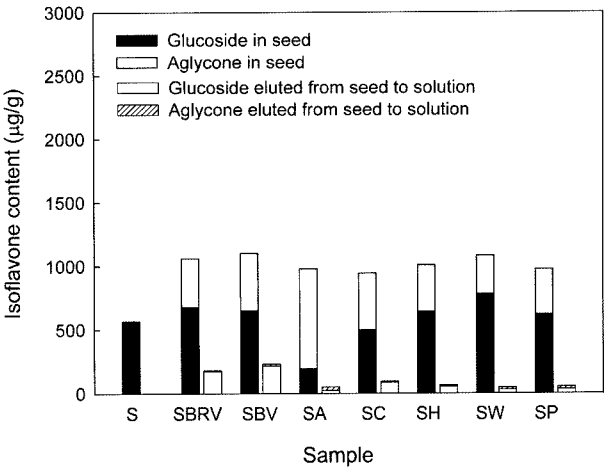

Fig. 3. Glucoside and aglycone contents of seeds and soaking solutions after pickling. BRV, brown rice vinegar; BV, brewing vinegar; S, raw seeds; SBRV, SBV, SA, SC, SH, SD, and SP: seeds and soaking solutions after pickling RNS in brown rice vinegar, brewing vinegar, acetic acid solution, citric acid solution, hydrochloric acid solution, distilled water, and phosphate buffer (50 mM, pH 7.0); respectively.

The increase of aglycones following pickling is presumed to be a result of activating the endogenous β-glycosidase in seeds by the pickling solution over the 2 week period (24, 39). The role of this enzyme is well documented for the hydrolytic cleavage of the glucose moiety from glucoside resulting in the production of aglycone in a temperature range of 10-50°C (optimum 45-50°C) and pH range 3.5-7.0 (optimum 4.5-5.5) (39-41). Considering the range of enzyme activity, the highest rate of conversion would be expected in seeds pickled in acetic acid solution since the pH reached 4.81, near the optimum pH for the enzyme. In the case of water, phosphate buffer, and hydrochloric acid, the pH levels were relatively higher

Table 2. Isoflavone contents of seeds and soaking solutions after pickling

Treatment	Isoflavones in seeds (μg/g)[1]				Isoflavones in soaking solution (μg/g)[2]			
	Daidzin	Genistin	Daidzein	Genistein	Daidzin	Genistin	Daidzein	Genistein
S[3]	190.9±4.3	373.0±0.6	nd	5.6±0.3	-	-	-	-
SBRV	183.0±5.8	498.8±16.5	203.1±8.6	180.2±1.7	96.7±20.5	76.9±4.2	5.0±0.5	2.7±0.1
SBV	158.1±2.2	497.7±6.9	230.3±5.9	219.1±7.3	145.5±24.3	69.2±4.7	10.7±0.8	5.3±1.0
SA	70.3±8.1	127.7±4.3	352.0±17.6	430.4±2.0	17.1±2.0	5.3±0.6	17.5±0.3	10.3±0.1
SA	150.6±44.6	352.4±53.8	214.2±8.4	228.6±8.0	56.2±19.2	28.6±10.7	5.3±0.3	3.4±0.1
SHCl	288.5±20.8	357.7±51.1	169.0±26.3	194.2±10.3	35.6±10.0	16.6±1.2	6.4±0.2	4.5±0.1
SW	315.3±15.7	466.6±55.0	134.0±29.6	169.3±2.2	18.5±1.1	9.6±3.4	9.7±0.8	6.8±1.2
SP	282.7±21.6	340.9±32.5	174.2±0.6	177.9±20.2	23.0±0.6	8.6±1.6	6.9±0.5	12.2±4.6

[1]Isoflavones retained in 1 g of seed powder after pickling.
[2]Isoflavones eluted from 1 g of seed powder into soaking solution after pickling.
[3]Isoflavones in raw seeds.

than other acidic solutions, which would confer lower enzyme activity and a lower rate of conversion. High conversion rates in *chokong* are plausible because vinegar can sustain a pH level appropriate for enzyme activity and induce osmotic cell disruption of the seeds causing the increased exposure of glucosides to the enzyme. Acid hydrolysis of the glucose moiety would not be feasible because acetic acid solution, with a higher pH, resulted in a higher rate of conversion than vinegar. A much stronger level of acidity is necessary for acid hydrolysis (7, 42). With the exception of acetic acid, seeds pickled in various solutions still contained glucosides at a similar or higher level than the raw seeds despite the conversion of glucosides to aglycones. This could be the result of hydrolytic cleavage of the malonyl group from malonyl glucosides, which produces the respective glucosides. The instability of the malonyl derivatives is apt to cause their conversion to glucosides by soaking as well as by heating and alkaline conditions (29, 43). Based on the results of this and other studies, the increase in aglycones without a severe decrease in glucosides could be possible during the pickling process through the conversion of malonyl derivatives to glucosides by soaking, and in turn to aglycones by β-glucosidase activity.

Analysis of residual pickling solutions showed that the vinegars contained more isoflavones, mainly glucosides, than the other soaking solutions, (Table 2 and Fig. 3). This indicates that vinegars cause greater isoflavone elution from seeds, similar to the elution of soluble solids and polyphenols. The sum of isoflavones remaining in and eluted from seeds was highest with the use of vinegars as pickling solutions; 1.25 and 1.33 mg/g with brown rice vinegar and brewing vinegar, respectively, compared to 1.03-1.13 mg/g with the other solutions tested. Considering both total isoflavone content and conversion to aglycones, these results indicate that vinegars are preferred for pickling over the other solutions tested.

The isoflavone profiles of seeds and soaking solutions were also analyzed after thermal processing, and compared with the pickling solutions. Thermal processing raised the glucoside content of seeds 2.6 (brown rice vinegar) and 2.7 (brewing vinegar) times compared to the same solutions used for pickling (Table 3 and Fig. 4). Heat treatment of dried seeds, however, did not result in increased glucosides.

Many researchers have reported that heat treatment under wet conditions promotes the hydrolysis of unstable malonylglucosides to glucosides (30, 44, 45) as in *tofu* and soymilk production (26, 46, 47), and that dry heat causes decarboxylation leading to the formation of acetyl-glucosides from malonylglucosides as in toasting or extrusion of soy (26, 48). Based on glucoside content, vinegars are better solutions for eluting isoflavones from seeds in the pickling process. In other words, the glucoside content was lower in seeds but higher in vinegars relative to other pickling solutions (Table 3 and Fig. 4). The sum of glucosides present in seeds and soaking solutions, however, was similar among the various treatments except for acetic acid solution; 2.36 and 2.52 mg/g in brown rice

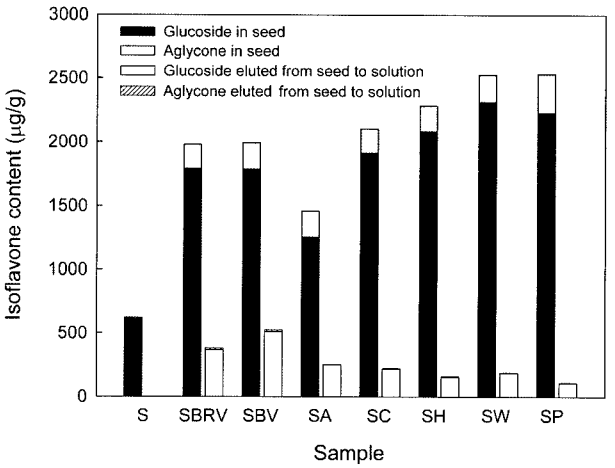

Fig. 4. Glucoside and aglycone contents of seeds and soaking solutions after thermal processing. BRV, brown rice vinegar; BV, brewing vinegar; S, raw seeds; SBRV, SBV, SA, SC, SH, SD, and SP: seeds and soaking solutions after pickling RNS in brown rice vinegar, brewing vinegar, acetic acid solution, citric acid solution, hydrochloric acid solution, distilled water, and phosphate buffer (50 mM, pH 7.0); respectively.

Table 3. Isoflavone contents of seeds and soaking solutions obtained after thermal processing

Treatment	Isoflavones in seeds (μg/g)[1]				Isoflavones in soaking solutions (μg/g)[2]			
	Daidzin	Genistin	Daidzein	Genistein	Daidzin	Genistin	Daidzein	Genistein
S[3]	208.0±17.7	409.6±20.3	Nd	8.1±0.8	-	-	-	-
SBRV	614.9±69.7	1181.5±31.2	90.5±19.4	94.1±2.2	190.7±21.4	177.1±23.3	9.5±2.3	4.8±0.1
SBV	662.3±90.3	1129.0±10.7	95.4±4.3	107.8±4.3	281.0±52.5	229.9±26.5	6.2±8.8	8.2±4.7
SA	436.1±73.3	825.8±141.4	135.6±24.5	65.7±11.7	144.8±30.0	104.3±28.0	4.5±2.5	1.6±0.3
SA	686.8±97.6	1232.5±11.2	86.4±7.4	101.3±5.1	124.7±9.1	92.6±12.5	2.8±0.1	3.7±3.2
SHCl	751.89±45.1	1337.7±45.7	97.6±2.8	98.0±0.6	91.7±9.1	62.6±4.1	1.7±0.1	3.4±3.6
SW	845.3±14.5	1473.2±10.9	99.5±2.8	111.0±2.2	97.6±2.4	89.6±3.2	2.5±0.1	1.4±0.0
SP	807.0±28.9	1425.4±35.7	206.5±1.5	95.0±0.8	56.7±5.8	49.0±4.6	5.3±3.1	0.8±0.1

[1]Isoflavones retained in 1 g of seed powder after thermal processing.
[2]Isoflavones eluted from 1 g of seed powder into soaking solution after thermal processing.
[3]Isoflavones in seeds thermally processed without soaking solution.

vinegar and brewing vinegar, respectively, 2.33-2.72 mg/g in other solutions except for acetic acid solution (1.72 mg/g). The low glucoside content in acetic acid solution might be due to the co-precipitation of isoflavones with other seed proteins since the pH is close to the isoelectric point of many soy proteins (49). In contrast to the production of glucosides, thermal processing was not as effective as pickling for aglycone production. Although aglycones were increased in the raw seeds by heat treatment, the aglycone content was 51 to 55% lower than *chokong*, and a similar phenomenon was observed for seeds soaked in other solutions. This shortcoming might be due to thermal deactivation of β-glucosidase which is necessary for the conversion of glucosides to aglycones. In fact, conversion to aglycones in this case would be possible only by the thermal degradation of malonylglucosides under wet conditions. Heat treatment of seeds under dry conditions was even less effective at producing aglycones as shown in Table 3 and Fig. 4. From these results, it is clear that thermal processing has some advantages for increasing glucoside content, however it resulted in poor aglycone contents compared to the pickling process. The vinegars, however, were still effective for the elution of isoflavones from seeds, as seen with the pickling process.

Acknowledgments

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MOST) (No. R01-2003-000-10790-0).

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