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# Heating Affects the Content and Distribution Profile of Isoflavones in Steamed Black Soybeans and Black Soybean Koji

RU-YUE HUANG AND CHENG-CHUN CHOU*

Graduate Institute of Food Science and Technology, National Taiwan University, Taipei, Taiwan

Steamed black soybeans and black soybean koji, a potentially functional food additive, were subjected to heating at 40−100 °C for 30 min. It was found that steamed black soybeans and black soybean koji after heating at 80 °C or higher generally showed reduced contents of malonylglucoside, acetylglucoside, and aglycone isoflavone and an increased content of β-glucoside. A lower reduction in malonylglucoside and acetylglucoside isoflavone but greater reduction in aglycone content was noted in steamed black soybeans compared to black soybean koji after a similar heat treatment. After 30 min of heating at 100 °C, steamed black soybean retained ca. 90.3 and 83.8%, respectively, of its original malonylglucoside and acetylglucoside isoflavone, compared to lower residuals of 80.9 and 78.8%, respectively, for black soybean koji. In contrast, the heated black soybeans showed an aglycone residual of 68.0%, which is less than the 80.0% noted with the heated black soybean koji.

**KEYWORDS: Black soybean koji; heat treatment; isoflavone**

## INTRODUCTION

Isoflavones, a subclass of flavonoids, have an extremely limited distribution in nature. They are mainly found in soybean and soy foods. In these foodstuffs, the isoflavones are present in four chemical forms, with daidzein, glycitein, and genestein serving as the three basic chemical structures for aglycones. On the other hand, there are three other forms, namely, β-glucoside, acetylglucoside, and malonylglucoside and derivatives from each aglycone (*1*).

Isoflavones are known as phytoestrogens, because they are able to interact with cellular receptors for estrogen due to similarity in their chemical structures (*2*). Additionally, various other health functions of isoflavones have been suggested. They were reported to reduce the level of low-density lipoprotein (LDL) cholesterol, total cholesterol, and LDL oxidation and thus reduce the development of cardiovascular diseases (*3*−*5*). Other health benefits that have been claimed include a reduction in the incidence of cancer, alleviation of postmenopausal symptoms, and reduced risk of osteoporosis in women (*6*).

Black soybean [*Glycine max* (L.) Merr.] is a rich source of protein, isoflavone, and vitamins B and E and is a nutritious food possessing functional properties (*7*). It is used to prepare traditional fermented food products such as *in-yu* black soybeans and *in-si* or *tou si*, the dried byproduct of the mash black soybean sauce (*8*). Recently, black soybean has been found to reduce the incidence of DNA damage by cyclophosphamide (*9*) and to inhibit LDL oxidation (*10*). It also has been proposed as a nutritious weaning food by using *Rhizopus*-fermented black soybean with rice (*11*). On the basis of studies conducted in our laboratory, we have observed that fungis-fermented black soybean (black soybean koji) possesses enhanced antioxidative and antimutagenic activities (*12, 13*). In addition, it has been noted that the content of the bioactive isoflavone, aglycone, increased in black soybean after fermentation with fungi (*14*). Due to these findings, the black soybean koji has been recommended as a useful ingredient for the formulation of healthy food.

Heating is commonly used in the manufacturing process of food. Changes in the content and distribution profile of isoflavones in foods due to heat treatments have been observed (*15*−*19*). This alteration may affect the functional properties of food (*20*). In the present study, we explore and compare the thermal stabilities of various isoflavones in unfermented steamed black soybeans and black soybean koji. The results could be useful when black soybeans or black soybean koji is further processed as a food product or a functional food additive.

## MATERIALS AND METHODS

**Preparation of Steamed Black Soybeans and Black Soybean Koji.** In the present study, black soybeans were obtained from a local market. A solid state fermentation as described by Lee and Chou (*14*) was performed to prepare the black soybean koji. Essentially, black soybeans, after washing, were soaked overnight in distilled water. After the water had been decanted, the black soybeans were steam-cooked in an autoclave (121 °C, 15 min). The steamed black soybeans were then inoculated with a spore suspension of *Aspergillus awamori* and incubated at 30 °C and 95% relative humidity for 3 days. The black

* Address correspondence to this author at the Graduate Institute of Food Science and Technology, National Taiwan University 59, lane 144, Keelung Rd., Sec. 4, Taipei, Taiwan (telephone 886-2-3366-4111; fax 886-2-2362-0849; e-mail fstcchou@ntu.edu.tw).

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**Table 1.** Isoflavone Contents of Steamed Black Soybeans after 30 min of Heating at Different Temperatures[a]

| isoflavone | control | content of isoflavone (μg/g of dried steamed soybeans) after heating | | | |
|---|---|---|---|---|---|
| | | 40 °C | 60 °C | 80 °C | 100 °C |
| β-glucoside | | | | | |
| daidzin | 695.4 ± 10.0b | 695.9 ± 9.64b | 696.4 ± 5.5b | 703.6 ± 21.0ab | 722.2 ± 15.3a |
| glycitin | 246.9 ± 3.6b | 247.9 ± 10.53b | 252.8 ± 11.7b | 264.7 ± 9.1b | 285.9 ± 14.0a |
| genistin | 705.1 ± 4.1b | 702.8 ± 2.68b | 706.3 ± 11.7b | 713.7 ± 21.1ab | 732.2 ± 12.7a |
| malonylglucoside | | | | | |
| daidzin | 218.2 ± 2.7a | 216.5 ± 3.8a | 213.4 ± 3.4a | 212.3 ± 5.0a | 212.4 ± 14.9a |
| glycitin | 72.1 ± 1.1a | 70.1 ± 1.5a | 67.2 ± 3.3ab | 63.4 ± 2.6bc | 58.8 ± 6.3c |
| genistin | 179.2 ± 14.3a | 182.2 ± 9.3a | 176.6 ± 6.1a | 169.3 ± 3.7a | 152.6 ± 3.1b |
| acetylglucoside | | | | | |
| daidzin | 91.7 ± 2.1a | 87.7 ± 2.7ab | 84.5 ± 4.01b | 82.2 ± 2.6bc | 76.7 ± 3.7c |
| glycitin | 23.0 ± 2.8a | 22.3 ± 2.4a | 21.4 ± 2.20ab | 19.9 ± 1.4ab | 14.9 ± 7.4b |
| genistin | 101.5 ± 0.3a | 99.1 ± 0.9ab | 98.1 ± 1.25bc | 96.5 ± 2.1ab | 89.3 ± 1.6d |
| aglycone | | | | | |
| daidzein | 23.8 ± 1.0a | 23.0 ± 0.9ab | 20.8 ± 2.2bc | 19.1 ± 1.2c | 15.9 ± 1.0d |
| glycitein | 8.7 ± 0.6a | 8.3 ± 0.5ab | 7.7 ± 0.7ab | 7.4 ± 1.0b | 5.8 ± 0.5c |
| genistein | 25.6 ± 0.7a | 24.8 ± 0.4ab | 24.1 ± 0.6b | 22.7 ± 1.1b | 17.9 ± 1.6c |
| total | 2391.1 ± 15.8a | 2380.6 ± 31.9a | 2369.3 ± 16.5a | 2374.6 ± 31.2a | 2384.4 ± 22.5a |

[a] Values are presented as means ± SD ($n = 3$). Means in the same row with different letters were significantly different by Duncan's multiple-range test ($p < 0.05$).

soybean koji and the unfermented steamed black soybeans were then freeze-dried by a freeze-dryer (77500-00 M. Labconco Co., Kansas City, MO) and homogenized.

**Heating of Steamed Black Soybeans and Black Soybean Koji.** The steamed black soybeans and the black soybean koji were heated by putting 10 g of the ground powder of the samples in a plate, which was then placed in an electric oven with the temperature controlled at 40, 60, 80, or 100 °C for 30 min. After heating, the sample-containing flasks were immediately cooled in an ice water bath for 30 min, and then the isoflavone content was analyzed.

**Analysis of Isoflavones.** Isoflavones, in both the samples of unfermented steamed black soybeans and black soybean koji, were extracted and analyzed according to the procedures described previously (*14*). In brief, dried powder (2.0 g) was extracted with 80% methanol (20.0 mL) by shaking (120 rpm) at 25 °C for 2 h and filtered through Whatman no. 42 filter paper. The filtrate was condensed to approximately 1−2 mL using a vacuum rotary evaporator at ca. 35 °C. It was then combined with fluorescein as an internal standard and readjusted with 80% methanol to a final volume of 20 mL. The samples were then filtered through a 0.45 μm Millipore PVDF filter (Schleicher & Schuell, GmbH, Dassel, Germany) and subjected to HPLC analysis for isoflavones. The HPLC equipment used was a chromatograph (model 7200, Jasco Co., Tokyo, Japan) equipped with a YMC-Pack ODS-AM-303 column (250 × 4.6 mm, 5 μm, YMC Co., Ltd., Kyoto, Japan), a UV−vis detector (model UV-970, Jasco), and a SISC chromatography data processor (SISC Co., Davis, CA). A linear HPLC gradient was composed of (A) 0.1% glacial acetic acid in $H_2O$ and (B) 0.1% glacial acetic acid in acetonitrile. After the 20 μL injection of sample onto the column (25 °C), solvent B was increased from 15 to 20% in 20 min, then increased to 24% in 10 min, held at 24% for 4 min, then further increased to 35% 10 min later, at which time it was held at 35% for 8 min, and then finally reduced to 15% after a further 5 min. The solvent flow rate was 1.0 mL/min. The content of the isoflavones was calculated from the standard curves of the area responses for authentic isoflavone standards normalized to the constant amount of fluorescein added to each sample. The contents were expressed as micrograms per gram of dried steamed black soybeans or koji.

**Determination of Dry Weight.** The dry weight of samples was determined according to an AOAC method (*21*).

**Statistical Analysis.** The mean values and the standard deviation were calculated from the data obtained from three separate experiments. These data were then compared by the Duncan's multiple-range test (*22*).

## RESULTS AND DISCUSSION

**Content and Profile of Isoflavones in Steamed Black Soybeans after Heating.** Generally, malonylglucosides were the major forms of soy iosflavone followed by β-glucosides, whereas the aglycones and acetylglucosides were present in only trace amounts (*23, 24*). However, β-glucosides became the predominant form of isoflavone along with a substantial amount of acetylglucosides and aglycones in the control sample of steamed black soybeans (**Table 1**). This phenomenon of alteration in isoflavone isomers observed in steamed black soybeans is in accordance with report of Wang and Murphy (*24*) and may be attributed to the effect of autoclaving, which was employed for the preparation of steamed black soybeans. As shown in **Table 1**, the content of total isoflavones of steamed black soybeans, regardless of further heating, ranged from 2391.1 to 2369.3 μg/g of dried steamed black soybeans. After heating, the residual of total isoflavone content ranged from 99.1 to 99.7%, showing no significant difference ($p > 0.05$) from the control.

Thermal stability of isoflavones varies with the isomers. Testing in the solution containing only isoflavones purified from soy flour, Xu et al. (*17*) indicated that the order of stabilities of β-glucoside isoflavones was glycitin, genistin, and daidzin, from lowest to highest, whereas among the aglycones, the stability of daidzen was higher than that of glycitein or genistein. We found that, generally, the content of individual aglycones (daidzein, glycitein, and genistein) decreased in steamed black soybeans as the heating temperature was raised to 60 °C or higher, whereas the content of daidzin, glycitin, and genistin, the β-glucoside isoflavones, increased in steamed black soybeans after heating at 60−80 °C or higher for 30 min. Meanwhile, a decreased content of the corresponding malonyl- and acetylglucoside isoflavone isomers was noted in the heated-steamed black soybeans. This phenomenon became more pronounced as the heating temperature was raised to 100 °C. For example, the content of daidzin increased from 695.4 to 722.2 μg/g of dried steamed black soybeans in the 100 °C heated−steamed black soybeans. Meanwhile, the content of malonyldaidzin, acetyldaidzin, and daidzein changed from 218.2, 91.7, and 23.8 μg/g of dried steamed black soybeans, respectively, in the unheated black soybeans to 212.4, 76.7, and 15.9 μg/g of dried steamed black soybeans noted in the 100 °C heated steamed black soybeans.

**Table 2** shows the total content and distribution profile of β-glucoside, acetylglucoside, malonylglucoside, and aglycone isoflavone in steamed black soybean before and after heat treatment. Despite the insignificant alteration ($p > 0.05$) of total

**Table 2.** Contents and Distribution Profile of Isoflavones in Steamed Black Soybeans after 30 min of Heating at Different Temperatures[a]

| heating temperature | β-glucoside | | | malonylglucoside | | | acetylglucoside | | | aglycone | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | content (μg/g) | residual[b] (%) | distribution[c] (%) | content (μg/g) | residual (%) | distribution (%) | content (μg/g) | residual (%) | distribution (%) | content (μg/g) | residual (%) | distribution (%) |
| control | 1647.4 ± 16.4b | 100.0 ± 0.0b | 68.9 ± 0.0d | 469.5 ± 11.3a | 100.0 ± 0.0a | 19.6 ± 0.0a | 216.1 ± 4.4a | 100.0 ± 0.0a | 9.1 ± 0.0a | 58.2 ± 2.3a | 100.0 ± 0.0a | 2.4 ± 0.0a |
| 40 °C | 1646.5 ± 16.5b | 100.5 ± 0.8b | 69.2 ± 0.0 cd | 468.8 ± 12.1a | 99.9 ± 2.4a | 19.7 ± 0.0a | 209.1 ± 5.7ab | 96.8 ± 1.6ab | 8.8 ± 0.0ab | 56.2 ± 1.7ab | 96.6 ± 1.7a | 2.4 ± 0.0ab |
| 60 °C | 1655.5 ± 7.8b | 100.5 ± 0.8b | 69.9 ± 0.0c | 457.8 ± 12.6ab | 97.4 ± 3.2a | 19.3 ± 0.0ab | 204.0 ± 6.2ab | 94.4 ± 2.0ab | 8.6 ± 0.0ab | 52.6 ± 2.9bc | 90.4 ± 4.9b | 2.2 ± 0.0bc |
| 80 °C | 1682.0 ± 32.0b | 102.1 ± 2.8b | 70.8 ± 0.0b | 444.9 ± 0.9b | 94.8 ± 2.2ab | 18.7 ± 0.0b | 198.5 ± 6.0b | 91.9 ± 2.7b | 8.4 ± 0.0b | 49.2 ± 1.8c | 84.6 ± 4.6b | 2.1 ± 0.0c |
| 100 °C | 1740.2 ± 19.4a | 105.6 ± 1.6a | 73.0 ± 0.0a | 423.8 ± 12.2c | 90.3 ± 4.7b | 17.8 ± 0.0c | 180.9 ± 11.0c | 83.8 ± 6.2c | 7.6 ± 0.0c | 39.6 ± 2.6d | 68.0 ± 1.9c | 1.7 ± 0.0d |

[a] Values are presented as means ± SD ($n = 3$). Means in the same column with different letters were significantly different by Duncan's multiple-range test ($p < 0.05$). [b] Residual percent was obtained by dividing the isoflavone content of the treated sample with the initial isoflavone content of the control sample. The isoflavone content of initial sample was regarded as 100%. [c] Distribution percent was obtained by dividing the content of individual isoflavone with the sum of isoflavone content of the sample.

isoflavone (**Table 1**), change in the content of these isoflavone isomers was observed in the steamed black soybeans depending on heating temperature (**Table 2**). Compared with that of the respective isoflavone content in the control, the total content of β-glucoside, malonylglucoside, and acetylglucoside isoflavone did not change significantly ($p > 0.05$) in the steamed black soybeans after heating at 60 °C or lower, whereas heating at 80 or 100 °C resulted in a significant ($p < 0.05$) increase in the content of β-glucoside isoflavone with a reduced content of acetylglucoside, malonylglucoside, and aglycone isoflavone in the heated−steamed black soybeans. The 100 °C heated−steamed black soybeans contained a glucoside isoflavone content of 1740.2 μg/g of dried steamed black soybeans compared to a lower content of 1647.4 μg/g of dried steamed black soybeans noted in the control steamed black soybeans. On the other hand, the contents of malonylglucoside, acetylglucoside, and aglycone isoflavone were reduced from, respectively, 469.5, 216.1, and 58.2 to 423.8, 180.9, and 39.6 μg/g of dried steamed black soybeans after 30 min of heating at 100 °C.

As shown in **Table 2**, the percentage of β-glucoside increased while the percentage of malonglucoside, acetylglucoside, and aglycone isoflavone decreased as the black soybeans were heated at 80 °C or higher for 30 min. After 30 min of heating at 100 °C, the percentage of β-glucoside increased from 68.9 to 73.0%, whereas the percentage of malonylglucoside decreased from 19.6 to 17.8%. This observation is partially in agreement with the report of Wang and Murphy (*24*), who observed that the cooking step in tofumaking altered the distribution of isoflavones by decreasing the malonylglucosides and increasing the β-glucoside form of isoflavones and aglycones. We noted that the percentage of total aglycone in steamed black soybeans decreased significantly ($p < 0.05$) after heating at 60 °C or higher for 30 min. This result is different from the report of Xu et al. (*17*), who indicated that aglycones in solution containing no nonisoflavone compound were relatively stable after heating at 200 °C over a period of 30 min. In the present study, steamed black soybeans, a rather complicated food system containing isoflavones and other constituents, were examined. Davis et al. (*25*) indicated that autodegradation and reaction of aglycone isoflavone such as genistein with an amino group to form Maillard reaction products might occur and lead to the loss of aglycones during heating. Therefore, the discrepancy between our results and those observed by Xu et al. (*17*) may be attributed to the difference of the testing system. On the other hand, the reduced content of aglycone observed in the heated−steamed black soybeans is in agreement with the report of Grun et al. (*26*), who found that the content of daidzein and gensitein decreased in tofu during thermal treatment. Furthermore, the observed alteration in the distribution profile of isoflavone in the heated−steamed black soybeans is similar to that reported by Coward et al. (*15*).

Xu et al. (*17*) reported that the β-glucoside isoflavones (daidzin, glycitin, and genistin) are stable at temperatures near the boiling point of water. Park et al. (*27*) and Kudou et al. (*28*) indicated that malonylated isoflavone glucosides were heat-liable, whereas thermal hydrolysis resulting from the de-esterification of malonyl and acetylglucoside isoflavones to their underivatized glucosides was observed by various investigators (*15, 27, 29*). Ungar et al. (*18*) found the degradation of daidzein and genistein incubated at 70−90 °C. In addition, Davis et al. (*25*) and Grun et al. (*26*) indicated that autodegradation and reaction of aglycone with glycine might occur during heating. These may all have contributed to the increased content of β-glucoside and the decreased level of aglycone and malonyl-

**Table 3.** Isoflavone Contents of Black Soybean Koji after 30 min of Heating at Different Temperatures[a]

| | | content of isoflavone ($\mu$g/g of dried koji) after heating | | | |
|---|---|---|---|---|---|
| isoflavone | control | 40 °C | 60 °C | 80 °C | 100 °C |
| $\beta$-glucoside | | | | | |
| daidzin | 323.4 ± 7.3bc | 315.9 ± 4.1c | 326.5 ± 4.5b | 330.3 ± 3.7b | 341.9 ± 1.4a |
| glycitin | 139.0 ± 4.9b | 138.3 ± 4.9b | 143.3 ± 2.4ab | 142.4 ± 3.3ab | 147.3 ± 1.9a |
| genistin | 520.6 ± 6.7c | 521.2 ± 8.8a | 527.4 ± 7.3a | 538.8 ± 9.9a | 557.7 ± 43.4a |
| malonylglucoside | | | | | |
| daidzin | 89.1 ± 2.0a | 87.0 ± 2.4a | 84.6 ± 2.3a | 81.8 ± 2.5bc | 79.3 ± 4.2c |
| glycitin | 47.2 ± 8.6a | 46.1 ± 7.7ab | 44.1 ± 8.0ab | 40.4 ± 7.8ab | 32.0 ± 3.4b |
| genistin | 52.0 ± 3.6a | 50.9 ± 0.5a | 49.2 ± 1.0ab | 46.3 ± 0.8b | 40.8 ± 1.0c |
| acetylglucoside | | | | | |
| daidzin | 52.9 ± 2.0a | 52.1 ± 0.8a | 50.7 ± 0.4a | 46.3 ± 2.8b | 41.7 ± 2.3c |
| glycitin | 13.6 ± 0.7a | 13.2 ± 0.7ab | 12.9 ± 0.6ab | 12.0 ± 0.8bc | 10.8 ± 0.8c |
| genistin | 91.9 ± 4.0a | 90.4 ± 2.4ab | 86.4 ± 0.7bc | 84.7 ± 1.2c | 72.3 ± 1.4d |
| aglycone | | | | | |
| daidzein | 174.9 ± 5.0a | 178.0 ± 0.3a | 157.9 ± 5.1b | 157.5 ± 2.6b | 154.1 ± 2.3b |
| glycitein | 28.2 ± 1.2a | 29.7 ± 1.4a | 27.3 ± 0.6a | 27.6 ± 1.9a | 19.4 ± 2.0b |
| genistein | 163.5 ± 7.0a | 164.2 ± 4.5a | 154.8 ± 6.5a | 134.3 ± 15.5b | 119.9 ± 0.7b |
| total | 1696.3 ± 36.0a | 1686.8 ± 30.4a | 1665.2 ± 19.3ab | 1642.4 ± 24.8ab | 1617.0 ± 53.5b |

[a] Values are presented as means ± SD ($n = 3$). Means in the same row with different letters were significantly different by Duncan's multiple-range test ($p < 0.05$).

and acetylglucoside isoflavones observed in the steamed black soybeans after heating. However, the exact cause merits further investigation.

**Content and Profile of Isoflavones in Black Soybean Koji after Heating. Table 3** shows the total and individual isoflavone contents of unheated black soybean koji and black soybean koji heated at various temperatures. Generally, a slightly lower content of total isoflavone was noted with the heated black soybean koji when compared with unheated black soybean koji (control). Statistical analysis revealed that the content of the 100 °C heated black soybean koji, retaining ca. 95.3% of the original total isoflavones, was significantly less ($p < 0.05$) than that of the unheated black soybean koji.

Consistent with our previous observation (*14*), the unheated black soybean koji had lower contents of daidzin, glycitin, and genistein (**Table 3**) than did the steamed black soybeans without fermentation (**Table 1**). Besides, the individual aglycone isomer (daidzein, glycitein, and genistein) content noted in the unheated black soybean koji (**Table 3**) is higher than the respective isoflavone isomer content of the unheated steamed black soybeans (**Table 1**). Apparently, this discrepancy was essentially due to the catalytic action of $\beta$-glucosidase produced by microorganisms as well as the hydrolysis and de-esterification of malonylglucosides, which occurred during the fermentation of black soybean (*14, 15, 26*). As shown in **Tables 3** and **4**, the trend in the changes of isoflavone content of black soybean koji caused by heating is generally similar to that observed for steamed black soybeans (**Tables 1** and **2**). Generally, the heated black soybean koji also exhibited an increased content of $\beta$-glucoside isoflavone with a reduced level of malonylglucoside, acetylglucoside, and aglycone isoflavone isomer after heating at 60−80 °C or higher for 30 min. After heating at 100 °C for 30 min, a significantly increased content of $\beta$-glucoside isoflavone of 1046.9 $\mu$g/g of dried black soybean koji was noted in the black soybean koji compared with the lower content of 982.9 $\mu$g/g of dried black soybean koji noted in the unheated black soybean koji. However, significantly lower ($p < 0.05$) contents of 152.1, 124.8, and 293.3 $\mu$g/g of dried black soybean koji, respectively, of malonylglucoside, acetylglucoside, and aglycone were observed in the 100 °C heated black soybean koji.

Despite the similarity in the trends of changing isoflavone content in the steamed black soybeans and black soybean koji after heating, generally, the reduction in the content of the individual or the total malonylglucoside and acetylglucoside isoflavone is more pronounced in black soybean koji than in steamed black soybeans after exposure to a similar heat treatment. For example, the 100 °C heated−steamed black soybeans contained ca. 90.3 and 83.8%, respectively, of their original total malonylglucoside and acetylglucoside isoflavones (**Table 2**), whereas lower residuals of 80.9 and 78.8%, respectively, were noted with the total malonylglucoside and acetylglucoside in the 100 °C heated black soybean koji (**Table 4**). In contrast, the heated black soybean koji showed a higher residual of aglycone (**Table 4**) than did the heated−steamed black soybeans (**Table 2**) after exposure to a similar heat treatment. Fermentation changes the texture and components of substrate due to the hydrolytic action of enzymes produced by microorganisms (*30*). It is reasonable to expect that the constituents of black soybean koji are more accessible to heat. This might enhance the de-esterification of malonylglucoside and acetylglucoside to their underivative glucosides. This may thus lead to the higher residual of $\beta$-glucoside with a lower residual of malonylglucoside and acetylglucoside observed in the heated black soybean koji (**Tables 3** and **Tables 4**) than in the heated−steamed black soybeans (**Tables 1** and **2**). In contrast to steamed black soybean samples, black soybean koji contained $\beta$-glucosidase produced by fungi during fermentation (*14*). As a result, catalytic liberation of aglycone from glucoside isoflavone by the activity of $\beta$-glucosidase during the heating process might occur. The liberated aglycone may compensate for the reduction of aglycone caused by heating and thus lead to a higher residual of aglycone noted with the heated black soybean koji than with the heated−steamed black soybeans. However, the exact reason remains to be further explored.

On the basis of the data obtained from the present study, it is concluded that heating may change the content and distribution profile of isoflavone by reducing the content of aglycone, malonylglucoside, and acetylglucoside isoflavones while increasing the content of $\beta$-glucoside isoflavone in steamed black soybeans and their fermented products. However, the change induced by heating is more profound in black soybean koji than in the unfermented steamed black soybeans. Although the heated black soybean koji retained less malonyl- and acetylglycoside isoflavone than did the heated−steamed black soybeans, it contained a higher residual of aglycone. Furthermore, the black soybean koji and steamed black soybeans retained a considerable amount of isoflavone after heating at 100 °C for 30 min. These observations provide valuable information related to the further utilization and processing of black soybean and black soybean koji as a food product or as an ingredient in the formulation of healthy food.

**Table 4.** Contents and Distribution Profile of Isoflavones in Black Soybean Koji after 30 min of Heating at Different Temperatures[a]

| heating temperature | β-glucoside | | | malonylglucoside | | | acetylglucoside | | | aglycone | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | content (μg/g) | residual[b] (%) | distribution[c] (%) | content (μg/g) | residual (%) | distribution (%) | content (μg/g) | residual (%) | distribution (%) | content (μg/g) | residual (%) | distribution (%) |
| control | 982.9 ± 18.8b | 100.0 ± 0.0b | 58.0 ± 0.0d | 188.3 ± 11.8a | 100.0 ± 0.0a | 11.1 ± 0.0a | 158.4 ± 6.3a | 100.0 ± 0.0a | 9.3 ± 0.0a | 366.66 ± 4.97a | 100.0 ± 0.0a | 21.6 ± 0.0a |
| 40 °C | 975.5 ± 16.2b | 99.2 ± 0.8b | 57.8 ± 0.0d | 184.0 ± 10.6a | 97.7 ± 2.6a | 10.9 ± 0.0a | 155.6 ± 3.3ab | 98.3 ± 2.3ab | 9.2 ± 0.0a | 371.75 ± 3.65a | 101.4 ± 0.7a | 22.0 ± 0.0a |
| 60 °C | 997.2 ± 10.3b | 101.5 ± 2.9b | 59.9 ± 0.0c | 177.9 ± 10.4a | 94.5 ± 3.9ab | 10.7 ± 0.0a | 150.0 ± 0.8bc | 94.8 ± 3.9b | 9.0 ± 0.0ab | 340.06 ± 11.18b | 92.8 ± 4.1b | 20.4 ± 0.0b |
| 80 °C | 1011.5 ± 10.2ab | 102.9 ± 1.3ab | 61.6 ± 0.0b | 168.6 ± 10.8ab | 89.5 ± 3.2b | 10.3 ± 0.0ab | 143.0 ± 4.7c | 90.3 ± 2.5c | 8.7 ± 0.0b | 319.41 ± 17.91c | 87.1 ± 4.3c | 19.5 ± 0.0b |
| 100 °C | 1046.9 ± 45.2a | 106.5 ± 3.7a | 64.7 ± 0.0a | 152.1 ± 6.5b | 80.9 ± 3.5c | 9.4 ± 0.0b | 124.8 ± 4.0d | 78.8 ± 1.4d | 7.7 ± 0.0c | 293.31 ± 2.86d | 80.0 ± 0.9d | 18.2 ± 0.0c |

[a] Values are presented as means ± SD ($n$ = 3). Means in the same column with different letters were significantly different by Duncan's multiple-range test ($p < 0.05$). [b] Residual percent was obtained by dividing the isoflavone content of the treated sample with the initial isoflavone content of the control sample. The isoflavone content of initial sample was regarded as 100%. [c] Distribution percent was obtained by dividing the content of individual isoflavones with the sum of isoflavone content of the sample.

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