Isoflavone Contents in Thai and Imported Soy-Based Beverages Commercially Available in Thailand

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Abstract

Objective To investigate and compare the contents of isoflavones in the form of β-glycosides (daidzin and genistin) as well as their respective aglycones (daidzein and genistein) in Thai versus imported soy-based beverages commercially available in Thailand.

Material and method Fifty-one brands (34 Thai brands and 17 imported products) of soy-based beverages, which used a sample code instead of their trade name, were analyzed for their isoflavone contents by using high performance liquid chromatography. The average coefficient of intraday and interday assay validation for daidzin, genistin, daidzein and genistein was less than 4.5%.

Results The total isoflavone contents in both soy-based beverages varied substantially from approximately 7 to 85 mg per serving, with β-glycosides dominating. Although the mean concentrations of aglycones in Thai soy-based beverages were significantly greater than those of the imported products, the mean concentrations of β-glycosides and total isoflavone contents per serving (either mg or μmol) between both products did not differ significantly. The average price per serving of imported products was more expensive statistically, despite comparable total isoflavone contents.

Conclusion Total isoflavone contents in both products varied substantially. The Thai soy-based beverages contained comparable average contents of isoflavones per serving when compared to the imported products, but they were less costly.

Keywords: isoflavones, daidzin, daidzein, genistin, genistein, soy-based beverages
In human food, soybeans are the usual source of isoflavones, which represent the most common group of phytoestrogens, as their structures resemble that of the potent synthetic estrogen, diethylstilbestrol. The basic structural feature of isoflavone aglycones consists of two benzene rings (A and B) linked through a heterocyclic pyran C ring (Fig. 1). Three isoflavone aglycones (daidzein, genistein and glycitin) exist in soy-based food and beverage. Most nonfermented soy-based products contain mixtures of β-glycosides as well as 6''-O-malonyl-β-glycosides (60MalGlc) and 6''-O-acetyl-β-glycosides (60AcGlc) conjugates (Fig. 1), whereas the unconjugated isoflavone aglycones, which derive from enzymatic hydrolysis during fermentation, are the predominant forms in fermented soy food such as miso and tempeh.

Isoflavones have received considerable attention because of their potential roles in relieving postmenopausal hot flushes as well as preventing and treating postmenopausal osteoporosis. A recent meta-analysis, which investigated 40–80 mg/day of calculated aglycones, found a statistical reduction in the frequency of menopausal hot flushes, and the extent of benefit appeared to associate positively with their frequency at baseline. Additionally, clinical studies in postmenopausal women, who were supplemented with 54-90 mg/day of soy isoflavones, demonstrated a favorable effect on markers of bone metabolism and preservation of bone density. A recent meta-analysis of randomized controlled trials also shown that daily isoflavone intervention

<table>
<thead>
<tr>
<th>β-glucosidase catalytic site</th>
<th>Isoflavone glycoside</th>
<th>Isoflavone aglycones</th>
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<tr>
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<td><img src="image2.png" alt="Isoflavone aglycones" /></td>
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### Table

<table>
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<th>R1</th>
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<th>Compound</th>
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<tr>
<td>H</td>
<td>H</td>
<td>-</td>
<td>Daidzein</td>
</tr>
<tr>
<td>OH</td>
<td>H</td>
<td>-</td>
<td>Genistein</td>
</tr>
<tr>
<td>H</td>
<td>OCH3</td>
<td>-</td>
<td>Glycitein</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>Daidzin</td>
</tr>
<tr>
<td>OH</td>
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<tr>
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<td>H</td>
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<tr>
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<td>OCOCH3</td>
<td>Acetylgenitin</td>
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<tr>
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<td>H</td>
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<td>Malonyldaidzin</td>
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<td>H</td>
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<td>OCOCH2COOH</td>
<td>Malonylglycitin</td>
</tr>
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</table>

**Figure 1.** Structure of isoflavones in the form of aglycones, β-glycosides, 6''-O-acetyl-β-glycosides and 6''-O-malonyl-β-glycosides.
Isoflavone contents in soy-based beverages

of >90 mg significantly attenuates bone loss of the spine in postmenopausal women.\(^{(9)}\) Furthermore, isoflavone intake of at least 80 mg/day also demonstrates favorable effects on lipid profiles in various populations, including postmenopausal women and subjects with hypercholesterolemia.\(^{(16)}\) In summary, the total dose of isoflavones that benefits menopausal health is generally up to approximately 126 mg/day.

Fresh soy milk, soy milk processed with Ultra High Temperature (UHT) technology and instant soy beverage powder have received more attention in Thailand because they contain isoflavones that might offer added health benefits together with low cost. The \(\beta\)-glycosides (daidzin and genistin) are found to be predominant forms in soy-based beverages\(^{(17-19)}\) However, since glycitein and its glycoside conjugates account for less than 5-10\% of the total isoflavones in soy-based food, most studies of soy isoflavones have focused on daidzein and genistein (and their respective glycosides), with little attention paid to glycitein.\(^{(20)}\) In 2002, we reported that the contents of isoflavones in UHT (10 brands) and fresh soy milk (20 samples), which were randomly purchased from different zones in Amphur Muang, Chiang Mai, Thailand, varied substantially from approximately 10 to 70 mg per serving.\(^{(21)}\) However, since the consumption of soy-based products has steadily increased worldwide during the past decade, there are currently more than 50 brands of soy-based beverages commercially available in Thailand in the form of UHT prepared soy milk and instant soy beverage powder. Approximately one-third of these brands are imported products from countries such as the United States of America and Australia. Therefore, the aim of this study was to investigate and compare the contents of isoflavones in the form of \(\beta\)-glycosides (daidzin and genistin) as well as their respective aglycones (daidzein and genistein) in Thai versus imported soy-based beverages commercially available in Thailand. This was done in order to establish a guideline for determining the daily amount of soy-based beverage intake that is sufficient to benefit postmenopausal health.

MATERIAL AND METHOD

Study design

This was a cross-sectional study to determine the contents of isoflavones in soy-based beverages commercially available in Thailand.

Samples for quantification of isoflavones

A total of 51 brands of soy-based beverages was purchased randomly from different department stores in Amphur Muang, Chiang Mai, Thailand. They included 34 Thai products (31 brands of UHT soy milk and 3 brands of instant soy beverage powder) and 17 brands of imported UHT soy milk. Each preparation was given a sample code instead of its trade name.

Quantification of isoflavones in soy-based beverages

The sample preparation and determination of isoflavone concentrations in both soy-based beverages were modified from the method described by Barnes et al\(^{41}\) and the AOAC official method 2001.10.\(^{(22)}\) Briefly, one mL of soy-based beverage was mixed with 9 mL of 80\% methanol in water. Then, 350 \(\mu\)L of 2 M NaOH were added and sonicated for 30 minutes. Two hundred and fifty \(\mu\)L of 100\% acetic acid was added and
mixed. Finally, one mL of the mixture was centrifuged, and 10 μL of clear supernatant was mixed with 30 μL of mobile phase and spiked with 10 μL of internal standard (IS, 50,000 ng/mL of fluorescein). Five μL of the mixture was injected into the high-performance liquid chromatography (HPLC) system.

The samples were analyzed on a C18 column (Inertsil, 150 mm x 4.6 I.D., 5μm, GL Science, Tokyo, Japan) with a C18 guard column (Inertsil ODS-3, 10 mm x 4.0 I.D., 5μm, GL Science, Tokyo, Japan). The chromatography condition consisted of two mobile phases. Mobile phase A was 55 mM ammonium acetate in distilled water/acetonitrile/methanol (250:45:45, v/v/v). Mobile phase B was 55 mM ammonium acetate in distilled water/acetonitrile/methanol (250:255:220, v/v/v). Both mobile phases contained 29 μL of perchloric acid and 250 μL of 1.44 mM sodium dodecyl sulfate. Separation was performed isocratically at 25 ºC. The flow rate of mobile was maintained at 1 mL/min and analyses were detected by UV absorption at 259 nm. Standard isoflavones (daidzin, genistin, daidzein and genistein) were spiked in serial dilution to obtain a standard calibration curve of 93.75, 187.5, 375, 750, 1500, 3000 and 6000 ng/mL. The regression equations for testing the linearity of standard calibration curves are as follows:

\[ y = 0.232x - 0.0478 \quad (r^2 = 1), \text{ for daidzin.} \]
\[ y = 0.4927x - 0.9139 \quad (r^2 = 1), \text{ for genistin.} \]
\[ y = 0.2868x + 0.2629 \quad (r^2 = 0.9998) \text{ for daidzein.} \]
\[ y = 0.3912x + 0.1016 \quad (r^2 = 1) \text{ for genistein.} \]

Chromatograms of isoflavones and their retention times are shown in Figure 2. The isoflavone content of unknown samples was determined by using a calibration curve of the peak height ratios of isoflavones and IS versus respective isoflavone concentrations, with the use of linear regression. Each

**Figure 2A.** Chromatogram of isoflavones in a standard mixture of daidzin, genistin, daidzein and genistein (6,000 ng/mL, each) and internal standard (IS, fluorescein 10,000 ng/mL).

**Figure 2B.** Chromatogram of isoflavones (daidzin, genistin, daidzein and genistein) in a Thai soy-based beverage sample (study code - OYH).
sample was analyzed in triplicate.

**Assay validation**
Five quality control (QC) samples spiked at 3 different concentrations of daidzein and genistein (281.25, 2,750 and 5,500 ng/mL) were analyzed for intraday and interday assay validation. The average coefficient of intraday assay validation for daidzin, genistin, daidzein and genistein was 1.91%, 1.73%, 2.92% and 2.01%, respectively. The average coefficient of interday assay validation for daidzin, genistin, daidzein and genistein was 4.22%, 3.05%, 2.85% and 2.09%, respectively.

**Statistical analysis**
The concentrations and contents of isoflavones in Thai and imported soy-based beverages, as well as their price per serving, were presented as mean (standard deviation, SD). The proportions of aglycones: β-glucosides in both soy-based beverages, were also reported and compared. The differences in the mean values of these variables between both soy-based beverages were analyzed by using the student’s t-test. The coefficient of determination value ($r^2$), calculated by linear regression analysis, was used to evaluate the correlation between total contents of isoflavones in 51 commercially available soy-based beverages and their price.

**RESULTS**
The concentrations and contents of isoflavones in soy-based beverages are shown in Table 1 and Figure 3. The total isoflavone contents among 51 soy-based beverages

Table 1. Concentrations and contents of isoflavones in investigated soy-based beverages

<table>
<thead>
<tr>
<th>Variables</th>
<th>This soy beverage (n=34)</th>
<th>Imported soy beverage (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrations of aglycones1 (μmol/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daidzein</td>
<td>0.05* (0.05)</td>
<td>0.01 (0.02)</td>
</tr>
<tr>
<td>Genistein</td>
<td>0.04* (0.03)</td>
<td>0.01 (0.01)</td>
</tr>
<tr>
<td>Summation of daidzein and genistein</td>
<td>0.09* (0.08)</td>
<td>0.02 (0.02)</td>
</tr>
<tr>
<td>Concentrations of β-glucosides1 (μmol/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daidzin</td>
<td>0.15 (0.12)</td>
<td>0.16 (0.07)</td>
</tr>
<tr>
<td>Genistin</td>
<td>0.19 (0.09)</td>
<td>0.22 (0.08)</td>
</tr>
<tr>
<td>Summation of daidzin and genistin</td>
<td>0.35 (0.20)</td>
<td>0.38 (0.15)</td>
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<td>Concentrations of total isoflavones2 (μmol/mL)</td>
<td>0.43 (0.23)</td>
<td>0.40 (0.15)</td>
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<tr>
<td>Total isoflavones per serving1 (mg/serving)</td>
<td>37.57 (22.26)</td>
<td>40.58 (14.93)</td>
</tr>
<tr>
<td>Maximal absorbable isoflavones per serving2 (μmol/serving)</td>
<td>96.23 (56.84)</td>
<td>97.50 (35.70)</td>
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<tr>
<td>Proportion of aglycones : β-glucosides5</td>
<td>1 : 4</td>
<td>1 : 19</td>
</tr>
<tr>
<td>Price per serving (Baht)6</td>
<td>14.05* (12.06)</td>
<td>26.96 (4.69)</td>
</tr>
</tbody>
</table>

1-6Data represents mean (SD). 1Isoflavone concentration (μmol/mL) divided by its respective molecular weight, 2Summative concentrations (μmol/mL) of daidzein, genistein, daidzin and genistin, 3Calculation from the concentration of total isoflavones (mg/mL) x serving size (mL), 4Calculation from the summation of individual isoflavones per serving (mg) divided by their respective molecular weight, 5Summative concentrations of daidzein and genistein (μmol/mL) divided by summative concentrations of daidzin and genistin (μmol/mL). *p<0.05 compared to imported soy beverages
Isoflavones in both soy-based beverages appeared predominately in the form of β-glycosides rather than aglycones. However, the proportion of aglycones: β-glycosides in Thai versus imported soy-based beverages, were approximately 1:4 versus 1:19, respectively. Although the mean concentrations of aglycones in Thai soy-based beverages were significantly greater than those of the imported products, the mean concentrations of β-glycosides and total isoflavone contents per serving (either mg or μmol) between both products did not differ significantly. Nevertheless, the wider variation (standard deviation) in total isoflavone contents per serving of Thai products was observed when compared to those of the imported products.

The price per serving in all 41 soy-based beverages ranged from approximately 5 to 72 Thai Baht. The average price per serving varied substantially from approximately 5 to 85 mg (14-208 μmol) per serving. In 34 Thai soy-based beverages, the three samples with the highest total isoflavones per serving were VS-N (85.03 mg), VM-J (82.55 mg) and VS-L (77.83 mg), consecutively. In contrast, the three samples with the lowest total isoflavones per serving were VM-C (5.30 mg), PEP-4 (6.68 mg), and DN-O (7.04 mg), consecutively (Fig. 4). Among the 17 imported soy-based beverages, the three samples with the highest total of isoflavones per serving were from the United States of America: W-UV (68.38 mg), W-OU (67.65 mg) and S-O (48.93 mg), consecutively. Whereas the three samples with the lowest total isoflavones per serving were SG-S (15.89 mg) and SG-L (19.92 mg) from Australia, and SS-CH from the United States of America (26.65 mg), consecutively (Fig. 5).

Figure 3. A) Concentrations of isoflavone aglycones (daidzein and genistein), B) Concentrations of isoflavone glycosides (daidzin and genistin), C) Total isoflavones per serving (mg), D) Maximal absorbable isoflavones per serving (μmol), in Thai and imported soy-based beverages. Horizontal bar represents mean value. * p<0.05 compared to imported soy beverages.
Figure 4. Chromatograms of isoflavones (daidzin, genistin, daidzein and genistein) in Thai soy-based beverages.  A) Examples of the three samples with the highest total isoflavones per serving (VS-N, VM-J and VS-L).  B) Examples of the three samples with the lowest total isoflavones per serving.
Figure 5. Chromatograms of isoflavones (daidzin, genistin, daidzein and genistein) in imported soy-based beverages. A) Examples of the three samples with the highest total isoflavones per serving (W-UV, W-OU and S-O). B) Examples of the three samples with the lowest total isoflavones.
of imported products was statistically more expensive than that of Thai soy-based beverages, despite the comparable total isoflavone contents. The total of isoflavone content in 51 commercially available samples did not correlate statistically with their price (Fig. 6).

Among the 17 imported soy-based beverages, four brands contained no labeled information regarding isoflavone contents per serving on the packaging. For the remaining 13 soy-based beverages, twelve out of 13 brands contained equal or even a greater amount of total isoflavones per serving when compared to the labeled values. The last product contained approximately 20% less total isoflavones per serving than the values declared by the manufacturing company. No isoflavone label existed on any of the 34 Thai soy-based beverages.

### DISCUSSION

The total isoflavone contents among 51 soy-based beverages varied substantially from 5 to 81 mg per 200-250 serving size, while Thai products provided a wider variation compared to that of the imported products. These findings are comparable to the values of 10-70 mg per serving size the same as previously reported by us,(21) but seemingly higher than those of approximately 12-96 mg/L(17) and 12-83 mg/L(18) reported by others. Additionally, when we compared the results from this study versus our previous one,(21) we also found variations in the total isoflavones per serving of the same Thai brands, for example VS-L (77.83 versus 67.94 mg), VM-J (82.55 versus 57.88 mg), LS-J (40.93 versus 37.96 mg), etc. These findings are consistent with Murphy et al,(23) who reported significant variation in iso-

![Figure 6. The coefficient of determination value ($r^2$) calculated by linear regression analysis between total contents of isoflavones in 51 commercially available soy-based beverages and their price.](image-url)
flavone contents among different brands of soy milk and between lots of the same brand. Indeed, this variation might be dependent on the variety of raw soybeans and seed characteristics (such as variety, growth location and crop year), processing conditions, dilution with non-soy ingredients, etc.\(^{(3,20,24,25)}\)

Isoflavone aglycones are found predominantly in fermented soy food, whereas most nonfermented soy-based products contain mixtures of the \(\beta\)-glycosides, 6OMalGlc and 6OAcGlc conjugates.\(^{(3,4)}\) However, since thermally unstable 6OMalGlc and 6OAcGlc in raw soy materials can be readily converted to their respective more heat stable \(\beta\)-glycosides,\(^{(26)}\) therefore, it is not surprising that isoflavones in our nonfermented soy-based beverages appeared predominantly in the form of \(\beta\)-glycosides rather than aglycones.

After oral administration, the glycoside forms of isoflavones are hydrolyzed by \(\beta\)-glycosidases to the aglycone forms in the jejunum.\(^{(27-29)}\) The released aglycones are either absorbed intact by the intestine or metabolized further by intestinal microflora into several other products.\(^{(30-32)}\) The mean concentrations of aglycones in Thai soy-based beverages were significantly greater than those of imported products, and the proportion of aglycones: \(\beta\)-glycosides in Thai and imported soy-based beverages, was quite different. However, these factors might not influence the absorption of total isoflavones considerably in different brands with such varied proportions, but equivalent \(\mu\)mol of total isoflavone contents per serving might; since the bioavailability of aglycones is approximately the same as that for \(\beta\)-glycosides.\(^{(2,33,34)}\) Nevertheless, it has been shown that the absorption of aglycones was faster and more extensive than that of the glycosides.\(^{(35)}\) Whereas, Setchell et al.\(^{(36)}\) reported a higher bioavailability of glycosides compared to aglycones.

The total isoflavone contents in the commercially available samples investigated did not correlate statistically with their prices. However, the average price per serving of Thai soy-based beverages was cheaper statistically than that of the imported products. Therefore, Thai consumers should consider Thai soy-based beverages, as first choice products on the basis of cost-effectiveness. Nevertheless, the Thai Food and Drug Administration (FDA) should encourage manufacturing companies to declare isoflavone content on the packaging of Thai commercial soy-based beverage, in the same way as imported products do, in order to provide nutritional details that are valuable for rational consumer choice.

As approximately 90 mg/day is the total dose of isoflavones that benefits menopausal health,\(^{(9,10)}\) adequate supplementations of calcium (1,200 mg/day) and vitamin D (400-800 IU/day) is recommended as a cornerstone for the prevention and treatment of postmenopausal osteoporosis.\(^{(37)}\) Consumers (especially perimenopausal and postmenopausal women) should therefore seek soy-based beverages providing adequate daily supplements of isoflavones, calcium and vitamin D in order to obtain maximum health benefits. For example, two daily servings of VSL at 77.83 mg, with 50% calcium, and 50% vitamin D per serving should be fully sufficient, especially in those who rarely consume other soy-based food or high calcium - vitamin D diets. However, as supplement of isoflavones requires at least 1.5-4 months to effectively alleviate hot flushes in postmenopausal women,\(^{(38-41)}\) a longer duration (>6 months) in needed for the prevention and
treatment of postmenopausal osteoporosis.(7,8) Therefore, not only sufficient daily amounts of isoflavone supplement, but also an appropriate duration of treatment should be taken into consideration. Generally speaking, these inexpensive soy-based beverages might be considered a more appropriate alternative food supplement than the more expensive soy extract capsules or hormonal replacement therapy (HRT) for Thai postmenopausal women, especially those with increased risk of deleterious effects from HRT.

The limitation of this study is insufficient quantification of isoflavones in the form of 6OMalGlc and 6OAcGlc conjugates as well as glycitein and its glycoside conjugates, which are expected to appear in soy-based beverages, despite their relatively low amount. Therefore, the total contents of isoflavones reported here probably under estimate the actual contents that exist. In addition, clinical studies that research the efficacy of soy-based beverages on postmenopausal health should investigate further and reconfirm in the Thai population.

**CONCLUSION**

Total isoflavone contents in both products varied substantially. The Thai soy-based beverages contained comparable average contents of isoflavones per serving when compared to the imported products, but they were less costly.

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**Potential conflict of interest**

The authors declare that they have no conflict of interests.

**REFERENCES**


ปริมาณของไอโซฟลาโวนในเครื่องดื่มถั่วเหลืองไทยและเครื่องดื่มถั่วเหลืองนำเข้าที่มีจำหน่ายในประเทศไทย


บทคัดย่อ
วัตถุประสงค์ เพื่อตรวจวัดและเปรียบเทียบปริมาณของไอโซฟลาโวนในเครื่องดื่มถั่วเหลืองไทย (เดดซินและเจนนิสทิน) รวมทั้งใกลโคไซด์ (เดดซินและเจนนิสทิน) ในเครื่องดื่มถั่วเหลืองของไทยที่มีเครื่องดื่มถั่วเหลืองนำเข้าที่มีจำหน่ายในประเทศไทย

วัสดุและวิธีการ เก็บตัวอย่างผลิตภัณฑ์เครื่องดื่มถั่วเหลือง 51 ตัวอย่าง (เครื่องดื่มถั่วเหลืองไทย 34 ตัวอย่าง และเครื่องดื่มถั่วเหลืองนำเข้า 17 ตัวอย่าง) และจะได้รับการตั้งรหัสเพื่อใช้เรียกแทนชื่อ

ผลการศึกษา ปริมาณไอโซฟลาโวนรวมในเครื่องดื่มถั่วเหลืองทั้งสองประเภทมีความแปรปรวนค่อนข้างมาก โดยมีค่าประมาณ 7-85 มิลลิกรัมต่อหนึ่งหน่วยบริโภค โดยไอโซฟลาโวนส่วนใหญ่อยู่ในเครื่องดื่มไทย อย่างไรก็ตาม ค่าเฉลี่ยของความชันขั้นของไอโซฟลาโวนในเครื่องดื่มถั่วเหลืองไทย มีค่าสูงกว่าเครื่องดื่มถั่วเหลืองนำเข้าอย่างมีนัยสtatย์สูง ซึ่งค่าเฉลี่ยของความชันขั้นของไอโซฟลาโวน และปริมาณไอโซฟลาโวนรวมกันหนึ่งหน่วยบริโภค (เมื่อพิจารณาในหน่วยมิลลิกรัม และไมโครโมล) ระหว่างเครื่องดื่มถั่วเหลืองไทยและเครื่องดื่มถั่วเหลืองนำเข้านั้นไม่แตกต่างกันอย่างมีนัยส tatย์ อีกทั้งพบว่าเครื่องดื่มถั่วเหลืองไทยมีปริมาณไอโซฟลาโวนรวมเทียบเท่าเครื่องดื่มถั่วเหลืองนำเข้าโดยมีค่าเฉลี่ยต่ำกว่าเครื่องดื่มถั่วเหลืองไทยอย่างมีนัยส tatย์ แม้ว่าปริมาณไอโซฟลาโวนรวมที่ได้ค่อนข้าง

สรุป ปริมาณไอโซฟลาโวนรวมในเครื่องดื่มถั่วเหลืองนำเข้าที่มีเครื่องดื่มถั่วเหลืองไทยที่มีเครื่องดื่มถั่วเหลืองนำเข้าที่มีเครื่องดื่มถั่วเหลืองนำเข้าต่ำกว่าเครื่องดื่มถั่วเหลืองไทย เรียกเครื่องดื่มถั่วเหลืองไทย มีปริมาณไอโซฟลาโวนรวมที่ได้ค่อนข้าง

คำสำคัญ: ไอโซฟลาโวน, เดดซิน, เดดซีน, เจนนิสทิน, เจนนิสทีน, เครื่องดื่มถั่วเหลือง