Isolavone Content and Anti-acetylcholinesterase Activity in Commercial Douchi (a Traditional Chinese Salt-fermented Soybean Food)

Yaqiong LIU1†, Lijun WANG1†, Yongqiang CHENG1, Masayoshi SAITO2, Kohji YAMAKI3,4, Zhihong QIAO1 and Lite LI1†

1 College of Food Science and Nutritional Engineering, China Agricultural University (Beijing 100083, P R China)
2 Research Planning and Coordination Division, Japan International Research Center for Agricultural Sciences (Tsukuba, Ibaraki 305–8686, Japan)
3 Post-harvest Science and Technology Division, Japan International Research Center for Agricultural Sciences (Tsukuba, Ibaraki 305–8686, Japan)
† These authors contributed equally to this work.

Abstract
The concentration and distribution of isoflavones in 19 representative commercial douchi products and their acetylcholinesterase (AChE) inhibitory activity were investigated in this study. Isoflavone aglycones are the predominant isoflavone forms in Chinese commercial douchi samples. The total content of isoflavones in douchi extracts were observed from 24 to 1,471 μg/g (dry matter). Results indicated that Aspergillus-type douchi had more isoflavone aglycones content than that of Mucor-type and Bacillus-type douchi. Nineteen samples also showed various extents of AChE inhibitory activity. The IC₅₀ value of AChE inhibitory activities of douchi extracts ranged from 0.040 to 2.319 mg/mL. Aspergillus-type douchi extracts exhibited significantly higher AChE inhibitory activity than that of Mucor-type and Bacillus-type douchi. Some brands of douchi which have low contents of isoflavone aglycones showed much lower inhibitory activity. However, high inhibitory activities did not mean high isoflavone content, maybe some other substances contributed to the inhibition which should be further studied.

Discipline: Functional food
Additional key words: Alzheimer’s disease, fermented food

Introduction
Traditional fermented soybean foods have been consumed by Asians for a significant part of their long history. There are various fermented soybean products such as douchi, sufu, natto, miso, soy sauce, and tempeh in Asian countries which play an important role in local diets. Douchi is a traditional salt-fermented soybean food which has been consumed for more than two thousand years in China1. Most douchi products are produced by a similar principle; the schematic diagram for production of douchi is shown in Fig. 1. Production of douchi involves four major steps: 1) soaking soybean; 2) steaming soybean; 3) inoculation; and 4) ripening. According to the micro-organism used, Chinese douchi can be divided into three types: Aspergillus-type, Mucor-type and Bacillus-type.

Recently, many physiological properties have been found in Chinese douchi, which include anti-oxidative activity26,30, thrombolytic effects30, anti-diabetic properties2, and anti-hypertensive effects35. In many respects, isoflavones are one of the most important compounds attributed to those healthful effects. Many investigations were made previously on a wide variety of soybeans, non-fermented soybean foods and fermented soybean food to evaluate the potential of isoflavones as a dietary anti-carcinogen by some researchers27,28. However, there is little information available regarding isoflavones content in Chinese douchi.
Soybeans \rightarrow \text{Soaking} \rightarrow \text{Steaming} \rightarrow \text{Cooling} \rightarrow \text{Inoculation} \rightarrow \text{Solid-substrate Fermentation} \rightarrow \text{Maturation} \rightarrow \text{Douuchi}

\text{Post-fermentation}

\text{Pre-fermentation}

\text{Pre-treatment}

\text{Douuchi}

\text{Straw mats or Pure mould cultures} \rightarrow \text{Dressing mixture} \rightarrow \text{Fermentation}

\text{Fig. 1. The schematic diagram for production of douchi}

douchi.

Isoflavones are a kind of flavonoid which have four chemical forms: the aglycones daidzein, genistein and glycitein; the glucosides daidzin, genistin and glycitin; the acetylgalactosides 6"-O-acetyl-daidzin, 6"-O-acetyl-genistin and 6"-O-acetyl-glycitin; and the malonylglucosides 6"-O-Malonyl-daidzin, 6"-O-malonyl-genistin and 6"-O-malonyl-glycitin.

Soybean isoflavones have been widely studied because of their physiological properties. They are known as phytoestrogens and have been shown to be effective in preventing osteoporosis, lowering breast cancer risk, inhibiting oxidative damage, and alleviating menopausal symptoms. The effects depend on the structures of isoflavones in various bioactivities. It has been reported that isoflavone aglycones were more effective than glucosides in regulating cholesterol and fatty acids metabolism in rats. Genistein was reported to have a higher anti-proliferative effect on the growth of human breast carcinomas compared with animals fed the control diet. Several lines of evidence showed that daidzein and genistein are more bioavailable than the conjugated forms in rat stomachs.

Owing to the specific structure, soy isoflavones can improve cognitive function in both humans and rats and prevent the degeneration of the central nervous system and the development of Alzheimer’s disease (AD). It has been reported that acetylcholinesterase activity in the soy isoflavones diet fed animals was significantly inhibited in the cortex, basal forebrain and hippocampus compared with animals fed the control diet. In a more recent study, soy isoflavones treatment could result in a significant decrease in acetylcholinesterase (AChE) activity and increase in the contents of some amino acid neurotransmitters such as glutamic and aspartic acids in the frontal cerebral cortex and hippocampus of mice. In a different study, a soy diet that was rich in isoflavones was able to reverse the increase of AChE in hippocampus of ovariectomized rats. In addition, Pal and Tandon (1998) reported that genistein extracted from the root–tuber peel of Flemingia vestita could significantly inhibit the activity of AChE in Raillietina echinobothrida. These findings show that soy isoflavones can influence the brain cholinergic system and reduce cognition decline in animal models.

In the present study, we investigated the content and composition of isoflavones in 19 samples of representative commercial douchi in China, as well as examining their AChE inhibitory activity in vitro for the first time. Moreover, the correlation between isoflavone content and AChE was also discussed. The purpose was to estimate the healthful effects of Chinese douchi in relation to isoflavones content and a potential AD-preventive agent derived from this food.

Materials and methods

1. Materials

Nineteen representative samples of douchi were obtained from local markets in different regions in China (Table 1). The types of douchi are shown according to the previous literature. Acetylcholinesterase from human recombinant (2,220 U/mg protein, E.C. No. 3.1.1.7), acetyltiophocholine iodide (ATChI), and 5,5'-dithiobis[2-nitrobenzoic acid] (DTNB) were purchased from Sigma Chemical Co. (St. Louis, MO). Galanthamine hydrobromide was obtained from NICBPB (National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China). All other chemicals were of analytical grade.

Daidzin, genistin, glycitin, daidzein, genistein, and glycitein were purchased from Sigma Chemical Co. (St. Louis, MO). 6"-O-Malonyl-daidzin, 6"-O-malonyl-genistin, 6"-O-malonyl-glycitin, 6"-O-acetyl-daidzin, 6"-O-acetyl-genistin, and 6"-O-acetyl-glycitin were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan).

2. Preparation of douchi extracts

The douchi samples were freeze-dried and pulverized into powder using a mortar and a pestle, and stored in brown glass bottles at −20°C until used.

One gram of lyophilized douchi powder was suspended in 10 mL 80% (v/v) ethanol. The mixture was then homogenized using a T25BS4 homogenizer (IKA Labortechnik, Staufen, Germany). The homogenized samples were extracted for 20 min in an ultrasonic water bath. Then the mixture was centrifuged at 3,000 × g for 15 min at 4°C, and the supernatant was filtered through a 0.45 μm syringe filter unit (Millex-HX, Millipore, USA). The filtrate was diluted by phosphate buffer (0.1M, pH 8.04).
Table 1. Brand names, origins, type of soybean used and types of commercial douchi samples

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Brands</th>
<th>Origins</th>
<th>Type of soybean</th>
<th>Types of douchi</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C.W</td>
<td>Sichuan (mid-west)</td>
<td>Yellow</td>
<td>Mucor-type</td>
</tr>
<tr>
<td>2</td>
<td>T.C</td>
<td>Sichuan (mid-west)</td>
<td>Black</td>
<td>Mucor-type</td>
</tr>
<tr>
<td>3</td>
<td>R.M</td>
<td>Chongqing (mid-west)</td>
<td>Yellow</td>
<td>Mucor-type</td>
</tr>
<tr>
<td>4</td>
<td>Y.C</td>
<td>Chongqing (mid-west)</td>
<td>Yellow</td>
<td>Mucor-type</td>
</tr>
<tr>
<td>5</td>
<td>Q.M</td>
<td>Chongqing (mid-west)</td>
<td>Yellow</td>
<td>Mucor-type</td>
</tr>
<tr>
<td>6</td>
<td>A.J</td>
<td>Chongqing (mid-west)</td>
<td>Yellow</td>
<td>Mucor-type</td>
</tr>
<tr>
<td>7</td>
<td>Y.F</td>
<td>Guangdong (south)</td>
<td>Black</td>
<td>Aspergillus-type</td>
</tr>
<tr>
<td>8</td>
<td>X.Q</td>
<td>Guangdong (south)</td>
<td>Black</td>
<td>Aspergillus-type</td>
</tr>
<tr>
<td>9</td>
<td>Y.J.Q</td>
<td>Guangdong (south)</td>
<td>Black</td>
<td>Aspergillus-type</td>
</tr>
<tr>
<td>10</td>
<td>Y.M</td>
<td>Guangxi (south)</td>
<td>Black</td>
<td>NC</td>
</tr>
<tr>
<td>11</td>
<td>Q.X</td>
<td>Guizhou (central)</td>
<td>Yellow</td>
<td>Bacillus-type</td>
</tr>
<tr>
<td>12</td>
<td>K.M</td>
<td>Yunnan (south-west)</td>
<td>Yellow</td>
<td>Bacillus-type</td>
</tr>
<tr>
<td>13</td>
<td>T.M.S</td>
<td>Hunan (central)</td>
<td>Black</td>
<td>Aspergillus-type</td>
</tr>
<tr>
<td>14</td>
<td>T.P.Q</td>
<td>Hunan (central)</td>
<td>Black</td>
<td>Aspergillus-type</td>
</tr>
<tr>
<td>15</td>
<td>L.Y.P</td>
<td>Hunan (central)</td>
<td>Black</td>
<td>Aspergillus-type</td>
</tr>
<tr>
<td>16</td>
<td>Q.W</td>
<td>Hunan (central)</td>
<td>Black</td>
<td>Aspergillus-type</td>
</tr>
<tr>
<td>17</td>
<td>D.X.Y</td>
<td>Jiangxi (mid-south)</td>
<td>Black</td>
<td>Aspergillus-type</td>
</tr>
<tr>
<td>18</td>
<td>K.W.K</td>
<td>Shanghai (east)</td>
<td>Black</td>
<td>Aspergillus-type</td>
</tr>
<tr>
<td>19</td>
<td>Y.B</td>
<td>Jilin (north-east)</td>
<td>Yellow</td>
<td>NC</td>
</tr>
</tbody>
</table>

NC: not clear.

to appropriate concentration for further AChE inhibitory activity assay.

3. Determination of isoflavones contents of douchi extracts

The contents of isoﬂavones in the douchi extracts were determined as described previously\(^5\). Freeze-dried powder (2 g) was extracted with 50 ml of 80% (v/v) methanol at 80°C for 4 h by a Soxhlet extractor and the extractants filtered through a 0.45 μm filter unit. Isoflavones were analyzed quantitatively by high pressure liquid chromatography (HPLC). The HPLC system used was a Shimadzu HPLC (Kyoto, Japan), consisting of an LC-10AT pump, a UV detector (SPD-10AVVP), and a Dikma Diamonsil C\(_{18}\) column (4.6 × 250 mm, Dima Co., Ltd., Orlando, FL). The mobile phases for HPLC consisted of solvent (A) 0.1% (v/v) acetic acid in filtered Milli-Q water, and (B) 0.1% (v/v) acetic acid in acetonitrile. The solvent gradient was as follows: Solvent B was increased from 15 to 25% over 35 min, then increased to 26.5% within the next 12 min, and finally increased to 50% within 30 s prior to being held for 14.5 min. The flow rate was 1.0 mL/min. The column temperature was 40°C and the absorption was measured at 254 nm. Quantitative data for each isoﬂavone was obtained by comparison to known standards. In order to estimate total isoﬂavone amounts, individual isoﬂavone glucosides and aglycones were normalized for their molecular weight differences and summed, and regarded as total isoﬂavones.

4. Determination of AChE inhibitory activity

The AChE inhibitory activities of douchi extracts were determined according to Ellman’s colorimetric method with some modifications\(^5\). In the 96-well plates, 150 μL of phosphate buffer (0.1M, pH 8.04) was added to the wells followed by 30 μL of sample solution, 50 μL of DTNB (756 μM) and 20 μL of AChE enzyme (0.54 U/mL). The mixture was incubated for 5 min at 37°C. Following pre-incubation, 50 μL of the substrate (ATChI, 3 mM) was added in the above mixture solution. The absorbance of the resulting solution was measured at 405 nm every 28 s for ten times using a 96-well plate reader (Model 680, Bio-Rad Laboratories, Tokyo, Japan). Absorbance was plotted against time and enzyme activity was calculated from the slope of the line so obtained and expressed as a percentage compared with an assay using a buffer without any inhibitor. The percent inhibition was calculated by the following formula.

\[
\text{inhibition(%) } = \frac{K_{\text{control}} - K_{\text{sample}}}{K_{\text{control}} - K_{\text{negative}}} \times 100
\]

The AChE inhibition activities were expressed as IC\(_{50}\), which is the inhibitory concentration of the test samples that inhibits 50% AChE by log-probit analysis. Galanthamine hydrobromide was used to benchmark the AChE inhibitory activity. Thirty μL of 10% ethanol in buffer was used as the negative control instead of the douchi extract. Tests were carried out in triplicate.
5. Statistical analysis

The mean values of three experiments were calculated. The SAS system (SAS for Windows 6.12, SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. Duncan’s multiple range tests were used to estimate significant differences among the mean values at the 5% probability level.

Results and discussion

1. Isoflavone content and composition of douchi extracts

Nineteen brands of douchi samples collected from various parts of China were investigated. The samples can be regarded as representative of the major types of douchi found in China. The brand names, origins, species types of soybean and microorganism species used for douchi preparation are shown in Table 1.

Isoflavone contents of douchi extracts are shown in Table 2. The total content of isoflavones in 19 brands of douchi samples ranged from 24 to 1,471 μg/g dry matter. Similar distribution patterns of isoflavones were observed among the 19 brands of douchi samples. Isoflavone aglycones accounted for 75% to 96% of total isoflavones contents (except No. 6 and No. 19). Daidzein and genistein were the major isoflavone constituents in douchi samples. The content of malonylglucosides was the lowest in isoflavone profiles. 6’-O-Acetylglycitin was not detected in any of the samples in the experiment. These results were consistent with previous literature.

Isoflavone content and distribution in soy-based foods were reported to depend on the variety of soybean, methods of processing, and addition of other components. Wang and Murphy evaluated the concentration and distribution of isoflavones in 29 commercial soybean foods. Traditional soybean foods showed differences between non-fermented and fermented food. Non-fermented foods had greater levels of glucosides, while in contrast, greater levels of aglycones were found in fermented foods.

Processing techniques, such as soaking, heat treatment and fermentation, not only influenced the content of isoflavones but also changed the forms of isoflavones. During soaking, the concentration of daidzin-genistin decreases accordingly to the daidzein-genistein formation. It was reported that the malonylated isoflavone glucosides were thermally unstable, and were converted into their corresponding isoflavone glycosides and aglycones at the higher temperature. In many respects, isoflavones convert mainly from glucosides to aglycones under the β-glucosidases from microbial source hydrolysis in the fermentation process.

From Table 2, the average content of isoflavone aglycones of Aspergillus-type douchi was two times higher than that of Mucor-type or Bacillus-type douchi, which had average contents of 857 μg/g, 384 μg/g and 380 μg/g, respectively. Although this might be relevant to the variety of soybean, the microorganism species and processing methods in douchi production may be the main factor of the difference. The different microorganism and inocula would provide different extents of hydrolysis activities during the fermentation. Moreover, in contrast with Aspergillus-type douchi, Mucor-type douchi was made during winter and had a year long fermentation time. Since the appropriate temperature of β-glucosidase was generally higher than 30°C, the fermentation season may affect the content of isoflavone aglycones. Meanwhile, fermentation time and non-soy ingredients might also influence the isoflavonoids contents.

The total contents of daidzein, glystein and genistein varied extremely in douchi samples which were made by the same microorganism. Compared with other fermented soybean food, such as tempeh (625 μg/g) and miso (895 μg/g), Chinese douchi has higher isoflavone contents especially Aspergillus-type douchi which ranged from 636 μg/g to 1,471 μg/g. Therefore, Chinese douchi is a better soybean food for consuming dietary isoflavone in a daily diet.

2. The AChE inhibitory activity of douchi extracts

In this study, the AChE inhibitory activities of 19 brands of Chinese douchi samples were examined. From Fig. 2., the IC50 values of douchi extracts ranged from 0.040 to 2.319 mg/mL. Among these samples, some showed high inhibitory activities (No. 18, 0.040 mg/mL and No. 2, 0.041 mg/mL) and some samples showed low inhibitory activities (No. 6, 2.319 mg/mL and No. 11, 0.992 mg/mL respectively).

Compared with the medicinal plants, douchi extracts had the better AChE inhibitory activity. Mukherjee reported the AChE inhibitory activity of six plant materials which are commonly used to improve memory function traditionally as central nervous system active plants in the Indian system of medicine. The IC50 values ranged from 106.55 μg/mL to 222.41 μg/mL. A similar research found that the IC50 values of several plants of the Amaryllidaceae family ranged from 16.74 μg/mL to 73.69 μg/mL. This indicated that douchi can be considered as a new potential specific food in preventing the onset of human AD.

In addition, the samples from the same region showed similar inhibitory activity (No. 7, No. 8 and No. 9). The samples from Sichuan and Chongqing showed low inhibitory activity (No. 1 and No. 6). Although the AChE in-
Table 2. Isoflavone contents of commercial douchi extracts* (μg/g dry matter)

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Din (μg/g)</th>
<th>Gin (μg/g)</th>
<th>Dein (μg/g)</th>
<th>Glin (μg/g)</th>
<th>Gin (μg/g)</th>
<th>Malonyl glucoside (μg/g)</th>
<th>Acetyl glucoside (μg/g)</th>
<th>Total isoflavones (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.46±0.24</td>
<td>0.46±0.03</td>
<td>2.27±0.13</td>
<td>190.07±10.70</td>
<td>21.19±1.73</td>
<td>30.27±4.41</td>
<td>6.23±0.39</td>
<td>1.32±0.03</td>
</tr>
<tr>
<td>2</td>
<td>2.95±0.57</td>
<td>1.05±0.07</td>
<td>5.62±0.85</td>
<td>324.02±2.45</td>
<td>21.36±0.13</td>
<td>93.12±4.12</td>
<td>1.11±0.11</td>
<td>0.78±0.03</td>
</tr>
<tr>
<td>3</td>
<td>4.60±0.21</td>
<td>nd</td>
<td>8.93±0.58</td>
<td>504.59±10.78</td>
<td>12.66±0.96</td>
<td>152.69±21.15</td>
<td>1.25±0.02</td>
<td>nd</td>
</tr>
<tr>
<td>4</td>
<td>19.22±2.75</td>
<td>1.39±0.45</td>
<td>37.07±1.06</td>
<td>436.94±23.09</td>
<td>19.28±2.26</td>
<td>146.29±22.18</td>
<td>3.77±0.25</td>
<td>7.12±0.86</td>
</tr>
<tr>
<td>5</td>
<td>44.61±6.00</td>
<td>3.02±0.20</td>
<td>87.21±10.70</td>
<td>250.46±20.41</td>
<td>13.11±0.41</td>
<td>76.12±1.96</td>
<td>nd</td>
<td>1.56±0.04</td>
</tr>
<tr>
<td>6</td>
<td>5.93±1.20</td>
<td>11.42±0.64</td>
<td>3.00±0.11</td>
<td>5.05±0.46</td>
<td>0.19±0.02</td>
<td>4.17±0.18</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>7</td>
<td>2.23±0.15</td>
<td>nd</td>
<td>5.02±0.18</td>
<td>429.18±11.30</td>
<td>30.71±1.41</td>
<td>142.06±24.45</td>
<td>1.43±0.03</td>
<td>1.06±0.04</td>
</tr>
<tr>
<td>8</td>
<td>38.88±3.16</td>
<td>7.28±1.56</td>
<td>80.20±7.55</td>
<td>416.93±14.55</td>
<td>33.82±1.52</td>
<td>132.64±4.81</td>
<td>5.50±0.64</td>
<td>3.44±0.35</td>
</tr>
<tr>
<td>9</td>
<td>7.25±0.67</td>
<td>2.19±0.31</td>
<td>10.00±0.64</td>
<td>468.29±29.13</td>
<td>29.69±1.50</td>
<td>152.80±6.72</td>
<td>0.82±0.65</td>
<td>0.87±0.13</td>
</tr>
<tr>
<td>10</td>
<td>10.62±0.89</td>
<td>4.83±0.77</td>
<td>5.52±1.13</td>
<td>225.75±20.02</td>
<td>15.02±1.17</td>
<td>29.52±2.24</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>11</td>
<td>0.82±0.06</td>
<td>0.38±0.02</td>
<td>3.58±0.29</td>
<td>203.25±7.31</td>
<td>21.21±0.54</td>
<td>45.50±7.46</td>
<td>4.66±0.02</td>
<td>nd</td>
</tr>
<tr>
<td>12</td>
<td>5.45±0.14</td>
<td>0.90±0.03</td>
<td>17.20±0.22</td>
<td>340.57±4.61</td>
<td>18.40±0.58</td>
<td>131.45±14.06</td>
<td>nd</td>
<td>1.81±1.19</td>
</tr>
<tr>
<td>13</td>
<td>67.79±2.11</td>
<td>6.64±0.21</td>
<td>119.40±4.24</td>
<td>1000.07±20.17</td>
<td>63.16±2.22</td>
<td>239.73±18.13</td>
<td>4.00±0.01</td>
<td>4.01±0.09</td>
</tr>
<tr>
<td>14</td>
<td>53.29±10.71</td>
<td>8.13±0.91</td>
<td>27.34±0.75</td>
<td>522.89±25.58</td>
<td>44.64±2.31</td>
<td>127.15±9.10</td>
<td>8.13±0.38</td>
<td>60.78±0.46</td>
</tr>
<tr>
<td>15</td>
<td>95.50±0.07</td>
<td>8.37±0.45</td>
<td>147.21±6.31</td>
<td>889.28±36.26</td>
<td>48.65±3.78</td>
<td>204.08±4.96</td>
<td>4.67±0.07</td>
<td>3.56±0.08</td>
</tr>
<tr>
<td>16</td>
<td>65.46±1.70</td>
<td>7.48±1.24</td>
<td>85.05±0.88</td>
<td>818.86±25.43</td>
<td>55.55±1.54</td>
<td>220.47±35.87</td>
<td>3.17±0.14</td>
<td>5.86±0.31</td>
</tr>
<tr>
<td>17</td>
<td>105.45±8.04</td>
<td>10.35±2.47</td>
<td>133.60±10.06</td>
<td>774.70±13.19</td>
<td>49.67±2.13</td>
<td>204.55±16.47</td>
<td>nd</td>
<td>22.00±0.15</td>
</tr>
<tr>
<td>18</td>
<td>23.94±1.29</td>
<td>1.64±0.60</td>
<td>53.57±3.32</td>
<td>552.52±23.05</td>
<td>23.35±1.77</td>
<td>121.24±14.49</td>
<td>3.39±0.11</td>
<td>1.17±0.31</td>
</tr>
<tr>
<td>19</td>
<td>473.88±20.28</td>
<td>22.41±1.39</td>
<td>450.63±21.01</td>
<td>293.41±9.72</td>
<td>9.37±0.50</td>
<td>77.65±19.86</td>
<td>nd</td>
<td>0.92±0.01</td>
</tr>
</tbody>
</table>

* Values represent the mean ± standard deviation; n = 3. ^Abbreviations: Din, daidzin; Gin, glycitin; Dein, daidzein; Glin, glycitein; Gein, genistein; nd, not detected. ^Sample code 1 to 19, the same samples as in Table 1. ^With normalization of molecular weight differences.
hibitory activities of the other douchi samples’s extracts were also influenced by producing region, there was no clear directional tendency between the region of production and high AChE inhibitory activity.

From this study, significant variations in AChE inhibitory activity of extracts from different types of douchi were also observed. Four brands of low AChE inhibitory activity (samples No. 1, No. 4, No. 5, and No. 6) all belonged to Mucor-type douchi, while almost all Aspergillus-type douchi extracts had significantly higher AChE inhibitory activity than that of Mucor-type and Bacillus-type douchi. Their average IC₅₀ values were 0.074 mg/mL (Aspergillus-type), 0.623 mg/mL (Mucor-type) and 0.526 mg/mL (Bacillus-type), respectively. In the experiment, we also discovered that some brands of douchi (samples No. 1, No. 11 and No. 6) exhibited high IC₅₀ values and low contents of isoflavone aglycones at the same time. However, high inhibition activities did not mean high isoflavone content, maybe some other substances contributed to the inhibition. Further studies to validate and determine the effect of isoflavone on AChE inhibitory activity, as well as other substances in douchi that may have an effect on inhibiting AChE should be considered.

Conclusions

In this study, the concentration and distribution of isoflavones and AChE inhibitory activity in 19 Chinese commercial douchi were evaluated. As a traditional fermented soybean food, aglycones were the main forms of isoflavones in Chinese commercial douchi. The total content of isoflavones varied significantly between the brands of douchi products. Aspergillus-type douchi is superior to the Mucor-type and Bacillus-type douchi in the isoflavones content. Furthermore, Chinese douchi showed different extents of AChE inhibitory activities, and the IC₅₀ values ranged from 0.040 to 2.319 mg/mL. Some of the douchi samples showed strong inhibitory effects on AChE with IC₅₀ values of 0.040 and 0.041 mg/mL. Aspergillus-type douchi exhibited significantly higher AChE inhibitory activity than that of Mucor-type and Bacillus-type douchi. Some brands of douchi which have a low content of isoflavone aglycones showed much lower inhibitory activity. However, high inhibition activities did not mean high isoflavone content, maybe some other substances contributed to the inhibition.

Acknowledgments

This study was conducted within the framework of the collaborative research project between Japan and China titled “Development of sustainable production and utilization of major food resources in China” supported by Japan International Research Center for Agricultural Sciences.
References
