Antioxidative Components of Sweet Potatoes

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Summary The antioxidative activity of a 70% methanol extract of sweet potatoes was estimated in a linoleic acid-aqueous system. The extract had a markedly strong antioxidative activity. Major phenolic components contained in the 70% methanol extract were identified as chlorogenic acid and isochlorogenic acid-1, -2 and -3 by using high-performance liquid chromatography. The other minor free phenolics were identified, or tentatively identified, as caffeic acid and 4-o-caffeoylquinic acid. Chlorogenic acid and/or isochlorogenic acids, however, had only slight antioxidative activity. From the results of the addition of chlorogenic acid, isochlorogenic acids and the other coexisting components contained in the sweet potato extract, the effective antioxidant activity of the sweet potato extract was proposed to be mainly based on the synergistic effect of phenolic compounds with amino acids.

Key Words sweet potato, antioxidative activity, chlorogenic acid, isochlorogenic acid, synergistic effect, free amino acids, tocopherol, high-performance liquid chromatography

Antioxidative components of plants used for food, for example potatoes (1), soy beans (2, 3), cacao bean husk (4) and so on, have been extensively investigated, following the requirement for the availability of an effective antioxidant from natural sources. The antioxidative effects were reported to be mostly due to phenolic compounds.

In sweet potatoes, however, the antioxidative effect has not been elucidated. Sweet potatoes have a high productive efficiency and can be widely cultivated in Japan. Investigation of the antioxidative activity of sweet potato extract is considered to be also important from the point of view of available utilization of waste fluid from the manufacturing process of sweet potato starch and from the production of ethanol from sweet potatoes as biomass. Since sweet potatoes contain various phenolic compounds (5, 6), antioxidative effects of these extracts are expected.

1 早瀬文孝，加藤博通
In the present study, the authors investigated the antioxidative components of sweet potatoes and discussed the development of the antioxidative effect.

MATERIALS AND METHODS

**Sweet potatoes.** Sweet potatoes of two varieties, Kintoki and Kokei No. 14, which were harvested at a farm in Chiba Prefecture, Japan, in 1980 and 1981 and in Ibaraki Prefecture, Japan, in 1980 respectively, were used. These varieties were used within one week of harvesting. The raw sweet potatoes were washed and prepared as follows. Whole sweet potato powder (WS) was prepared from sweet potatoes which were sliced and freeze-dried. Peeled sweet potato powder (PS) was prepared from sweet potatoes which were peeled, sliced and freeze-dried. Peel powder of sweet potatoes (PP) was prepared from the peel of sweet potatoes which was cut into small pieces and freeze-dried. The center part of sweet potatoes (CS) was obtained by slicing and freeze-drying after elimination of the peel and primary cambium of sweet potatoes.

**Chemicals.** Almost all the chemicals used in this investigation were commercially available. Chlorogenic acid and caffeic acid were purchased from Nakarai Chemicals Ltd. Isochlorogenic acid was purchased from ICN Pharmaceutical Inc. Isochlorogenic acid was confirmed to be the mixture of isochlorogenic acid-1, -2 and -3 by high-performance liquid chromatography. The constitution of isochlorogenic acid-1, -2 and -3 was in the molar ratio 1:2:4. Tocopherol was purchased from Eizai Co., Ltd., and was the mixture of α-, β-, γ- and δ-tocopherols.

**Peroxide value (PV) test.** One gram of linoleic acid (Wako Pure Chemical Industries, Ltd.) dissolved in 20 ml of ethanol was placed in an Erlenmeyer flask with a stopper of ground glass. Twenty-five ml of 0.2 M phosphate buffer (pH 7.0) and test solution (2 ml of 70% methanol solution) were added to the above solution. The flask was tightly stoppered and stored in an incubator at 45°C for 48 h. Oxidized linoleic acid was extracted with chloroform by using a separatory funnel and the peroxide value (PV) was measured by iodometry (7). Antioxidative activity was indicated as follows: PV% = PV of test samples/PV of control × 100. Accordingly, the lower the PV% is, the stronger the antioxidative activity of the sample.

**Extraction of phenolic compounds, free amino acids and sugars.** A 70% methanol solution of coumarin (1 mg) as an internal standard was added to 5 g of sweet potato powder. The mixture was extracted twice with 200 ml of 70% methanol by stirring for 2 h at room temperature and filtered. Alcoholic filtrate was combined and evaporated in vacuo at 30°C. The remaining syrup was taken up in 70% methanol and diluted to 10 ml in a volumetric flask. The resulting extract was passed through a SEP-PAK C18 cartridge (Water Associates, Inc.) prewetted with acetonitrile and distilled water. The eluate was subjected to analysis of free sugars by high-performance liquid chromatography (HPLC). The cartridge was treated with 5 ml of acetonitrile–methanol–water (40:40:20) to elute the absorbed phenolics and the eluate was analyzed by HPLC.

Furthermore, extraction of phenolic acid bound to proteins and other components, was performed. Fifty grams of whole sweet potato powder were extracted with 70% methanol and filtered. The filtrate was concentrated, adjusted to pH 7 and extracted with diethyl ether. The aqueous layer was hydrolyzed with 2N NaOH at 20°C for 4 h, adjusted to pH 3 and extracted with ether. The ether extract was dehydrated with anhydrous sodium sulfate and concentrated. The concentrate was dissolved in methanol and analyzed by HPLC.

**High-performance liquid chromatography (HPLC).** The HPLC analysis was done using a Hitachi Model 638-30 liquid chromatograph as follows. Columns: a) for analysis of free phenolic compounds and b) for analysis of phenolic acids, a stainless column (25 cm x 4 mm) pre-packed with Lichrosorb RP-18 was used, c) for analysis of sugars, a stainless column (25 cm x 4 mm) pre-packed with Hitachi #3013-N was used. Detector: a), b) Hitachi Model 100-50 Spectrophotometer (a, 254 and 313 nm; b, 280 nm), c) Shodex RI SE-11. Mobile phase: a) Methanol-0.033M potassium phosphate-acetic acid (40 : 59 : 1, pH 3.0), b) butanol-actic acid–water (14 : 1 : 342), c) acetonitrile–water–ethanol (80 : 15 : 5). Flow rate: a), b) 0.5 ml/min, c) 2 ml/min. The peak area on the chromatograms was measured by a Shimadzu Model Chromatopac El-A integrator, and the quantity of free phenolic compounds was calculated from the ratio-to-peak area of coumarin as an internal standard.

**Amino acid analysis.** After removal of proteins with trichloroacetic acid from 70% methanol extract of sweet potato, the determination of free amino acid composition was carried out using a Hitachi Amino Acid Analyzer 835-30. Measurement of the chromatographic peak area was done using a sic intelligent integrator Model 7000A connected to the amino acid autoanalyzer.

**Thin-layer and paper chromatography.** Phenolic compounds in 70% methanol extract of sweet potato were also analyzed by thin-layer chromatography on plates of silica gel 60 F254 and by paper chromatography. The solvent system used was butanol–actic acid–water (4 : 1 : 2).

**CMR spectroscopy.** The CMR spectrum was recorded using a Jeol-FX-100 NMR Spectrometer operating at 25 MHz in the pulsed Fourier-transform mode. This spectrum was recorded in water by using 8,000 data points and a spectral width of 6 kHz. C13-chemical shifts are expressed using methanol as an internal standard.

**RESULTS AND DISCUSSION**

**Estimation of solvent used for extraction**

Table 1 shows the antioxidative activity of a test extraction solution of sweet potatoes. PV of the linoleic acid control solution incubated at 45°C for 48 h was 330 and PV% of test solutions with added ethyl ether extract and hexane extract were 33 and 46, respectively. The antioxidative activity of slightly yellow hexane extract is considered to be based on \(\beta\)-carotene. \(\beta\)-Carotene is a major carotenoid pigment in sweet potatoes (8) and is known to react as a quencher of singlet oxygen.
Table 1. Antioxidative activity of test extract solutions.

<table>
<thead>
<tr>
<th>Extract</th>
<th>PV %&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48 h</td>
</tr>
<tr>
<td>70% MeOH extract</td>
<td>0</td>
</tr>
<tr>
<td>Ethyl ether control</td>
<td>54</td>
</tr>
<tr>
<td>Ethyl ether extract</td>
<td>33</td>
</tr>
<tr>
<td>Hexane control</td>
<td>86</td>
</tr>
<tr>
<td>Hexane extract</td>
<td>46</td>
</tr>
</tbody>
</table>

<sup>a</sup>PV of linoleic acid control solution was 330 and 740 for 48 h and 4 days respectively, and PV% was calculated as percentage of PV (sample) for PV (control). Peroxide value of linoleic acid for 0 time was 11.<sup>b</sup> Each extract was obtained from 20 g of sweet potatoes.

Table 2. Antioxidative activity for each concentration of 70% methanol extract obtained from peeled sweet potato (Kintoki and Kokei No. 14).

<table>
<thead>
<tr>
<th>Extract&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PV%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kintoki</td>
</tr>
<tr>
<td>2 ml</td>
<td>3</td>
</tr>
<tr>
<td>1 ml</td>
<td>5</td>
</tr>
<tr>
<td>0.5 ml</td>
<td>7</td>
</tr>
<tr>
<td>0.1 ml</td>
<td>7</td>
</tr>
<tr>
<td>0.05 ml</td>
<td>33</td>
</tr>
<tr>
<td>0.01 ml</td>
<td>85</td>
</tr>
</tbody>
</table>

<sup>a</sup>Sweet potato powder (5 g) was extracted with 200 ml of 70% methanol. Alcoholic filtrate was evaporated and 10 ml placed in a volumetric flask.

for lipid oxidation under photo-irradiation (9).

On the other hand, PV% of test solution with added 70% methanol extract of sweet potato incubated at 45 °C for 2 and 4 days was 0. Accordingly, the existence of markedly strong antioxidants was expected in the 70% methanol extract, and the present investigation of antioxidative compounds was focussed on this extract.

Comparison between sweet potato varieties and among each portion of sweet potato as to antioxidative activity

No difference in antioxidative effect was observed between Kintoki and Kokei No. 14 as shown in Table 2. The antioxidative effect of each 70% methanol extract obtained from WS, PS, CS and PP was tested. As shown in Table 3, the
Table 3. Antioxidative activity of 70% methanol extract obtained from sweet potato (Kintoki).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peroxide value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48 h</td>
</tr>
<tr>
<td>Control</td>
<td>320</td>
</tr>
<tr>
<td>WS</td>
<td>22</td>
</tr>
<tr>
<td>PP</td>
<td>25</td>
</tr>
<tr>
<td>PS</td>
<td>24</td>
</tr>
<tr>
<td>CS</td>
<td>50</td>
</tr>
</tbody>
</table>

WS, whole sweet potato powder (1 g); PP, peel powder of sweet potato (0.5 g); PS, peeled sweet potato powder (1 g); CS, powder of secondary cambium of sweet potato (1 g).

antioxidative activity of a part of the peel was stronger than that of the inner portion, because the antioxidative activity of WS was stronger than that of PS. Pratt and Watts (1) studied the antioxidative activity of hot-water extract of several plant tissues, and reported that potato-peel extracts had a greater protective effect than extracts from potatoes.

Isolation and determination of phenolic compounds

The 70% methanol extract obtained from 5 g of WS was analyzed by HPLC. Six peaks were separated, as shown in Fig. 1. Peak 2 was similar to the authentic chlorogenic acid as regards the retention volume by HPLC and the UV spectrum. Moreover, separation and purification of peak 2 by HPLC were attempted, and the fraction was analyzed by CMR as follows: δ^TMS, ppm: 38.2 (2), 38.7 (6), 71.3 (5), 71.8 (4), 73.5 (3), 76.1 (1), 115.0 (12, 15), 116.4 (9), 122.8 (16), 127.5 (11), 146.7 (13), 146.9 (10), 149.5 (14), 168.5 (8), 176.9 (7).
Peaks 1, 5, 6 and 7 were similar to the authentic caffeic acid and isochlorogenic acid-1, -2 and -3 as regards the retention volume by HPLC and the UV spectrum respectively. Chlorogenic acid and isochlorogenic acid-1, -2 and -3 were verified also by TLC and paper chromatography. Peak 3 was tentatively identified as 4-o-caffeoylquinic acid from the data of HPLC and paper chromatography reported by Walter et al. (6) and Thompson (10). From these results, six peaks were identified or tentatively identified as caffeic acid, chlorogenic acid, 4-o-caffeoylquinic acid, isochlorogenic acid-1, -2 and -3, respectively.

Table 4 indicates the quantitative results for phenolic compounds determined by HPLC using coumarin as an internal standard. Chlorogenic acid and isochlorogenic acid-2 were the most abundant components, comprising more than 80% of the total phenolic compounds in WS, PP and PS. This was very similar to the results for sweet potato phenolics as determined by Walter et al. (6). Each sample of caffeic acid and 4-o-caffeoylquinic acid was trace in 70% methanol extract (Fig. 1). The phenolic compounds identified in the present study, with the exception of isochlorogenic acid-3, have already been reported in sweet potatoes (5, 6, 10, 11). Neochlorogenic acid has already been found in sweet potatoes (5, 6, 10), but it was not detected in the present investigation. Walter et al. (6) found that neochlorogenic acid was not present at harvest time, but became detectable about 4 weeks thereafter, and Thompson (10) reported that it was not detectable in many sweet

Table 4. Phenolic composition* of sweet potato as determined by high-performance liquid chromatography.

<table>
<thead>
<tr>
<th>Sample</th>
<th>CA</th>
<th>ICA-1</th>
<th>ICA-2</th>
<th>ICA-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kintoki</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WS</td>
<td>27.6</td>
<td>7.3</td>
<td>17.3</td>
<td>3.5</td>
</tr>
<tr>
<td>PP</td>
<td>70.7</td>
<td>11.6</td>
<td>50.1</td>
<td>9.1</td>
</tr>
<tr>
<td>PS</td>
<td>12.0</td>
<td>1.5</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Kokei No. 14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS</td>
<td>12.1</td>
<td>2.0</td>
<td>3.1</td>
<td></td>
</tr>
</tbody>
</table>

*Milligrams in 100 g fresh weight. CA, chlorogenic acid; ICA, isochlorogenic acid; —, not detected; WS, whole sweet potato powder; PP, peel powder of sweet potato; PS, peeled sweet potato powder.
Table 5. Antioxidative activity of chlorogenic acid (CA) and isochlorogenic acids (ICA).

PV% of 70% methanol extract obtained from peeled sweet potato powder (PS, 0.25 g) was 7. Chlorogenic acid and isochlorogenic acids content of PS (0.25 g) was 87 and 33.5 µg, respectively.

<table>
<thead>
<tr>
<th>CA (µg)</th>
<th>ICA (µg)</th>
<th>PV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>—</td>
<td>19</td>
</tr>
<tr>
<td>200</td>
<td>—</td>
<td>17</td>
</tr>
<tr>
<td>50</td>
<td>—</td>
<td>63</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
<td>102</td>
</tr>
<tr>
<td>1</td>
<td>—</td>
<td>102</td>
</tr>
<tr>
<td>—</td>
<td>160</td>
<td>30</td>
</tr>
<tr>
<td>—</td>
<td>80</td>
<td>52</td>
</tr>
<tr>
<td>—</td>
<td>40</td>
<td>75</td>
</tr>
<tr>
<td>—</td>
<td>8</td>
<td>109</td>
</tr>
<tr>
<td>50</td>
<td>40</td>
<td>38</td>
</tr>
</tbody>
</table>

Potato cultivars.

**Antioxidative activity of phenolic compounds**

Table 5 shows the antioxidative activity of chlorogenic acid and isochlorogenic acids in various concentrations. This activity was weaker than that of 70% methanol extract. Accordingly, such a strong antioxidative activity of 70% methanol extract is considered to be based on the presence of unknown antioxidants or the synergistic effect of coexisting components.

Pratt (12) reported that caffeic acid was an effective antioxidant but that chlorogenic acid had no antioxidant activity. On the other hand, Naito et al. (4) indicated that catechin and chlorogenic acid had strong antioxidative activity. These discrepancies may depend on the difference in the method of measurement of antioxidative activity and in the ratio of the concentration of chlorogenic acid to lipid.

**Determination of bound-type phenolic acid**

Since the antioxidative activity of major phenolic compounds identified in 70% methanol extract of sweet potato was weak, the bound-type phenolic acids were analyzed. The 70% methanol extract was hydrolyzed with 2 N NaOH, extracted with ethyl ether and analyzed by TLC and HPLC. The results are shown in Fig. 2. Caffeic acid, trans-p-coumaric acid and trans-p-ferulic acid were detected, but the last two compounds were present in very small amounts in 70% methanol extract. Caffeic acid is considered to be mostly formed from chlorogenic acid and isochlorogenic acids on alkaline hydrolysis.
Fig. 2. Thin layer chromatogram and high-performance liquid chromatogram of phenolic acids in whole sweet potato powder after alkaline hydrolysis. A: alkaline hydrolysate of 70% methanol extract of sweet potato. B: standard; 1, caffeic acid; 2, trans-p-coumaric acid; 3, trans-p-ferulic acid. * Numbers are percent constitution of phenolic acids.

Table 6. Free sugars and amino acids contained in sweet potato (Kintoki)*.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Fructose</th>
<th>Glucose</th>
<th>Sucrose</th>
<th>Glc</th>
<th>Asn</th>
<th>Thr</th>
<th>Ser</th>
<th>Glu</th>
<th>Gln</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.50</td>
<td>0.58</td>
<td>5.18</td>
<td>1/2Cys</td>
<td>trace</td>
<td>7.2</td>
<td>13.2</td>
<td>50.2</td>
<td>11.7</td>
</tr>
<tr>
<td>Amino acid</td>
<td>Asp</td>
<td>Ala</td>
<td>1/2Cys</td>
<td>Val</td>
<td>Met</td>
<td>56</td>
<td>4.6</td>
<td>7.2</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>trace</td>
<td>9.6</td>
<td>trace</td>
<td>3.7</td>
<td>0.7</td>
<td></td>
<td>Ile</td>
<td>Leu</td>
<td>trace</td>
</tr>
</tbody>
</table>

*Percentage of water content of sweet potato (Kintoki) was 65.5%. * Grams in 100 g fresh weight. * Milligrams in 100 g fresh weight. * The increments of Glu and Asp by hydrolysis with 2N HCl for 3 h of 70% methanol extract of peeled sweet potato powder were regarded as the amounts of Gln and Asn, respectively.

Synergistic effect of phenolic compounds with amino acids and of 70% methanol extract with tocopherols

As described above, free phenolic compounds identified in 70%, methanol extract had only a slight antioxidative effect, and bound-type phenolic acids, with the exception of caffeic acid, were minor components in this extract. The synergistic effects of phenolic compounds with various components coexisting in the extract were then surveyed.

Table 6 shows the composition of free amino acids and sugars in the 70% methanol extract. Model solutions based on the determination of phenolic.
compounds (Table 4), free amino acids and sugars were prepared. An amino acid mixture was prepared as follows. Threonine, methionine, histidine and arginine, which are known to have effective antioxidative activity (13), were mixed in the concentrations as found in the 70% methanol extract, and all the other amino acids were replaced with aspartic acid; the mixture was then dissolved in 0.2 M phosphate buffer (pH = 7.0). Table 7 shows each respective value for antioxidative activity as obtained by the combination of test model solutions. Both PV% of 70% methanol extract obtained from 0.5 g of PS and PV% of the test solution of phenolic compounds and amino acid mixture corresponding to the extract of 0.5 g of PS, gave the same value of 5. Accordingly, a synergistic effect of phenolic compounds with amino acid mixture was clearly observed. However, PV% of the test solution corresponding to the 70% methanol extract obtained from 0.05 g of PS was 33 (Table 7). This value was greater than PV% (= 7) of the 70% methanol extract from 0.05 g of PS (Table 2). In this case, the other components are supposed as being contributors to the antioxidative effect of the sweet potato extract. On the other hand, the PV% of the 70% methanol extract obtained from 0.05 g of PS was 33 (Table 7). This value was greater than PV% (= 7) of the 70% methanol extract from 0.05 g of PS (Table 2). In this case, the other components are supposed as being contributors to the antioxidative effect of the sweet potato extract.

Table 7. Antioxidative activity in combination with chlorogenic acid (CA), isochlorogenic acid (ICA), amino acid mixture (AA), sugar mixture (SM) and citric acid (CI). PV% of 70% methanol extract obtained from 0.5 g and 0.05 g of peeled sweet potato powder (PS) was 5 and 7, respectively. CA, ICA, AA and SM content of 0.5 g of PS was 174 μg, 67 μg, 2,650 μg and 90.7 mg, respectively.

<table>
<thead>
<tr>
<th>CA (μg)</th>
<th>ICA (μg)</th>
<th>AA (μg)</th>
<th>SM (mg)</th>
<th>CI (μg)</th>
<th>PV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>174</td>
<td>67</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>39</td>
</tr>
<tr>
<td>174</td>
<td>67</td>
<td>2,650</td>
<td>—</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>2,650</td>
<td>—</td>
<td>—</td>
<td>84</td>
</tr>
<tr>
<td>174</td>
<td>67</td>
<td>2,650</td>
<td>90.7</td>
<td>730</td>
<td>7</td>
</tr>
<tr>
<td>17.4</td>
<td>6.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>56</td>
</tr>
<tr>
<td>17.4</td>
<td>6.7</td>
<td>265</td>
<td>—</td>
<td>—</td>
<td>33</td>
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<tr>
<td>—</td>
<td>—</td>
<td>265</td>
<td>—</td>
<td>—</td>
<td>108</td>
</tr>
</tbody>
</table>

* Thr (100 μg)+ Met (10 μg)+ His (40 μg)+ Arg (50 μg)+ Asp (2,450 μg).  
* Glucose (8.4 mg)+ fructose (7.2 mg)+ sucrose (75.1 mg).

Table 8. Antioxidative activity of 70% methanol extract* of peeled sweet potato powder (5 g) and the synergistic effect with tocopherol.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Tocopherol</th>
<th>PV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05 ml</td>
<td>—</td>
<td>33</td>
</tr>
<tr>
<td>—</td>
<td>50 μg</td>
<td>36</td>
</tr>
<tr>
<td>0.05 ml</td>
<td>50 μg</td>
<td>9</td>
</tr>
</tbody>
</table>

* 70% methanol extract was prepared by a method similar to that shown in Table 2.
hand, a synergistic effect of glucose, fructose and sucrose was not observed, as shown in Table 7. Citric acid also did not appear to show a synergistic effect because of the non-addition of metallic ions in the present solution system.

From the results obtained above, the strong antioxidative activity of the sweet potato extract is considered to be mainly based on the synergistic effect of phenolic compounds with amino acids.

Moreover, the synergistic effect of 70% methanol extract with the tocopherol mixture is shown in Table 8. The antioxidative activity of the sweet potato extract obtained from 0.025g of PS nearly corresponded to that of 50 micrograms of tocopherol mixture.

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REFERENCES
