Antioxidative Activity of Avocado Epicarp Hot Water Extract

Naoko TERASAWA1*, Miki SAKAKIBARA1 and Masatsune MURATA2

1 Faculty of Education, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa 920–1192, Japan
2 Department of Nutrition and Food Science, Ochanomizu University, 2–1–1 Otsuka, Bunkyo-ku, Tokyo 112–8610, Japan

Received July 21, 2005; Accepted October 13, 2005

The radical scavenging activity of avocado epicarp extract was investigated and found to be about 2 times higher than those of α-tocopherol and ascorbic acid. Radical scavenging activity was substantially retained after heat treatment at 180°C for 60 min. The antioxidative activities of the avocado epicarp extract and α-tocopherol were measured by the thiocyanate method with a linoleic acid system. The activity of the avocado epicarp extract was higher than α-tocopherol, but a mixture of the extract and α-tocopherol did not show a synergistic effect on activity. The avocado epicarp extract was analyzed by HPLC, with 4 peaks, (+)-catechin, (−)-epicatechin, and 2 unknown ones, being detected. These 4 peaks contributed to about 15% of the total antioxidative activity.

Keywords: antioxidative activity, radical scavenging activity, avocado, epicarp, polyphenol

Introduction

Many investigations of antioxidative activity in vegetables and fruits have been reported, and such components as vitamins C and E, catechins and anthocyanins have been revealed as antioxidants. As the antioxidants contained in vegetables and fruits are generally present in the epicarp, the antioxidative activities of waste portions of plants such as the bamboo shoot sheath (Katsuzaki et al., 1999) and barley bran (Tamagawa et al., 1997) have also been studied. Avocado is abundant in nutritional components such as vitamins E, B, and B, pantothenic acid, potassium, and dietary fiber (Kagawa, 2001), and antioxidants are contained in its epicarp (Nose and Fujino, 1982) and seed (Geissman and Dittmar, 1965; Matsusaka et al., 2003). However, the chemical properties of antioxidants other than vitamin E in avocado epicarp have not been investigated.

Antioxidative activity sometimes increases when two antioxidants are used together. For example, the stability of lard was found to be highest when both 0.5% ascorbic acid and 0.1% nordihydroguaiaretic acid were added to the lard sample (Sakurai et al., 1986). In this study, we extracted the polyphenol fraction from avocado epicarp and investigated the synergistic effect of avocado epicarp extract and α-tocopherol.

Materials and Methods

Sample Avocado was purchased from a local market in the city of Kanazawa, Ishikawa prefecture (Japan), in 2002–2003.

Sample preparation Avocado epicarp was minced and then extracted with hot distilled water at 90°C for 10 min. The resulting extract was filtered with No. 2 filter paper, and the filtrate was washed 8 times with chloroform and then extracted 4 times with ethyl acetate (Terasawa et al., 2001). The combined ethyl acetate layer was evaporated in vacuo.

Determination of the total phenol content The total phenol content of the avocado epicarp extract was determined with Folin-Ciocalteau reagent assay (Tsimidou et al., 1992) as follows. The sample solution (2 ml) was transferred into a 25-ml volume mess flask and Folin-Ciocalteau reagent (0.5 ml) was added. After 3 min, 1 ml of saturated sodium carbonate solution was added and the flask was made up to volume with water and stored in the dark for 1 hr. The absorbance of the solution was then measured at 725 nm. The total phenol content was expressed as gallic acid equivalent weight (g), using a calibration curve of gallic acid.

Radical scavenging assay The radical scavenging activities of the avocado epicarp extract, dl-α-tocopherol, and L-ascorbic acid were determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (Terasawa et al., 2001). The avocado epicarp extract and dl-α-tocopherol were dissolved separately in ethanol, and L-ascorbic acid was dissolved in 0.1 M acetate buffer (pH 5.5). Two ml of these solutions, 2 ml of the acetate buffer or ethanol, and 1 ml of 0.5 mM DPPH/ethanol were mixed in a test tube. In the control experiment, ethanol or the acetate buffer was added to a test solution instead of the sample solution. The absorbance of the control at 517 nm was measured immediately after mixing (0 min), and that of each test solution was measured 30 min after mixing (30 min). Radical scavenging activity was calculated using the following formula:

*To whom correspondence should be addressed.
E-mail: terasawa@ed.kanazawa-u.ac.jp
Each sample was measured in triplicate, and the average value was calculated. The radical scavenging activity of dibutyl hydroxytoluene (BHT) was also measured, and the relationship between the activity and the concentration of BHT was used to produce a standard curve. The activity of avocado epicarp extract is expressed as the BHT equivalent weight (g) from this standard curve.

**Effect of heat treatment on antioxidative activity of avocado epicarp extract** The avocado epicarp extract was dissolved in ethanol at a concentration of 10 μg/ml. Ten milliliter aliquots of this solution were taken into screw-capped test tubes and heated in a block heater for 15, 30 or 60 min at a temperature of 120°C or 180°C. The radical scavenging activity of the avocado epicarp extract was then determined by the DPPH method, each sample being measured in triplicate.

**Synergistic effect of avocado epicarp extract and α-tocopherol on antioxidative activity** Antioxidative activity was evaluated using the thiocyanate method with a linoleic acid system (Osawa and Namiki, 1981). Avocado epicarp extract (0.1, 1.0 or 10 mg) or d,l-α-tocopherol (0.1, 0.2 or 0.5 mg) was dissolved in 10 ml of ethanol. Avocado epicarp extract (0.1 mg) and d,l-α-tocopherol (0.1, 0.2 or 0.5 mg) were also dissolved together in 10 ml of ethanol. The sample solution (10 ml) was added to a mixture of linoleic acid (0.13 ml), 99.5% ethanol (10 ml), and a 50 mM phosphate buffer (pH 7.0, 10 ml), and the total volume was adjusted to 25 ml by adding distilled water. The solution was incubated at 40°C in the dark, and the degree of oxidation was measured according to the thiocyanate method (Mitsuda et al., 1966). This mixture (0.1 ml) was added to 4.7 ml of 75% ethanol, 0.1 ml of 30% ammonium thiocyanate, and 0.1 ml of 20 mM FeCl₃ in 3.5% HCl. After 3 min, the absorbance of the reaction mixture at 506 nm was measured.

**Identification of phenolics in avocado epicarp extract** Several phenolics were used as HPLC standards: gallic acid monohydrate, salicylic acid, resorcinol, 2,5-dihydroxybenzoic acid, hydroquinone, chlorogenic acid, and phenol were purchased from Wako Pure Chemicals (Osaka). Pyrocatechol was purchased from Nacalai Tesque (Kyoto), and (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate, (-)-epigallocatechin, and (-)-epigallocatechin gallate were purchased from Funakoshi (Tokyo). Analytical HPLC conditions were as follows: column, LUNA 5 μ C₁₈ No. 2 (4.6 i.d. × 150 mm; Phenomenex, Torrance, CA); pump, LC-9A (Shimadzu, Kyoto); column oven temperature, 40°C; flow rate, 2.0 ml/min; eluent, water-acetonitrile-50 mM phosphoric acid 95:5:0.05 (v/v/v); detection, OD₂ₘ₅₀ (Terasawa et al., 2002). The absorption spectra of isolated peaks were measured with a Sefi IUV-1240 spectrophotometer (Asone, Osaka).

**Results and Discussion**

**Determination of total phenol content and radical scavenging activity of avocado epicarp extract** About 570 mg of extract was obtained from 100 g of avocado epicarp. The total phenol content of the extract, measured by Folin-Ciocalteau reagent assay, was 0.8 g of gallic acid equivalent/g, converted to 4.57 mg of gallic acid equivalent/g of avocado epicarp. Golan et al. (1977) reported that the total phenol content in avocado mesocarp was about 28–290 μg of chlorogenic acid equivalent/g, and Soong et al. (2004) reported that the total phenol contents in freeze-dried avocado seed and flesh were 88 mg and 1 mg of gallic acid equivalent/g, respectively. These results show that avocado epicarp contains more polyphenols than avocado flesh.

The radical scavenging activities of the avocado epicarp extract, α-tocopherol, and ascorbic acid were 1.46 g, 590 mg, and 880 mg of BHT equivalent/g, respectively. The activity of the avocado epicarp extract was about 2 times higher than those of α-tocopherol and ascorbic acid. Soong et al. (2004) investigated the antioxidative activity of fresh avocado seeds and flesh, and reported activities of 236 μmol and 55 μmol ascorbic acid equivalent/g, respectively. In our study, the antioxidative activity of avocado epicarp extract was 7.6 mmol ascorbic acid equivalent/g, which was converted to 43 μmol ascorbic acid equivalent/g of fresh avocado epicarp.

**Effect of heat treatment on avocado epicarp extract on radical scavenging activity** The radical scavenging activities of avocado epicarp extract heated at 120°C and at 180°C were almost the same as that of the unheated sample (Fig. 1). This extract was thus stable to heating at 180°C for 60 min, which means that the radical scavenging activity of avocado epicarp would not decrease as a result of high-temperature cooking methods such as oven heating. Murakami et al. (2004) have reported that the contents of quercetin and epigallocatechin gallate in samples heated at 180°C decreased much more rapidly than the radical scavenging activity, and more than 80% of radical scavenging activity was retained after 15 min of heating at 180°C. It was suggested that the degradation products of these compounds also show radical scavenging activity. Some of the polyphenols in avocado epicarp may have degraded with retention of radical scavenging activity of avocado epicarp extract. (n=3)
scavenging activity.

Synergistic effect of avocado epicarp extract and \( \alpha \)-tocopherol on antioxidative activity The individual antioxidative activities of avocado epicarp extract and \( \alpha \)-tocopherol, as measured by the thiocyanate method, are shown in Fig. 2. The peroxidation of linoleic acid was inhibited in those samples to which avocado epicarp extract or \( \alpha \)-tocopherol was added, although the antioxidative activity of the avocado epicarp extract decreased with increasing concentration. Next, the possibility of a synergistic effect between avocado epicarp extract and \( \alpha \)-tocopherol was examined, but no effect was observed (Fig. 2). The activity of a mixture of avocado epicarp extract (0.1 mg) and \( \alpha \)-tocopherol (0.5 mg) was lower than those of the avocado epicarp extract (0.1 mg) and \( \alpha \)-tocopherol (0.2 mg) measured individually. These results indicate that a higher concentration of avocado epicarp extract and \( \alpha \)-tocopherol is not as effective against the peroxidation of linoleic acid.

Nakamura et al. (2001) investigated the modifying effect of a toxic dose of protocatechuic acid (PA), a strong antioxidant, on glutathione levels in mouse liver and kidney. It was suggested that overdoses of PA can disturb the detoxification of other electrophilic toxicants, including ultimate carcinogens. They stated that as antioxidants may be considered a double-edged sword for cancer control, there should be increased focus on the safety of antioxidants administered excessively.

Separation of phenolics in avocado epicarp extract The polyphenols in avocado epicarp extract were analyzed by reversed-phase HPLC; four main peaks could be separated (Fig. 3). Two of these peaks coincided in their retention times and UV absorption spectra with authentic samples of the phenolics \((+)-catechin\) and \((-)-epicatechin\). \((+)-Catechin\) and \((-)-epicatechin\) have been detected in avocado seed (Matsusaka et al., 2003), and \((-)-epicatechin\) was detected in avocado epicarp (Nose and Fujino, 1982). Ramirez-Martinez and Luh (1973) extracted phenolic compounds from frozen avocado and analyzed them by paper chromatography. Fifteen spots were found, which were tentatively identified by their Rf values, color reactions, etc. as chlorogenic acid, \( p \)-coumarylquinic acid, catechin, epicatechin, leucaanthocyanidin, isoflavone, caffeic acid, and \( p \)-coumaric acid. The two unknown peaks separated from avocado epicarp extract in this study are under investigation. These four
peaks made up about 38% of the total avocado epicarp extract on the basis of weight and contributed to about 15% of the total antioxidative activity (Table 1). Epicatechin contributed the most among the four peaks.

In conclusion, avocado epicarp extract containing polyphenols such as (-)-epicatechin showed high radical scavenging and antioxidative activities that were stable to heating. For this reason, avocado epicarp is considered a promising resource.

References

<table>
<thead>
<tr>
<th>Recovery (%)</th>
<th>Radical scavenging activity (g of BHT equivalent/g for each peak)</th>
<th>Contribution ratio of each peak to radical scavenging activity of avocado epicarp extract (%)</th>
<th>$\lambda_{\text{max}}$</th>
<th>Tentative identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avocado epicarp extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak 1</td>
<td>7.6</td>
<td>1.46</td>
<td>0.9</td>
<td>279.5</td>
</tr>
<tr>
<td>Peak 2</td>
<td>9.5</td>
<td>0.85</td>
<td>5.5</td>
<td>280.0</td>
</tr>
<tr>
<td>Peak 3</td>
<td>13.3</td>
<td>0.84</td>
<td>7.7</td>
<td>279.5</td>
</tr>
<tr>
<td>Peak 4</td>
<td>7.6</td>
<td>0.24</td>
<td>1.2</td>
<td>279.5</td>
</tr>
<tr>
<td>Total</td>
<td>38.0</td>
<td>15.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>