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Note

Antioxidative Activity of Bound-Form Phenolics in Potato Peel

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Free and bound-form phenolics were isolated from potato (cv. Toyoshiro) flesh and peel. The free and bound-form phenolics in the peel showed high DPPH radical scavenging activity, while those in the flesh showed low activity. The total amount of chlorogenic acid and caffeic acid in the free-form phenolics from the peel was highly correlated with the DPPH radical scavenging activity. Ferulic acid was identified as the active radical scavenging compound in the bound-form phenolics from the peel. The potato peel may therefore offer an effective source of an antioxidative.

Key words: potato; peel; DPPH radical scavenging activity; bound-form phenolics; ferulic acid

The phenolic compounds commonly found in many plants are involved in many biological activities, including the chelation of metals, scavenging active oxygen species, and antioxidative activity.[1,2] Phenolic compounds are generally either in the free-form or bound-form. Most research has determined free soluble phenols by using aqueous methanol, ethanol, and acetone, either separately or mixed together.[3,4]

The presence of free-form phenolic compounds (such as chlorogenic acid and caffeic acid) has been reported in potato, and recent studies have been conducted on the antioxidative activity of a potato extract.[5,6] However, detailed information about the bound-form of phenolics has not yet become available.

Such bound-form phenolics as ferulic acid and p-coumaric acid are ester-linked to the cell wall polysaccharides in several plants.[7] Ferulic esters are a subject of great interest, given their functional role in cell adhesion and thermal stability of the texture in food plants.[8,9] Ferulic acid and its sugar ester have high antioxidative potential. Moreover, ferulic acid has less of a suppressive effect on LDL oxidation than its sugar ester.[10] We described here the phenolic content and antioxidative activity in the free- and bound-form phenolics extracted from potato. We also analyze the compounds related to radical scavenging activity.

Potatoes (*Solanum tuberosum* cv. Toyoshiro) of similar size and appearance were obtained for the experiment from a field in Hokkaido, Japan. The potatoes were cleaned with tap water and then peeled in strips about 1-mm thick with a common vegetable peeler. The remaining tissue is designated as flesh. The peel and flesh were immediately freeze dried, and each then ground to a fine powder (flesh = 21.34 g/100 g of fresh weight; peel = 13.76 g/100 g of fresh weight).

The powder (2 g) was homogenized five times with 80% (v/v) ethanol (20 ml) at room temperature (20 ± 3 °C), and the homogenate centrifuged. The precipitate was washed in acetone and then dried. The dried material is designated as the alcohol-insoluble solid (AIS). The supernatant thus obtained was combined and is designated as the alcohol-soluble fraction (free-form phenolics). The yields of AIS from the flesh and peel were 1.95 g and 1.76 g, respectively.

To extract the bound-form phenolics, AIS (200 mg) was treated with 20 ml of 4 M NaOH for one hour at room temperature (20 ± 3 °C), and the solution centrifuged. The supernatant was acidified with HCl and extracted three times with ethyl acetate. The ethyl acetate phase was evaporated to dryness at 40 °C under a vacuum. The bound-form phenolics were dissolved in 50% methanol for subsequent determination. The free- and bound-form phenolics were then determined by the Folin-Ciocalteu method.[11] Table 1 shows the total phenolic content of free- and bound-forms in potato. The content of free and bound-form phenolics in the peel was more than that in the flesh. In respect of the distribution of free and bound-form phenolics, the bound-form in the peel was more widely distributed (26%) than that in the flesh (1%). Chu *et al.* have reported that the bound-form accounted for 40% of the total phenolic content in the edible part of potatoes.[1] The total amount of phenolics in potato also varies

Table 1. Total Contents of Free- and Bound-Form Phenolics in Potato

Sample	Total phenolics (mg of ferulic acid equiv/g of dry matter)	
	Free	Bound
Flesh	1.68	0.02
Peel	3.66	1.26

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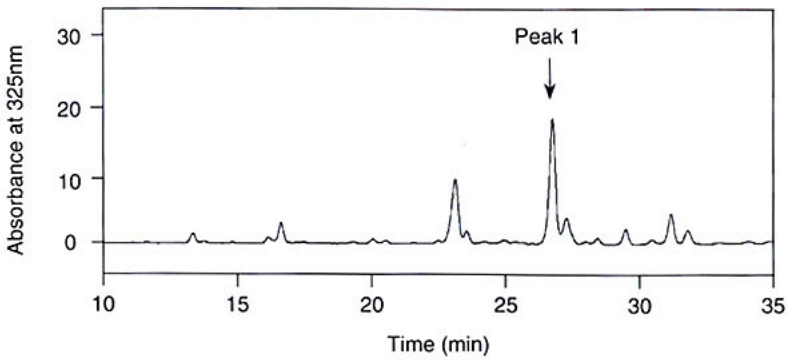

Fig. 1. HPLC Chromatograms of the Bound-Form Phenolics in Potato Peel.

significantly among different varieties of potato.[12] It is conceivable that there would be a difference in the amount of bound-form phenolics.

In order to obtain useful information about the components of the free-form phenolics, on HPLC analysis was conducted in an Inertsil ODS-3 column (4.6 × 250 mm; GL Science, Tokyo, Japan) with photodiode array detection. Elution was done at 40 °C by using a linear gradient of 10% to 25% acetonitrile containing a 20 mM sodium phosphate buffer (pH 3.3) at a flow rate of 1.0 ml/min. Two major peaks were identified in the peel as chlorogenic acid and caffeic acid by co-chromatography with a standard (data not shown). The chlorogenic acid and caffeic acid contents of the free-form phenolics in the peel were 6.75 µmol/g of dry matter and 3.00 µmol/g of dry matter, respectively. The amount of chlorogenic acid found in potato has been reported to vary among different varieties of potato.[12]

In addition, chlorogenic acid has 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity. Therefore, the radical-scavenging activity was determined by using the DPPH stable radical. Briefly, 150 µl of a 50% ethanol solution was added to 50 µl of a sample solution in a 96-well microplate. The reaction was initiated by adding 100 µl of 20 mM DPPH to the ethanol solution. After the solution had been left standing for 15 minutes, a microplate reader (Bio-Rad, CA, USA) was used to measure the absorbance at 540 nm. The DPPH radical-scavenging activity was estimated from the decrease in absorbance at 540 nm and is expressed as the trolox equivalent per g of dry matter, using a standard curve for trolox. The antioxidative activity in the peel extract (20.72 µmol of trolox equiv/g of dry matter) was higher than that in the flesh (7.03 µmol of trolox equiv/g of dry matter).

The DPPH radical-scavenging activities of chlorogenic acid and caffeic acid of the free-form phenolics in the peel were respectively calculated as 7.87 and 3.95 µmol of trolox equiv/g of dry matter. These totals from the chlorogenic acid and caffeic acid are equivalent to about 57% of total activity (20.72 µmol of trolox equiv/g of dry matter). These findings suggest that the level of antioxidative activity and the amount of phenolics were closely related. Therefore, chlorogenic acid and caffeic acid are important components of the free-form phenolics in potato peel.

The bound-form phenolics (extracted with NaOH) were diluted, and a UV–vis spectrophotometer (Shimadzu, Kyoto, Japan) was used to determine the UV–vis spectrum in the range of 200 nm to 500 nm. The bound-form phenolics in the peel exhibited a spectrum near 325 nm (data not shown). Such phenolic acids as chlorogenic acid, caffeic acid and ferulic acid are generally found in a typical UV–vis spectrum, with a maximum absorption peak at 325 nm. Therefore, the peel exhibited a spectrum with maximum absorption near 325 nm due to the presence of cinnamic acid. The components of the bound-form phenolics were then subjected to an HPLC analysis. The chromatogram shows several peaks (Fig. 1), including a particularly large one at Rt = 26.62 (peak 1). Peak 1 corresponded to cinnamic acid-related compounds with a maximum absorption near 325 nm. The MS/MS data showed the peak 1 ion at m/z 134, 149, 178 and 193, as well as ferulic acid. Finally, peak 1 was identified as ferulic acid by co-chromatography with an authentic standard (data not shown). The total amount of ferulic acid in the peel was estimated to be 0.37 µmol/g of dry matter.

Ferulic acid is a major phenolic acid that is ester-linked in the cell wall polysaccharides of several plants.[8] Ferulic acid has recently been reported to be a good antioxidative, the oxidation of human plasma LDL induced by $CuSO_4$ being inhibited by the ferulic sugar ester.[10] Figure 2 shows the total antioxidative activity in the bound-form phenolics as determined by a DPPH radical-scavenging assay. The bound-form phenolics in the peel showed much stronger activity (5.70 µmol of ferulic acid equiv/g of dry matter) than those in the flesh (0.28 µmol of ferulic acid equiv/g of dry matter). These results indicate that the bound-form phenolics in the peel had strong activity as judged from the estimated amount of ferulic acid in the peel.

In conclusion, we have clearly demonstrated DPPH radical-scavenging activity in potato peel. Given the large quantity of potato peel waste generated by potato processing, the results obtained in the present study

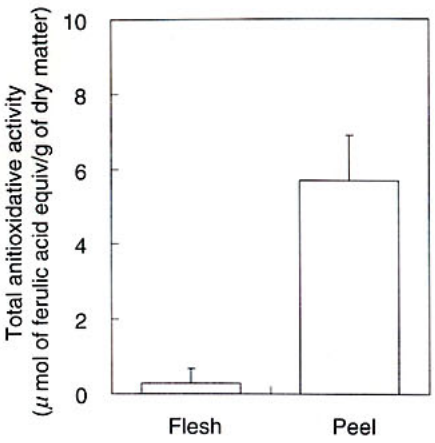

Fig. 2. DPPH Radical-Scavenging Activity of Bound-Form Phenolics in Potato Flesh and Peel.
Vertical bars indicate the standard deviation.

suggest that potato peel can be effectively used as a new source of a natural antioxidative. Moreover, ferulic acid (ester-linked to the cell wall polysaccharides) oxidizes to form diferulic acid. Such oxidative coupling of wall matrix polysaccharides has been considered to drastically affect cell wall extensibility and its growth rate.[7–9] Based on these findings, the ferulic acid found in potato is expected to play an important role in such growth. It is therefore necessary to examine the detailed structure of cell wall polysaccharides in the future.

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