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Sweet potato (*Ipomoea batatas* [L.] Lam 'Tainong 57') storage root mucilage with antioxidant activities in vitro

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Abstract

Sweet potato storage root mucilage was extracted and purified by SDS and heating treatments. Total antioxidant activity, DPPH (1,1-diphenyl-2-picrylhydrazyl) staining, reducing power method, metal ion-dependent hydroxyl radical, FTC (ferric thiocyanate) method, and protection of calf thymus DNA against hydroxyl radical-induced damage were studied. Half-inhibition concentrations, IC_{50}, were 0.08 mg/ml and $IC_{50} > 0.1$ mg/ml, respectively, for the crude and purified mucilage in the total antioxidant activity test. In the DPPH staining, the crude and purified mucilage appeared as white spots when they were diluted to 50 and 100 μg per application, respectively. Like total antioxidant activity, reducing power, scavenging capacity against hydroxyl radical, FTC activity and protection of calf thymus DNA against hydroxyl radical-induced damage were found in the mucilage. It is suggested that the mucilage might contribute its antioxidant activities against both hydroxyl and peroxyl radicals.

Keywords: Sweet potato; Antioxidant; Mucilage; Hydroxyl radical; Peroxyl radical; 1,1-Diphenyl-2- picryl hydrazyl (DPPH)

1. Introduction

It is commonly accepted that, in a situation of oxidative stress, reactive oxygen species, such as superoxide ($O_2^{\bullet}$), hydroxyl ($OH^{\bullet}$) and peroxyl ($^{\bullet}OOH$, $ROO^{\bullet}$) radicals, are generated. The reactive oxygen species play an important role related to the degenerative or pathological processes of various serious diseases, such as aging (Burns et al., 2001), cancer, coronary heart disease, Alzheimer's disease (Ames, 1983; Gey, 1990; Smith et al., 1996; Diaz, Frei, Vita, & Keaney, 1997), neurodegenerative disorders, atherosclerosis, cataracts, and inflammation (Aruoma, 1998). Traditional medicine is widespread and plants still presents a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several anti-inflammatory, digestive, anti-necrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have an antioxidant and/or anti-radical scavenging mechanism as part of their activity (Perry, Pickering, Wang, Houghton, & Perru, 1999; Lin & Huang, 2002; Repetto & Llesuy, 2002). In the search for sources of natural antioxidants and compounds with radical scavenging activity during recent years, some have been found, such as echinacoside in *Echinaceae* root (Hu & Kitts, 2000), anthocyanin (Espin, Soler-Rivas, Wichers, & Viguera-Garcia, 2000), phenolic compounds (Rice-Evans, Miller, & Paganga, 1997), water extracts of roasted *Cassia tora* (Yen & Chuang, 2000), whey proteins (Allen & Wrieden, 1982; Tong, Sasaki, McClements, & Decker, 2000), and thioredoxin *h* protein from sweet potato (Huang, Lin, Chen, Hou, & Lin, 2004a).

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The major components of plant mucilage are pectins. Pectins are largely acidic polysaccharides that form gels in the extracellular matrix and are present in all cell walls. The two most common pectins found in dicotyledonous plants are polygalacturonic acid (PGA) and rhamnogalacturonan I (RG I) (Brett & Waldron, 1990; Carpita & Gibeaut, 1993; Cosgrove, 1997). PGA is an unbranched chain of α-1,4-linked galacturonic acid (GalUA) residues, while RG I is a highly substituted, branched polysaccharide with a backbone of alternating α-1,4-linked GalUA and α-1,2-linked rhamnose residues (Brett & Waldron, 1990). There are reports concerning the physiological activities of pectins on the interactions between fibroblast growth factors and receptors (Liu et al., 2001), on the modulation of lung colonization of B16-F1 melanoma cell (Platt & Raz, 1992), and on the inhibition of human cancer cell growth and metastasis in nude mice (Nangia-Makker et al., 2002). Pectin diets could also reduce the incidence of colon cancer in rats (Hardman & Cameron, 1995). Yam (*Dioscorea batatas*, Dioscoreaceae) is a major tuber crop and its mucilages are mainly composed of mannan-protein macromolecules with antioxidant activities (Tsai & Tai, 1984). A G009 fraction (polysaccharide fraction) of *Ganoderma lucidum* showed inhibitory activity against iron-induced lipid peroxidation and dose-dependent hydroxyl radical scavenging activity (Lee et al., 2001). The arabinogalactan polysaccharide showed good protection against iron-mediated lipid peroxidation of rat brain homogenate, as revealed by the thiobarbituric acid-reactive substances (TBARS) and lipid hydroperoxide (LOOH) assays (Subramanian, Chintalwar, & Chattopadhyay, 2002).

No report on the antioxidant activities of mucilage of sweet potato is presently available. In this work we report (for the first time) that purified mucilage from sweet potato displayed antioxidant activities in comparison with chemicals such as butylated hydroxytoluene (BHT), reduced glutathione, or ascorbic acid in a series of in vitro tests.

2. Materials and methods

2.1. Mucilage extractions and purifications

Mucilage extractions and purifications were done according to the method of Hou, Hsu, and Lee (2002). Fresh sweet potato (*Ipomoea batatas* [L.] Lam 'Tainong 57') storage roots were purchased from a local market. After being cleaned with water, the storage roots were cut into strips for crude mucilage extractions. Sweet potato storage root was homogenized with two volumes (w/v) of 50 mM Tris–HCl buffer (pH 8.3) containing 1% vitamin C. After centrifugation at 14,000*g* for 30 min, the supernatants were mixed with isopropanol to a final concentration of 70%, and stirred quickly at 4 °C overnight. The precipitates were filtered and dehydrated with 100% isopropanol, then rinsed with acetone. After drying at 40 °C in an oven, the crude mucilage was ground and collected for further purification by both SDS and heating procedures. About 1.0 g crude mucilage powder was dissolved in 200 ml of distilled water and kept in a 50 °C water bath. Forty ml of 5% SDS solution (dissolved in 45% ethanol) were added to the crude mucilage solution. The mixture was kept with gentle stirring at 50 °C for 30 min, then at room temperature for another 2 h. After that, the mucilage solution was placed in an ice bath to quickly lower the temperature in order to precipitate the SDS-protein complex. After centrifugation at 14,000*g* at 0 °C for 30 min, the supernatants were precipitated with isopropanol and dried at 40 °C in an oven as described earlier. The semi-purified mucilage was again ground, dissolved, and then heated in boiling water for 20 min. After centrifugation at 14,000*g* at 0 °C for 30 min, the supernatants were mixed with isopropanol to a final concentration of 70%. The purified mucilage was filtered, dehydrated, rinsed with acetone, dried, and then collected for further uses.

2.2. Protein and PAS stainings on 10% SDS–PAGE gels

Samples were mixed with sample buffer, namely 60 mM Tris–HCl buffer (pH 6.8) containing 2% SDS, 25% glycerol, and 0.1% bromophenol blue with 2-mercaptoethanol, and subjected to electrophoresis according to the method of Laemmli (1970). Coomassie brilliant blue G-250 was used for protein staining (Huang, Chen, Hou, Chen, & Lin, 2004b). Periodic acid–Schiff (PAS) staining was used for oligosaccharide staining. After electrophoresis, the gel was placed in fixative (7.5% acetic acid), and shaken gently for 60 min. To oxidize the oligosaccharides, the gel was treated with 0.2% periodic acid for 45 min at 4 °C. The gel was washed with distilled water and stained with Schiff's reagent in the dark at 4 °C (Deepak, Thippeswamy, Shivakameshwari, & Salimath, 2003).

2.3. Total antioxidant status

Total antioxidant status of the mucilage was measured using 2,2′-azinobis[3-ethylbenzthiazoline]-6-sulfonic acid (ABTS) assay (Re et al., 1999). ABTS was dissolved in deionized water to 7 mM concentration, and potassium persulphate added to a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature overnight (12–16 h) in the dark before use. The resultant intensely-coloured $ABTS^{\cdot+}$ radical cation was diluted with 0.01 M PBS (phosphate buffered saline), pH 7.4, to give an absorbance value of ~0.70 at 734 nm. The test compound was diluted 100 × with the ABTS solution to