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Effect of low temperature storage on phenolic composition and antioxidant activity of sweetpotatoes

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Abstract

Low temperature stress-induced phenolic compounds may increase the antioxidant activity and hence the nutraceutical value of sweetpotatoes [*Ipomoea batatas* (L.) Lam]. Cured and non-cured roots of 'Beauregard' sweetpotatoes were exposed to low temperature storage (5 °C) for up to 4 weeks. A significant increase in total phenolic content in cured and non-cured roots was observed after 2 weeks of low temperature exposure. However, an increase in the antioxidant activity after 3 weeks of storage at 5 °C was noticed only in non-cured roots. After 4 weeks of storage at 5 °C, non-cured roots accumulated higher total phenolics and antioxidant activity than cured roots. Among tissue locations, the highest phenolic content and antioxidant activity were found in the periderm tissue and the lowest in the pith tissue. A 3-day exposure period to ambient temperature (~22 °C) following low temperature storage resulted in a significant increase in antioxidant activity in periderm tissue. The highest total phenolic content (7.55 g kg^{-1}) and antioxidant activity (8.05 g kg^{-1}), on a fresh weight basis, were found in periderm tissue from roots transferred to ambient temperature after 4 weeks of low temperature storage. The most abundant individual phenolic acid in sweetpotato roots was chlorogenic acid followed by 3,5-dicaffeoylquinic acid. Chilling injury symptoms such as pitting and internal discoloration were apparent after 3 weeks of storage and were noticeably more severe in non-cured roots than cured root tissue.
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Keywords: *Ipomoea batatas*; Total phenolics; Chlorogenic acid; Caffeic acid; Chilling

1. Introduction

Sweetpotato is indigenous to tropical regions and is susceptible to chilling injury when subjected to low temperature storage. Chilling injury may occur during on-farm storage, long distance transport, in wholesale and retail storage facilities, in supermarket display racks, or/and in consumer refrigerators. Long-term exposure to low temperature results in internal tissue darkening, which has been attributed to an increased content of phenolic compounds in sweetpotato roots (Lieberman et al., 1958, 1959; Porter et al., 1976). However, Minamikawa et al. (1961) found no significant increase in chlorogenic acid content or polyphenols in 'Okinawa No. 100' sweetpotato roots stored at 0 °C. These apparent contradictions indicate chilling injury and phenolic content in sweetpotatoes are influenced by cultivar and other pre- and/or postharvest conditions. The external chilling injury symptoms and internal tissue darkening in non-cured sweetpotato roots was greater than in cured roots (Picha, 1987). Transferring the cured 'Porto Rico' sweetpotato roots to 15 °C after 6 weeks of storage at 7.5 °C resulted in a higher buildup of chlorogenic acid (Lieberman et al., 1959). Cantwell et al. (2002) reported an accelerated buildup of phenolics when jicama roots were transferred to 20 °C after 2 weeks of storage at 10 °C. These studies suggested that a transfer to warmer temperature intensifies the changes induced by low temperature storage. The change in phenolic compound metabolism in response to low temperature also depends on the tissue sensitivity. Yamaki and Uritani (1972) reported that some regions of the sweetpotato root tissue showed vacuolar membrane degradation in response to low temperature stress. This may have resulted in a greater influx of phenolic compounds into the cytosol from their vacuolar storage location.

Phenolic compounds have attracted attention due to their antioxidant properties and ability to protect the human body from oxidative stress (Kaul and Khanduja, 1998). A recent review indicated the dietary polyphenols may help to protect the human body against many chronic diseases such as diabetes, cancer and cardiovascular ailments (Scalbert et al., 2005). Thus,

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an increase in phenolic compounds due to low temperature exposure may enhance the nutraceutical value of sweetpotato roots. However, an extended period of low temperature exposure may result in chilling injury, which decreases the market value of sweetpotatoes. The previous studies provided no information pertaining to the effect of limited time low temperature exposure on levels of individual phenolic acids and antioxidant activity in different tissues of sweetpotato roots. This study was initiated with an objective to determine the effect of low temperature storage on phenolic acid composition and antioxidant activity in different tissues of sweetpotato roots.

2. Materials and methods

2.1. Reagents

Chlorogenic acid and caffeic acid standards, Folin–Denis reagent and DPPH (1,1-diphenyl-2-picrylhydazyl) reagent were purchased from Sigma–Aldrich (St. Louis, MO). Standards of three isomers of dicaffeoylquinic acid (4,5-diCQA, 3,5-diCQA, 3,4-diCQA) were kindly provided by Dr. Makoto Yoshimoto (Department of Upland Farming, Kyushu National Agricultural Experiment Station, Miyakonojo, Miyazaki, Japan).

2.2. Plant material

'Beauregard' sweetpotatoes were grown at the LSU AgCenter Sweetpotato Research Station in Chase, LA in 2004. The roots were harvested during the first week of October and divided into two lots. One lot was cured at 30 °C and 90% relative humidity for 7 days and then stored at 5 °C. The second lot was stored at 5 °C without curing. The roots were sampled at harvest, after curing, and at specified intervals during a 4-week exposure to low temperature.

2.3. Tissue preparation and extraction

In an initial experiment, cured and non-cured sweetpotato roots were stored at 5 °C for 4 weeks. The stored roots were analyzed for total phenolic content at weekly intervals. Randomly selected roots were washed and allowed to dry at ambient temperature before tissue preparation. Peeled whole roots were blended using a Cuisinart model DLC-2AR food processor (Cuisinart Inc., Windsor, NJ). Blended tissue was passed through a 1 mm sieve and 1 g fresh weight sample of sieved tissue was placed in a 15 mL centrifuge tube and homogenized in 8 mL of 80% methanol using a VirTishear homogenizer (Virtis Co., Gardiner, NY). The tubes were capped and immersed in a water bath at 80 °C for 10 min. After vigorously shaking the heated samples manually for 30 s, the tubes were cooled and centrifuged at $4500 \times g$ for 15 min. The final volume of clear supernatant was made to 10 mL with 80% methanol and analyzed for total phenolic content.

A second experiment was initiated to study the effect of low temperature storage on total phenolics and individual phenolic acid content in different tissues of non-cured roots. Roots were analyzed after 2 and 4 weeks of storage at 5 °C. At each sampling, some of the chilled roots were held at ambient temperature (~22 °C) for an additional period of 3 days. The four tissue types analyzed at each sampling were: (1) periderm (skin), the outer tissue, (2) cortex, secondary tissue immediately internal to the periderm (3) cambium, the tissue forming a ring partitioning internal and external tissue (4) pith, the secondary tissue internal to the cambium. It has been previously reported that the periderm comprised approximately 4% of the total root weight, the cortex and cambium together approximately 35% of the total root weight and the pith tissue the reminder (Walter and Schadel, 1981). Exactly 1 g of tissue, excised with a scalpel, was obtained from each tissue type from the same root. The individual tissues were put in 15 mL centrifuge tubes and homogenized in 8 mL of 80% methanol. The capped tubes were immersed in a water bath at 80 °C for 10 min and manually shaken for 30 s. The supernatant was collected after centrifugation and analyzed for total phenolics, individual phenolic acids, and antioxidant activity.

2.4. Total phenolics

Total phenolic content was determined by a slight modification of the Folin–Denis method (Swain and Hillis, 1959). Exactly 0.5 mL of supernatant was placed in a 25 mL test tube and mixed with 8 mL of Megapure water (Barnstead MP-12A, Haverhill, MA) followed by the addition of 0.5 mL of Folin–Denis reagent. After 3 min, 1 mL of 2 N Na_2CO_3 was added and the solution was allowed to stand for 2 h at 22 °C. Absorbance of the resulting blue color complex was measured at 750 nm using a Lambda 35 UV–vis spectrophotometer (Perkin-Elmer Inst., Norwalk, CT). A standard curve of chlorogenic acid (50–300 mg/L) was used for quantification and the total phenolic content was expressed as g of chlorogenic acid equivalents per kg fresh weight, $g\,kg^{-1}$.

2.5. Individual phenolic acids

Separation, analysis, and quantification of individual phenolic acids were accomplished using reversed-phase HPLC with a Gemini C18, 5 μm, 250 × 4.6 mm (Phenomenex, Torrance, CA) column. An aliquot of the supernatant was filtered through a 0.45 μm Nylaflo membrane filter (Pall Corp., East Hills, NY). A sample volume of 20 μL was injected onto the column using a Waters 717 autosampler connected to a Waters 600E pump (Waters Corp., Milford, MA). Phenolic acids were eluted using a mobile phase consisting of 1% (v/v) formic acid in aqueous solution:acetonitrile:2-propanol (70:22:8) at pH 2.5 with an isocratic flow rate of $12.5\,\mu L\,s^{-1}$. Peaks detected were identified and quantified by comparing the retention time and peak area to that of known standards. Quantification was based on absorbance at 320 nm using a Waters 2487 dual wavelength UV absorbance detector.

2.6. Antioxidant activity

The antioxidant activity was measured according to the method developed by Brand-Williams et al. (1995), with slight modifications. DPPH (1,1-diphenyl-2-picrylhydazyl) was used

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