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Plants as biofactories: Stress-induced production of chlorogenic acid isomers in potato tubers as affected by wounding intensity and storage time

Ana Mariel Torres-Contreras[a], Vimal Nair[b], Luis Cisneros-Zevallos[b,**], Daniel A. Jacobo-Velázquez[a,*]

[a] Department of Biotechnology and Food Engineering, School of Biotechnology and Food, Centro de Biotecnología-FEMSA, Tecnológico de Monterrey-Campus Monterrey, E. Garza Sada 2501 Sur, C.P. 64849, Monterrey, N.L., Mexico
[b] Department of Horticultural Sciences, Texas A&M University, Vegetable & Fruit Improvement Center, College Station, TX 77843-2133, United States

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ABSTRACT

In the present study, the feasibility of using wounded-potato tubers as biofactories of antioxidant phenolic compounds (PC) was evaluated. Potato tubers were wounded to obtain different wounding intensities (slices, pie-cuts, and shreds) and stored for 144 h at 10 °C. The accumulation of total and individual PC was determined during storage of the wounded-tissue. Results indicated that the highest accumulation of total PC was obtained at 96 h of storage for slices and pie-cuts. At this storage time, slices and pie-cuts showed 100% and 65% higher total PC content, respectively, whereas shredded-potatoes showed 40% lower PC content as compared with wholes before storage. The main PCs identified in wounded-potatoes were chlorogenic acid (CGA), neo-chlorogenic acid (neo-CGA), and crypto-chlorogenic acid (crypto-CGA). Results also indicated that selecting the wounding intensity to apply and storage time could manipulate the accumulation of specific PC in potato tubers. For instance, if the production of CGA or crypto-CGA were desirable, potato tubers should be wounded to obtain slices and pie-cuts. Likewise, to produce neo-CGA the appropriate wounding intensity should be pie-cuts. Furthermore, the highest accumulation of CGA and neo-CGA in wounded-potatoes was obtained at 96 h of storage, whereas for the crypto-CGA its highest accumulation was observed in wounded-potatoes stored for 144 h at 10 °C. The stressed-potato tubers with increased concentrations of chlorogenic acids could be used as a starting material for the extraction of high value antioxidant PC with potential applications in the pharmaceutical and dietary supplements industries.

1. Introduction

Potato (*Solanum tuberosum* L.) is one of the most important crops around the world. In the year 2012, ~324 million tons were produced worldwide (FAOSTAT, 2012). However, in the US supply chain ~6% of potato production is lost due to poor postharvest practices (USDA, 2012). Alternative uses for potatoes considered as waste should be of interest for the potato industry. In the past few years, the use of stressed-plants as biofactories of phenolic compounds (PC) has been proposed as an alternative use for horticultural crops (Jacobo-Velázquez and Cisneros-Zevallos, 2012).

When potato tubers are subjected to wounding stress, chlorogenic acids (CGAs) are produced and converted into lignin and suberin during the wound-healing process (Gamborg, 1967; Dean and Kolattukudy, 1977; Friedman, 1997; Lulai and Corsini, 1998; Bernards et al., 2000; Bernards and Razem, 2001; Mattila and Hellström, 2007). The CGAs are PC with potential applications for the treatment and prevention of Alzheimer (Kim et al., 2005), obesity (Thom, 2007), hepatitis B (Wang et al., 2009), and cancer (Yagasaki et al., 2000; Aggarwal and Shishodia, 2006; Kurata et al., 2007; Noratto et al., 2009; Weng and Yen, 2012). Therefore, the application of wounding-stress in potato could be used as an emerging technology to induce the production of CGAs.

* Corresponding author at: Tecnológico de Monterrey-Campus Monterrey, E. Garza Sada 2501 Sur, Centro de Biotecnología-FEMSA, 303-A, C.P. 64849 Monterrey, N.L., Mexico. Tel.: +52 818 358 20 00x4820; fax: +52 818 328 4136.
** Corresponding author at: Department of Horticultural Sciences, Texas A&M University, HFSB Building, Room 202, MS 2133, College Station, TX 77843-2133, United States. Tel.: +1 979 845 3244; fax: +1 979 845 0627.
E-mail addresses: lcisnero@tamu.edu (L. Cisneros-Zevallos), djacobov@itesm.mx (D.A. Jacobo-Velázquez).

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The stressed-tissue can be subjected to downstream processing to recover and purify CGAs with potential applications in the pharmaceutical and dietary supplements industries.

However, depending on the wounding intensity applied in plant tissues, the biosynthesis and conversion rates of CGAs will change during storage time, and thus it is important to optimize the abiotic stress conditions that lead with the highest accumulation of CGAs. Therefore, the present project objective was to evaluate the effect of wounding intensity and storage time (144 h at 10 °C) on the accumulation of total and individual PC in potato tubers.

2. Materials and methods

2.1. Chemicals

Ferulic acid (FA), *p*-coumaric acid (*p*-CA), chlorogenic acid (CGA), methanol (HPLC grade), water (HPLC grade), orthophosphoric acid and formic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Plant material, processing and storage study

White potatoes (*S. tuberosum* L.) were obtained from a local market (HEB, Monterrey, N.L. Mexico), sorted, washed and disinfected with chlorinated water (200 ppm, pH 6.5). Three different wounding intensities were prepared (slices, pie-cuts and shreds) as shown in Fig. 1. Whole potatoes were used as control samples. To obtain potato slices, whole potatoes were cut (thickness ~3 mm) using a commercial serrated knife. To obtain pie-cuts, potato slices were cut in four pieces making two perpendicular cuts. Finally, to obtain the shredded-potatoes whole tubers were wounded with a commercial vegetable shredder (diameter of 0.7 cm). Whole and wounded-potatoes (~200 g) were placed in plastic containers with capacity of 5.7 L (Sterilite, Townsend, USA). Samples treated with different wounding intensities were stored in an incubator (VWR, Radnor, USA) at 10 °C for 144 h and samples were collected every 48 h during the storage period. The containers were opened every 12 h in order to avoid CO_2 accumulation (Reyes et al., 2007).

2.3. Sample preparation for phytochemical analyses

For the extraction of PC, potato tissue (5 g) was homogenized with methanol (20 mL) using a tissuemizer (Advanced homogenizing system, VWR, Radnor, PA, USA), and then centrifuged (10,000 × *g*, 15 min, 4 °C). The clear supernatant (further referred as methanol extract) was microfiltered using nylon membranes (0.45 μm, VWR, Radnor, PA, USA) prior to injection to the chromatographic system.

2.4. Identification and quantification of phenolic compounds (PC) by high-performance liquid chromatography-diode array detector (HPLC-DAD) and HPLC-electrospray ionization (ESI)-MS^n

Phenolic compounds were analyzed according to the method reported by Becerra-Moreno et al. (2012). Briefly, 10 μl of the methanol extract were injected in the HPLC system, which was composed of a quaternary pump, an autosampler, and a diode array detector (1260 Infinity, Agilent Technologies, USA). Compounds were separated on a 4.6 mm × 250 mm, 5 μm, C18 reverse phase column (Luna, Phenomenex, Torrance, CA, USA). The mobile phases consisted of water (phase A) and methanol:water (60:40, v:v, phase B) adjusted to pH 2.4 with orthophosphoric acid. The gradient solvent system was 0/100, 3/70, 8/50, 35/30, 40/20, 45/0, 50/0, and 60/100 (min/% phase A) at a constant flow rate of 0.8 mL/min. Chromatographic data was processed with OpenLAB CDS ChemStation (Agilent Technologies, Santa Clara, CA, USA).

The identification of individual PC was based on their DAD spectra and ESI-MS fragmentation patterns as compared with authentic standards and previous reported data (Clifford et al., 2003; Im et al., 2008; Jacobo-Velázquez et al., 2011; Ieri et al., 2011; Luthria, 2012). Mass spectra were obtained on a MS Finnigan LCQ Deca XP Max, Ion trap mass spectrometer coupled at the exit of the DAD and equipped with a *Z*-spray ESI source, and run by Xcalibur version 1.3 software (Thermofinnigan-Surveyor, San José, USA). Separations were conducted using the Phenomenex (Torrance, CA) Synergi 4 μ Hydro-RP 80A (2 mm–150 mm) with a C18 ward column, and a flow of 200 μL/min from the DAD eluent was directed to the ESI interface using a flow-splitter. Mobile phases consisted of water (phase A) and methanol:water (60:40, v:v, phase B) adjusted to pH 2.4 with formic acid. The gradient solvent system was 0/100, 3/70, 8/50, 35/30, 40/20, 45/0, 50/0, and 60/100 (min/% phase A). Nitrogen was used as desolvation gas, at 275 °C and a flow rate of 60 L/h, and no cone gas was used. ESI was performed in the negative ion mode using the following conditions: sheath gas (N_2), 50 arbitrary units; auxiliary gas (N_2), 0 arbitrary units; spray voltage, 1.5 kV; capillary temperature, 250 °C; capillary voltage, 21 V; and tube lens offset, 60 V.

To quantify the individual PC identified, standard curves of authentic standards (CGA, FA, and *p*-CA) at a range 5–250 ppm were obtained. The concentration of the phytochemicals was expressed as mg of each individual compound per kg of potatoes dry weight (DW). In the specific case of the CGA derivatives neochlorogenic acid (neo-CGA), cryptochlorogenic acid (crypto-CGA) and 3,5-dicaffeoylquinic acid (3,5-diCQA), results were expressed as chlorogenic acid (CGA) equivalents. Likewise, the sum of all individual PC identified was expressed as total PC. To calculate the concentration of PC in DW, the moisture content of the samples was determined by the air-oven method.

2.5. Statistical analysis

Statistical analyses were performed using 5 repetitions. Data represents the mean value of samples and their standard error. Analyses of variance (ANOVA) were conducted using JMP software version 11.0 (SAS Institute Inc. Cary, NC), and mean separation was performed using the LSD test ($p < 0.05$).

3. Results

3.1. Effect of wounding stress and storage time on the accumulation of total phenolic compounds (PC) of potato tissue

The total PC content of samples was determined before and during 144 h of storage (Fig. 2). The application of wounding stress to obtain slices and pie-cuts produced a significant increase ($p < 0.05$) on the total PC content of potato tubers during storage at 10 °C. However, when potato tubers were treated with the highest wounding intensity (shredded-potatoes), samples showed a significant decrease ($p < 0.05$) on the concentration of total PC. Likewise, no significant change ($p > 0.05$) on the total PC content was observed during storage of whole potatoes (Fig. 2). For slices and pie-cuts, the highest accumulation of PC was observed at 96 h of storage. At this storage time, slices and pie-cuts showed 100% and 65% higher total PC content, whereas shredded-potatoes showed 40% lower PC content as compared with wholes before storage.

3.2. Effect of wounding stress and storage time on the accumulation of individual phenolic compounds (PC)

The tentative identification and quantification of PC in potato tubers was performed by HPLC-DAD and HPLC-ESI-MS^- (Fig. 3 and Table 1). The individual PC identified in whole and wounded-potato