Food Chemistry 210 (2016) 473–480

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Potato wound-healing tissues: A rich source of natural antioxidant molecules with potential for food preservation

Keyvan Dastmalchi, Isabel Wang, Ruth E. Stark *

Department of Chemistry and Biochemistry, The City College of New York, City University of New York Graduate Center Ph.D. Programs in Biochemistry and Chemistry, and CUNY Institute for Macromolecular Assemblies, New York, NY 10031, USA

ARTICLE INFO

Article history:
Received 10 January 2016
Received in revised form 22 April 2016
Accepted 26 April 2016
Available online 27 April 2016

Keywords:
Solanum tuberosum
Potato
LC–MS
TOF-MS
Wound periderm
Antioxidants
$ABTS^{\cdot+}$ scavenging activity

ABSTRACT

The need for safe, effective preservatives is a prominent issue in the food and drug industries, reflecting demand for natural alternatives to synthetic chemicals viewed as harmful to consumers and the environment. Thus, this study determined the identities and scavenging capacities of antioxidant metabolites produced as a response to potato tuber wounding, using activity-guided fractionation of polar extracts from a Yukon Gold cultivar that had previously exhibited exceptionally high radical-scavenging activity. Activity-guided fractionation using the $ABTS^{\cdot+}$ radical scavenging assay and LC–MS with TOF-MS for compositional analysis of the most potent antioxidant fractions yielded identification of nine constituents: coumaroylputrescine; feruloylquinic acid; isoferuloylputrescine; ferulic acid; 22,25-dimethoxy-3-[[2,3,4-tri-*O*-methyl-6-O-(2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]oxy]-(3β)-lanost-9(11)-en-24-one; 4-(2*Z*)-2-decen-1-yl-5-[1-(4-hydroxyphenyl)decyl]-1,2-benzenediol; 8-[(2*E*)-3,7-dimethyl-2,6-octadien-1-yl]-5-hydroxy-2,8-dimethyl-6-(3-methyl-2-buten-1-yl)-2*H*-1-benzopyran-4,7(3*H*,8*H*)-dione; 3-[(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-20-[(6-*O*-β-D-xylopyranosyl-β-D-glucopyranosyl)oxy]-dammar-24-en-19-al; (3β)-28-oxo-28-(phenylmethoxy)oleanan-3-yl 2-*O*-β-D-galactopyranosyl-3-*O*-(phenylmethyl)-, butyl ester β-D-glucopyranosiduronic acid. A positive correlation was observed between the scavenging activities and the polarities of the active fractions. The antioxidant capacities of the fractions were also characterised by monitoring the activity throughout a 45-minute assay period.

1. Introduction

The development of safe, effective preservatives poses a significant challenge in the food and drug industries. The demand for sustainable, natural alternatives to synthetic preservatives, many of which are reported to have a negative impact on the environment as well as potentially carcinogenic effects on consumers, has been growing rapidly over the past decade (Nychas & Tassou, 1999). Plant-based antioxidants have potential applications as natural preservatives, but their sources and potencies remain to be optimised. In particular, the potato (*Solanum tuberosum*) has been found to be a rich source of antioxidants with high capacities for free radical scavenging (Madiwale, Reddivari, Holm, & Vanamala, 2011). In previous studies, the extracts from potato peels have also been reported to inhibit oxidative processes in food products (Kanatt, Chander, Radhakrishna, & Sharma, 2005).

As the world's fourth largest crop after wheat, rice, and corn, the potato also ranks third in global consumption, demonstrating its importance as a staple in the human diet as well as a mainstay of the global economy (Al-Weshay & Rao, 2012; Madiwale et al., 2011; Thompson et al., 2009). Wounding and mechanical damage of the potato tuber together with suboptimal healing conditions result in desiccation and allow for bacterial or fungal infection. These problems render as many as 40–50% of harvested potatoes unsuitable for human consumption and generate substantial waste by-products (Schieber & Saldana, 2008). However, under proper conditions potatoes form a layer called the wound periderm to guard against pathogen invasion and dehydration, progressing through different stages of healing at successive time points: three days after wounding, the suberised closing layer is fully formed; by the seventh, development of the wound periderm is initiated (Dastmalchi et al., 2014; Neubauer, Lulai, Thompson, Suttle, & Bolton, 2012). Moreover, in conjunction with periderm formation, wounded potato tissues generate reactive oxygen species (ROS) such as superoxide, peroxide, and hydroxyl radicals, containing infection by triggering cell death and thus damaging the tuber tissue (Reyes, Villarrel, & Cisneros-Zevallos, 2007).

It has been proposed that tubers produce antioxidant compounds to scavenge for ROS in response to such stress (Reyes

* Corresponding author.
E-mail address: rstark@ccny.cuny.edu (R.E. Stark).

http://dx.doi.org/10.1016/j.foodchem.2016.04.123
0308-8146/

et al., 2007), displaying antioxidant activity (AOA) levels comparable to high-antioxidant herbs such as ginkgo and ginger (Kähkönen et al., 1999). The reported phytochemicals are of significant nutritional and pharmacological interest: they can interfere with oxidative processes by disrupting chain-breaking reactions and scavenging for free radicals; and they are associated with antibacterial, antiallergic, and anticarcinogenic properties (Friedman, 1997; Madiwale, Reddivari, Holm, & Vanamala, 2012; Madiwale et al., 2011; Thompson et al., 2009). Moreover, the periderm from potato tubers represents a major industrial by-product that offers a potentially rich source of natural antioxidants. To realistically assess their efficacy as preservatives, the antioxidants must be isolated and identified so that their activities in different food systems can be determined. Published reports of free radical scavenging activities typically utilise assays that are subject to shortcomings such as limited pH range, polarity, steric hindrance, and spectral interference (Magalhaes, Segundo, Reis, & Lima, 2008). Therefore, we sought a versatile assay that could overcome these limitations. The resulting antioxidant activities then formed the basis for activity-guided fractionation and identification of the most potent extracts from the wound-healing potato tuber periderm tissues.

The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) ammonium salt (ABTS) decolorisation assay measures the ability of antioxidants to scavenge free radicals in potato wound periderm by means of a single electron transfer reaction. Its advantages stem from the solubility of $ABTS^{\cdot+}$ in both hydrophilic and lipophilic systems and its ability to monitor AOA over a wide pH range as well as over time. The ABTS assay also offers improvements compared with the traditional 2,2-diphenyl-1-picrylhidrazyl (DPPH) assay reported previously (Reyes & Cisneros-Zevallos, 2003; Reyes et al., 2007), which can suffer from steric hindrance or spectral interference from other phytochemicals (Re et al., 1999).

A reverse-phase high-performance liquid chromatography (RP-HPLC) strategy using a non-linear gradient system was then employed for separation of phytochemical constituents present in the antioxidant-rich fractions of the wound tissue extract. Finally, liquid chromatography–mass spectrometry (LC–MS) and time-of-flight mass spectrometry (TOF-MS) made it possible to identify the chemical constituents present in the most active fractions. Using the techniques outlined above, this study aimed to isolate and identify the most important antioxidants produced during the wounding response of potato tubers by determining the AOA in Day-7 Yukon Gold polar extracts and the metabolites responsible for scavenging activity. This study builds upon the findings of Dastmalchi et al. (2014), who found polar wound periderm extracts of the Yukon Gold cultivar at Day 7 to have the highest activity among four tubers with distinct russeting patterns, reflecting the strong antioxidant ability of a number of secondary metabolites (polyphenolic amines, flavonoid glycosides, and phenolic acids) involved in potato tissue wound healing.

Specifically, we subjected the polar fractions of the Day-7 Yukon Gold extract obtained through RP-HPLC to the ABTS assay, in order to evaluate scavenging activity over a time period of 45 min, permitting detection of slow- as well as fast-acting antioxidants. To isolate the constituents present in the fractions obtained by HPLC, we used reverse-phase liquid chromatography–mass spectrometry (LC–MS), since the extract being fractionated is polar and its constituents are non-volatile. Hence, through the structural elucidation of highly active fractions and the characterisation of their bioactivities, we hoped to identify promising new sources of natural preservatives with dietary value. We also obtained new information about the phytochemical constituents produced during the potato tuber wound-healing process, uncovering several compounds that had not been identified previously in their native or wound periderms (Dastmalchi et al., 2014, 2015; Narvaez-Cuenca, Vincken, Zheng, & Gruppen, 2012; Yang & Bernards, 2007).

2. Materials and methods

2.1. Chemicals and reagents

HPLC–MS grade acetonitrile, water, methanol (J. T. Baker, Phillipsburg, NJ), and formic acid (Sigma-Aldrich, St. Louis, MO) were used in HPLC, LC–MS, and TOF-MS analyses. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulphonic acid ammonium salt) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (TCI, Tokyo, Japan) and potassium peroxosulfate (Sigma-Aldrich, St. Louis, MO) were used for the antioxidant assay.

2.2. Plant material

Potato tuber cultivars of the Yukon Gold variety, 2011 crop year, were provided by Joe Nuñez, University of California Cooperative Extension (Davis, CA).

2.3. Sample preparation

Procedures for this work followed the methods described by Dastmalchi et al. (2014, 2015). Briefly, Yukon Gold potato tubers were peeled and sectioned longitudinally under sterile conditions using a mandolin slicer to obtain disks ~5 mm in thickness. Disks were placed on wet cellulose filter paper and left for 7 days at 25 °C on wire netting supports within closed plastic boxes in a dark enclosure. Humidity was maintained by adding water to the bottom of the boxes. The newly formed brown surface layer of easily detached wound tissue was collected using a flat spatula. Harvested wound tissues were frozen immediately in liquid nitrogen and stored at −80 °C for further processing.

The wound tissues were extracted using a modification of the protocol employed byChoi et al. (2004). A 10-mg portion of the freeze-dried material was extracted with 2 mL of 60% (*v*/*v*) methanol-water by ultrasonication (Branson Ultrasonics, Danbury, CT) for 1 min, followed by addition of 2 mL chloroform and sonication again for 1 min. Each extract was then incubated at room temperature in a shaker for 10 min, followed by tabletop centrifugation (Beckman Coulter, Fullerton, CA) at 3000 rpm to produce three separate phases: soluble polar, soluble nonpolar, and an interphase of suspended particulates. The upper soluble polar extracts were removed carefully with a glass Pasteur pipette and dried under a flow of nitrogen gas.

2.4. Fractionation of the extracts

LC separation was performed using a 150 × 4.6 mm, 3.0 μm AscentisR C18 column (Supelco, Bellefonte, PA) operated by an Agilent 1200 Series HPLC liquid chromatograph equipped with a G1311A quaternary pump, G1322A degasser, G1316A temperature controller, and G1315B diode array detector coupled to a G1364C analytical fractionator (Agilent, Santa Clara, CA). Each analysis was performed by injecting a 30-μL sample into the column and eluting with a flow rate of 0.4 mL/min. The mobile phase was composed of 0.1% aqueous formic acid (**A**) and 0.1% formic acid in acetonitrile (**B**). The following program of nonlinear gradient elution was used: 2% **B** (0–5 min), 10% **B** (5–8 min), 15% **B** (8–25 min), 100% **B** (25–38 min), and 2% **B** (38–50 min). Fractions were collected in time-based mode at 30-s intervals between 14 and 41 min during a 50-min chromatographic run that was repeated twenty times to accumulate enough sample for concentration and analysis by LC–MS and TOF-MS.