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Original research article

Identification and quantification of phenolic compounds responsible for the antioxidant activity of sweet potatoes with different flesh colours using high performance thin layer chromatography (HPTLC)

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ABSTRACT

The objectives of the present study were to develop a simple high performance thin layer chromatography (HPTLC)-based protocol: (i) to allow high-throughput profiling of phenolic compounds of microwaved roots from 295 sweet potato varieties and breeding lines, (ii) to quantify the content of anthocyanins and caffeoylquinic acid (CQA) derivatives, and (iii) to determine their respective contributions to the antioxidant activity of sweet potato methanolic extracts using the DPPH test. Analysed accessions were separated into three groups: white-fleshed ($n = 100$), orange-fleshed ($n = 64$) and purple-fleshed ($n = 131$). Purple-fleshed accessions presented the highest mean CQA content. After DPPH treatment and transmittance scanning of the plate at 517 nm, the most active free radical scavengers were found to be the four CQAs (CGA, 3,4-, 4,5- and 3,5-diCQA) while the anthocyanins were found to be less active. The total antioxidant capacity of the sweet potato methanolic extracts was mostly linked to total CQAs content. This method can now be used for fast routine analysis and selection of sweet potato breeding clones.

1. Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam.) phenolic compounds of purple-fleshed varieties have attracted great attention. The intention has been to add value to the crop by finding new uses such as natural food colorants with antioxidant activity (Zhao et al., 2013). Anthocyanins extracted from these varieties have been shown to be interesting for the prevention of lifestyle-related diseases (Harada et al., 2004). They have been classified into two groups, cyanidin or peonidin types (Yoshinaga et al., 1999) and shown to be selectively absorbed after ingestion (Suda et al., 2002). These anthocyanins are stable after heat treatment (Xu et al., 2015) and their antioxidant effects have been shown to be variety-dependent (Kano et al., 2005). The total anthocyanin content of different varieties has been used as an indicator of the antioxidant activity of their extracts (Kubow et al., 2016). It was found to be highest for purple-fleshed and lowest for white-fleshed varieties (Teow et al., 2007) but was also related to the cyanidin- and peonidin-based chemical composition (Hu et al., 2016). The protocols for anthocyanin extraction and HPLC analysis have been optimised (de Aguiar Cipriano et al., 2015) to support improvement programmes of purple-fleshed sweet potatoes (Truong et al., 2012).

However, anthocyanins alone could not account for all the antioxidant activity of root extracts which has been shown to result from both anthocyanins and hydroxycinnamic acids, additively (Philpott et al., 2003). Several phenolic acids have been isolated including chlorogenic acid (CGA) and dicaffeoylquinic acids (diCQAs) (Dini et al., 2006). The highest total phenolic acid content and antioxidant activity were observed in purple-fleshed varieties but the highest CGA content was found in a white-fleshed variety (Padda and Picha, 2008). Unfortunately, these studies were conducted on raw freeze-dried tissues and not with cooked sweet potatoes. CGA was found to be higher in the roots while 3,5-diCQA and 4,5-diCQA were predominant in the leaves (Islam et al., 2002). In the roots, CGA, 3,4-, 4,5- and 3,5-diCQA content was highest in the cortex and lowest in the periderm (Harrison et al., 2008). Bioassays revealed that these compounds contributed to the allelopathic potential and resistance to pathogens of sweet potatoes (Peterson et al., 2005).

The effects of various cooking techniques have also been investigated. Microwaving did not reduce the antioxidant capacity

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of cooked sweet potatoes compared to raw roots (Bellail et al., 2012) and no differences in antioxidant activity were detected between purées made from whole or peeled roots (Truong et al., 2007). In cooked sweet potatoes, CGA and 3,5-diCQA were found to be the most abundant (Zhao et al., 2014). Evaluation at harvest indicated that curing did not significantly affect total phenolic acid content. However, in a purple-fleshed variety, anthocyanin degradation occurred during storage and so antioxidant activity also gradually declined. Freshly harvested sweet potatoes were therefore shown to present a higher anti-inflammatory capacity (Grace et al., 2014).

In a nutshell, variation in individual and total phenolic compounds, and their correlation with the antioxidant activity of extracts have shown great differences between plant parts, curing and storage processes, but the most significant effects were variety-dependent. However, the respective contributions of anthocyanins and phenolic acids to the overall antioxidant activity of varieties with different flesh colours still deserve further research. Most studies have been conducted with raw root samples and it is currently unknown which of CGA, diCQAs or anthocyanins contributes most to the antioxidant activity of cooked sweet potatoes. Consequently, it is not clear if the antioxidant activity of cooked sweet potatoes is significant only in purple-fleshed varieties or if white- and orange-fleshed varieties can also present comparable activities. As most analytical methods are time consuming, a limited number of varieties have previously been analysed, and the focus was mostly on purple-fleshed varieties, hampering progress on this matter. It would, therefore, be useful to develop simple protocols allowing direct evaluation of the antioxidant capacities of phenolic compounds in cooked sweet potato prepared through microwaving to allow faster cooking. Thin layer densitometry has been shown to be a reliable and efficient tool for the quantitative evaluation of the free radical scavenging activity of antioxidants by measuring the inhibition of 2,2-diphenyl-1-picrylhydrazyl, DPPH (Abourashed, 2005). Bleaching of the oxidised DPPH purple colour caused by antioxidants on a thin layer chromatography (TLC) plate can be measured with computerised image processing (Olech et al., 2012) to allow the simultaneous quantitative evaluation of the antioxidant activity of different samples (Hosu et al., 2015).

The aim of the present research was to analyse a broad sample of genotypes (n = 295) to clarify the relationships between sweet potato flesh colours and their anthocyanins and diCQAs antioxidant activities in cooked sweet potatoes. The objectives of our study were to develop a simple protocol: (i) to allow high-throughput profiling of cooked sweet potato varieties and breeding lines using high-performance thin layer chromatography (HPTLC), (ii) to quantify the content of anthocyanins and diCQA, and iii) to determine their respective contributions to the antioxidant activity of sweet potato methanolic extracts using the DPPH test. Potential applications for selecting sweet potatoes with high antioxidant content are discussed.

2. Materials and methods

2.1. Plant materials

All accessions (295 varieties and breeding lines) analysed were maintained and evaluated by the Vanuatu sweet potato breeding programme, jointly conducted with the Vanuatu Agricultural Research and Technical Centre (VARTC) located on Santo Island, northern Vanuatu, the Department of Agricultural and Rural Development (DARD) and the NGO Farmers Support Association (FSA) located on Efate Island, central Vanuatu. All sweet potatoes were harvested when plants were four months old. For each accession, one root was selected for its good appearance, with no visible insect damage. In addition, four commercial varieties were studied (*Baby*,*Salili*, *Bankis* and *Teouma*); for each, 20–40 roots were purchased from the Port-Vila market and analysed to assess intra-variety variation. All accessions were grouped according to their flesh colour: 1—white (100 accessions), 2—yellow and/or orange (64 acc.) and 3—a combination of any colour with purple (131 acc.). Overall, 295 different accessions were analysed between August and November 2015.

After harvest, the fresh roots were hand-washed under cool running water, peeled, and then a slice of 20 g was cut from the central part of the root. This slice was wrapped in a full-sized paper towel and damped with cold water. The excess water was gently squeezed out and the 20 g portion was put in a 100 mL beaker. Eight samples at a time were placed in a circle on a glass turntable and cooked at 80% heat in a microwave oven (Sharp model R-42 B ST) for 4 min. This procedure was standardised for taking a constant preparation time of less than 1 h. Microwaved samples were then unwrapped and 20 mL of methanol was poured into the beaker. The samples were mashed gently with a fork for 5 min until the cooked sweet potato was transformed into a fine and homogenous purée. The whole purée was then transferred into a 50 mL plastic centrifuge tube and the eight samples were centrifuged together at 4500 rpm for 10 min. The supernatant was then transferred to 5 mL vials and stored in a fridge at 4 °C in the dark until analysis.

2.2. Standards and reagents

CGA, 3,4-, 4,5- and 3,5-diCQA HPLC grade standards were purchased from Sigma-Aldrich (St Quentin, France). Standard stock solutions were prepared by dissolving the appropriate amount of each compound in methanol (1.0 mg/mL). Stock solutions were stored at 4 °C in the dark and were stable for several weeks. All solvents were of analytical grade. Ethyl acetate, methanol, acetic acid, formic acid and DPPH were also purchased from Sigma-Aldrich (St Quentin, France).

2.3. High-performance liquid chromatography (HPLC)

Five accessions (VU12, 49, 68, 99, 138) selected for their contrasting flesh colours were analysed by reversed-phase liquid chromatography (RP-HPLC) using an Agilent 1290 Infinity series HPLC (Agilent, Santa Clara, CA, USA) equipped with a gradient pump, a cooled autosampler and a UV–vis photodiode-array detector (210, 254, 280, 300, 320, 366, 420 and 520 nm with full scan from 190 to 600 nm). Samples were injected through a column (5 μm, 250 × 4.6 mm, Kromasil™ C18, AkzoNobel, Separation Products, Bohus, Sweden) coupled to a guard column (10 × 4.6 mm, Kromasil C18) at 30 °C. The mobile phase was aqueous acetic acid (0.4% v/v, eluent A) and acetonitrile (eluent B) at 1 mL/min. After photometric analysis, chromatographed material was subjected to mass analysis by ESI-QTOF (Agilent 6530) in positive and negative modes. The ESI source was optimised as follows: scan spectra from *m*/*z* 100–2000, capillary voltage 3.5 kV, fragmentor at 120 V, fixed collision-induced dissociation (CID) energy at 20 eV. Nitrogen was used as nebulising gas with a flow rate of 11 L/min and a temperature of 310 °C at 40 psi. Compounds were identified by analysis of their UV, HRMS and MS/MS characteristics using MassHunter Quantitative Analysis software (Agilent Technologies, USA).

2.4. High-performance thin layer chromatography (HPTLC)

Analyses were performed on Merck (Darmstardt, Germany) silica gel 60 F_{254} plates (glass Plates 20 × 10 cm, reference 1.05642.0001), using a Camag (Muttenz, Switzerland) HPTLC system equipped with an automatic TLC sampler (ATS4),