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A comparative metabolomics study of flavonoids in sweet potato with different flesh colors (*Ipomoea batatas* (L.) Lam)

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ARTICLE INFO

Chemical compounds studied in this article:
Caffeic Acid (PubChem CID:689043)
Cinnamic Acid (PubChem CID: 444539)
Quercetin(PubChem CID: 5280343)
Kaempferol (PubChem CID: 5280863)
Chrysoeriol (PubChem CID: 5280666)
Quinic acid (PubChem CID: 6508)
ρ-Coumaric acid (PubChem CID: 637542)
Ferulic acid (PubChem CID: 445858)
O-feruloylquinic acid (PubChem CID:9799386)
Cyanidin (PubChem CID: 128861)

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ABSTRACT

To study the diversity and cultivar-specific of phytochemicals in sweet potato, Liquid Chromatography-Electrospray Ionization-Mass Spectrometry was used to analyze the metabolic profiles of five sweet potato cultivars exhibiting different flesh colors: purple, yellow/orange, and white. A total of 213 metabolites, including 29 flavonoids and 27 phenolic acids, were characterized. The flavonoid profiles of the five different cultivars were distinguished using PCA, the results suggested the flesh color accounted for the observed metabolic differences. In addition to anthocyanins, quinic acids and ferulic acids were the prominent phenolic acids, O-hexoside of quercetin, chrysoeriol were the prominent flavonoids in sweet potato tubers, and they were all higher in the OFSP and PFSP than WFSP. The main differential metabolic pathways between the OFSP, PFSP and the WFSP included those relating to phenylpropanoid and flavonoid biosynthesis. This study provides new insights into the differences in metabolite profiles among sweet potatoes with different flesh colors.

1. Introduction

Polyphenolic compounds in edible plants have gained attention due to their antioxidant activity and other health-beneficial properties. Sweet potato (*Ipomoea batatas* (L.) Lam.), the world's seventh most important crop, is not only a rich source of energy, but also contains many useful by-products (Mussoline & Wilkie, 2017). Its tuber is the main organ harvested that functions primarily in nutrient storage and reproduction. Many sweet potato cultivars exist globally, and exhibit differences in size, skin color (e.g., white, cream, yellow, orange, pink, and red) (Rose & Vasanthakaalam, 2011), and flesh color (e.g., white, cream, orange, yellow, and purple). Sweet potato is a rich source of carbohydrates, dietary fiber, β-carotene, minerals, and other nutrients, the quantities of which are cultivar-dependent (Endrias Dako, 2016). Consumer acceptance mainly concerns the external appearance of the tubers as well as taste, both of which are primarily influenced by their biochemical composition. The nutritional composition of many varieties has been investigated, especially the white-fleshed sweet potato (WFSP) and yellow- or orange-fleshed sweet potato (OFSP) varieties. The amount of moisture, ash, protein, crude fiber, total reducing sugars, and β-carotene differed among two yellow and two white varieties cultivated in Rwanda (Rose & Vasanthakaalam, 2011). Changes in carbohydrates including individual sugars (sucrose, fructose, and glucose), total sugars, and crude starch during tuber development in WFSP and OFSP cultivars have been reported (Bonte, Picha, & Johnson, 2000). Park et al. (Park et al., 2016) detected 41 polar primary metabolites including 7 sugar alcohols, 17 amino acids, 1 amine, and 13 organic acids in the three different sweet potato cultivars exhibiting differences in flesh color.

Secondary metabolites (such as terpenes, polyphenolics, and alkaloids) from various plant sources are receiving increasing attention due to their remarkable physiological functions. Researches in many areas

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indicate that sweet potato contains high levels of carotenoids, which may have several beneficial functions in the human diet. The β-carotene content of 19 sweet potato genotypes exhibiting distinct flesh colors (white, cream, yellow, orange, and purple), ranged from 0.2 to 226 μg β-carotene/g FW (Teow, et al., 2007). Furthermore, carotenoid content and composition were also analyzed in the leaves and tubers of sweet potato cv. WS (WFSP) and W71 (OFSP) (Khan, Takemura, Maoka, Otani, & Misawa, 2016). The leaf carotenoid composition was approximately 45–50% lutein and 30% β-carotene, constituting the two predominant carotenoids, which was the same as the leaves of other plants. However, the tubers of WFSP and OFSP were found to contain some unique carotenoids, including β-carotene-5,8-epoxide (9.4% and 4.6% of the total carotenoids, respectively), β-carotene-5,6,5′,8′-diepoxide (35.7%, 9.2%), and β-carotene-5,8,5′,8′-diepoxide (25.1%, 13.8%).

Polyphenolic substances mainly include phenolic acids, flavonoids, stilbenes and lignans (Hua, Wang, Yong, Li, & Hua, 2009). Many reports have been published on polyphenolic compounds because of their high antioxidant activity. The content of phenolic compounds varies considerably among sweet potato genotypes, and the amount and composition were found to differ dramatically in the periderm, cortex, and the stele tissue of the tuber (Harrison et al., 2008).

Phenolic acids usually refer to hydroxybenzoic acids (HBAs) and hydroxycinnamic acids (HCAs) (Robbins, 2003). Chlorogenic acid was identified as the major constituents and were the dominant DPPH radical-scavengers in sweet potato (Oki et al., 2002). Phenolic content ranged from 192.7 to 1159.0 mg gallic acid equivalent (GAE)/100 g dry sample among five Philippine sweet potato varieties and significant negative correlation was observed between total phenolic content and the EC50 for DPPH radical scavenging activity, which indicted that phenolic compounds were the primary contributors to their radical scavenging activity (Rumbaoa, Cornago, & Geronimo, 2009). Caffeoylquinic acids (CoAs), the prominent phenolic acids presented in leaf, were identified and quantified for the first time by NMR, FABMS, and RPHPLC analysis in sweet potato leaves (Chunying et al., 2013) .

Flavonoids, including anthocyanins, flavanes, flavones, flavanones, flavonols, and chalcones, constitute another important group of plant secondary metabolites. They are found in numerous colored fruits and flowers (Gould, 2012) and play an important role in multiple plant functions, for instance pigmentation, protection against damage by dormancy, and fertility, ultraviolet light and phytopathogens, and protection against biotic and abiotic stresses (Jia, 2012). Previous reports have revealed that they also offer protection against chronic diseases including cardiovascular disease, certain types of cancer (Sehitoglu, Farooqi, Qureshi, Butt, & Aras, 2014), and inflammatory diseases (Lee et al., 2015) due to their antioxidant activities (Harborne & Williams, 2000). In recent years, anthocyanins, which are water-soluble pigments belonging to an important subgroup of flavonoids, have attracted attention due to their notable antioxidative (Wang, et al., 2013).

Nevertheless, a little information is available on phenolic acids and non-anthocyanin flavonoids composition in sweet potato tubers. In addition, there is rare study of large-scale detection, identification and quantification on flavonoids in sweet potato.

Qualitative and quantitative variations in the phytochemical profiles and nutrients, and especially the bioactive compounds of sweet potato could contribute to differences in their health-promoting properties. However, researchers have tended to focus on specific compounds, rather than thoroughly assessing all of the phytochemical constituents present in sweet potato. Liquid Chromatography-Tandem Mass Spectrometry (LC–MS/MS)-based non-targets metabolomics analysis is a rapid and highly sensitive method for detecting as many plant metabolites as possible by taking all information present in database into account (Chen et al., 2013), and was used in this study to identify and quantify metabolites, particularly phenolic acid esters and flavonoids, from five sweet potato cultivars exhibiting white, yellow/orange, and purple flesh. Multivariate analyses were used to determine the phenotypic differences and relationships between the metabolite contents. Our work aims to contribute toward an elucidation of the metabolic differences among sweet potato cultivars with different colored flesh and provides useful data for evaluating the nutritive value to inform future breeding strategies.

2. Materials and methods

2.1. Plant materials

Five sweet potato cultivars were cultivated in a randomized field plot according to standard agricultural practices in a field at the Xuzhou experimental station (E 117°17.48′, N 34°16.95′) of the Sweetpotato Research Institute of the China Agriculture Academy of Science in 2015. One white-fleshed sweet potato cv. Xushu 28 (“Xu28”), two yellow-fleshed sweet potatoes cv. Xushu 32 and Xushu 34 (“Xu32” and “Xu34”), and two purple-fleshed sweet potatoes cv. JiZishu 1 and XuZishu 3 (“JiZ1” and “XuZ3”) were cultivated. They were planted in March and transplanted in May. The developing tubers (90 d after transplantation) and the mature tubers (120 d after transplantation) exhibiting a cross-sectional diameter of 5–6 cm were harvested and washed with tap water and stored at −80 °C until further analysis. The developing tubers were used for quantitative real-time (qRT)-PCR analysis, whereas mature tubers were used in the assessment of physiological and metabolic characteristics.

2.2. Analysis of physiological parameters

Total anthocyanin contents (TAC) were extracted from the five cultivars and quantified as previously described (White, Howard, & Prior, 2011). Briefly, samples was extracted twice with 5 mL ultrapure water (containing 5% formic acid). After centrifuging 4,000 rpm for 10 min, the combined and filtered suspensions were measured at OD_{530} (DU730UV VIS, Beckman Coulter, USA). The content was calculated according the formula: TAC = 0.1 * OD_{530} * DR mg/g FW, where DR is the dilution rates and FW is the fresh weight of the tissue in grams. Cyanidin 3-O-sophoroside was also measured at OD_{530} as an equivalents.

Quantification of total proanthocyanidin content (PC) was performed using the vanillin-HCl method as reported previously (Huang et al., 2012). The total phenolic content (TPC) was determined according to the Folin-Ciocalteu method, using gallic acid as a reference standard (Bursal & Gulcin, 2011).

2.3. Expression analysis of key genes involved in flavonoid metabolism pathways

The expression of genes encoding key phenylpropanoid (PP) pathway enzymes, including phenylalanine ammonia lyase (*IbPAL*), cinnamate 4-hydroxylase (*IbC4H*), chalcone synthase (*IbCHS*), chalcone flavanone isomerase (*IbCHI*), flavanone 3-hydroxylase (*IbF3H*), flavonoid-3′-O-hydroxylase (*IbF3′H*), dihydroflavonol 4-reductase (*IbDFR*), anthocyanidin synthase (*IbANS*), UDP-glucose flavonoid 3-O-glucosyl transferase (*IbUGT*), anthocyanidin reductase (*IbANR*), and one regulatory gene MYB transcription factor 1 (*IbMYB1*) were analyzed by qRT-PCR. Total RNA was extracted from the tubers of the five sweet potato cultivars using TRIzol Reagent (Invitrogen, Waltham, MA, USA) and treated with DNase I (Promega, Madison, WI, USA) according to the manufacturer’s instructions. First-strand cDNA synthesis was performed using a SYBR PrimeScript RT-PCR kit (Perfect Real Time; Takara, China). Three biological replicates per sample were performed during the qRT-PCR reactions according to the SYBR Green I Master Mix protocol (Takara) on a BIO-RAD CFX Connect™ System (Bio-Rad, Hercules, CA, USA). The *IbActin* gene was used as an internal reference standard. For each gene analysis, an NRT (no reverse transcription