



Changes in anthocyanidin levels during the maturation of color-fleshed potato (*Solanum tuberosum* L.) tubers



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ABSTRACT

Certain potato cultivars are capable of producing anthocyanin pigments in the potato skin and flesh and those pigments have been shown, together with other phytochemicals, to promote good health. Six common anthocyanidins (cyanidin, delphinidin, petunidin, pelargonidin, malvidin and peonidin) were analyzed weekly for 15 weeks in red- and purple-fleshed potato cultivars (Red Emma, Königsapur, Valfi and Blaue de la Mancha) grown in field conditions using a validated LC-(+ESI)MS/MS method. Pelargonidin was the major type detected in red-fleshed cultivars whereas petunidin was the major type detected in the purple ones. Neither cyanidin nor delphinidin were found in any of the cultivars. The anthocyanidin levels observed were as high as 78 mg/100 g FW during tuber growth; however, fully matured tubers contained only 10–39 mg anthocyanidins/100 g FW. Anthocyanidin levels were moderately correlated with global solar irradiation ($r < 0.6252$) but not with rainfall or daily temperature.

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1. Introduction

For a long time there has been a trend of increased interest in food health benefits as well as in raising the availability of the nutrients supplied by highly consumed crops (Ayvaz et al., 2016). The nightshade family (*Solanaceae*) includes several important agricultural crops like potato, tomato, bell pepper, eggplant, tobacco, and other predominantly horticultural plants. Potatoes (*Solanum tuberosum* L.) are a staple for a significant part of the world's population, providing carbohydrates as a source of energy. They are consumed in myriad ways such as boiled, steamed, fried, baked, roasted, mashed or converted into ethanol or potato starch, which are further utilized by the food and beverage industry.

According to FAOSTAT 382 million tons of potatoes were produced worldwide on 19 million ha of land in 2014.

Besides their very important role in human nutrition, potato tubers contain modest amounts of phytochemicals (secondary plant metabolites) such as glycoalkaloids, carotenoids, phenylpropanoids and polyamines. Because of the high consumption of potatoes, even those modest levels are significant sources of phytochemicals in the human diet. These compounds play an important role in the plant itself (enabling it to cope with abiotic and biotic stress) and have been shown to promote health in those who consume them by affecting physiological functions and thus increasing the nutritional value of potatoes beyond being a mere source of energy (Andre et al., 2009; Navarre, Payyavula, Shakya, Knowles, & Pillai, 2013). Phytochemicals (most of which are effective antioxidants) in colored potatoes act as free radical scavengers, have been shown to reduce breast cancer incidence in rats, and have anti-proliferative, antifungal and antimicrobial activities *in vitro* (Bontempo et al., 2013; Thompson et al., 2009). The range of disorders anthocyanins (mostly derived from berries) have been

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effective in combating is much wider and comprises: obesity, cardiovascular diseases, diabetes and neurodegenerative disorders (Espin, Garcia-Conesa, & Tomas-Barberan, 2007).

Purple- and red-fleshed potato cultivars are a novelty that is attractive to consumers. Besides their exotic pigmentation, colored genotypes show three to four times higher contents of phenolic compounds when compared to the white cultivars. Anthocyanins, apparent even to the naked eye, are the most obvious group of phenolic compounds present in the color-fleshed potato tubers, next to chlorogenic acid. Therefore a high anthocyanin potato has potential as a new cultivar with enhanced health benefits (Tierno et al., 2016).

Color-fleshed potatoes have equal anthocyanin amounts to those found in red cabbage, red onion or strawberry (Brown, Culley, Yang, Durst, & Wrolstad, 2005). According to Lewis, Walker, Lancaster, and Sutton (1998), red-fleshed potatoes contain pelargonidin and peonidin, while purple potatoes contain petunidin and malvidin. Tierno et al. (2016) found the highest anthocyanin content in cv. Highland Burgundy Red, Purple Peruvian, Rogue de Flandres, Violet Queen and Vitelotte. Generally, potato peel contains significantly more phenylpropanoids compared to the flesh. The values for anthocyanins in potato reported by scientists can be as high as 7 mg/g FW (Ezekiel, Singh, Sharma, & Kaur, 2013). However, there is extensive variance in phenylpropanoid content and profile among potato germplasm (flesh) reflecting the considerable genetic diversity of potatoes and the capacity of tubers to synthesize these compounds (Navarre et al., 2013). It is worth mentioning that anthocyanins can be produced anywhere in the potato plant (Lu & Yang, 2006) and that they occur in an array of glycosylated and acylated forms in the plant as in the case of petunidin 3-O-[6-O-(4-O-*E*-caffeoyl-O- α -rhamnopyranosyl)- β -glucopyranoside]-5-O- β -glucopyranoside (Fossen, Ovstedal, Sliemstad, & Andersen, 2003).

Phenylpropanoid compounds are produced in plants through the phenylpropanoid pathway by transforming phenylalanine (by phenylalanine ammonia lyase, PAL) into a large family of compounds. In particular, anthocyanidins are formed by utilizing activated *p*-coumaroyl-CoA and converting it into dihydrokaempferol, dihydroquercetin and dihydromyricetin, which are precursors for further anthocyanidin formation using anthocyanidin synthase, which is followed by glycosylation carried out by a transferase to form anthocyanins (Navarre et al., 2013; Payyavula, Navarre, Kuhl, Pantoja, & Pillai, 2012; Payyavula, Singh, & Navarre, 2013). Different aglycones and sugar moieties determine anthocyanin bioavailability and potential health benefits (Tierno et al., 2016) yet the complex glycosylation patterns make the identification of individual anthocyanins difficult even when aided with LC-MS (Li et al., 2012).

In potato, gene expression seems to be coordinated and reflected at the metabolite level, supporting the hypothesis that regulation of gene expression plays an essential role in phenylpropanoid production. Red- and purple-fleshed genotypes were observed to have a much higher expression of PAL compared to white-fleshed potatoes (Andre et al., 2009). Expression of the phenylpropanoid pathway genes is controlled by a complex of (R2R3) MYB, basic helix-loop-helix transcription factors and WD-repeat proteins (Payyavula, Singh et al., 2013). Most natural variation in potato tuber color is a result of tissue-specific genes localized on various chromosomes. The potato R locus codes for dihydroflavonol 4-reductase, which produces red anthocyanins (pelargonidin derivatives), whereas P locus produces purple ones (petunidins) by coding for flavonoid 3',5'-hydroxylase, and I (D) locus produces both types of anthocyanins in tuber skin (De Jong, Eannetta, De Jong, & Bodis, 2004; Zhang et al., 2009). Overexpression of the gene encoding dihydroflavonol 4-reductase can result

in an increase in tuber anthocyanins, a four-fold increase in petunidin and a three-fold increase in pelargonidin derivatives (Stobiecki, Matysiak-Kata, Franski, Skala, & Szopa, 2003).

Anthocyanins are induced by light, temperature and water stress. Anthocyanins are proposed to be light attenuators induced in high-light conditions and their biosynthesis is increased by colder temperatures and repressed by higher temperatures via MYB transcription factors (Navarre et al., 2013; Payyavula et al., 2012). Tuber-specific overexpression of the MYB transcription factor leads to elevated amounts of phenylpropanoids. It has been further shown that tuber sucrose content is capable of modulating anthocyanin synthesis (Payyavula, Navarre, Kuhl, & Pantoja, 2013). Phytonutrients in potatoes are influenced by the growth stage of the plant and the temperature whereas fertilization by N-P-K does not have any impact. Potatoes harvested earlier tend to have higher contents of some phytonutrients like folate and chlorogenic acid (Ezekiel et al., 2013).

Engineering the tuber phenylpropanoid pathway has the potential to further increase the nutritional value of potatoes because red and purple genotypes with genetically increased PAL enzyme activity have markedly higher levels of phenylpropanoids. As Navarre et al. (2013) mentioned, a more comprehensive understanding of phenylpropanoid metabolism during tuber maturation in field conditions could provide insights about approaches to produce more nutritious potatoes. There is not much information in the scientific literature to date about anthocyanidin content during tuber maturation in colored genotypes, contrary to the knowledge of how storage conditions (Jansen & Flamme, 2006; Lachman et al., 2012) and various food processing techniques impact the anthocyanin levels (Lachman et al., 2013). Understanding the fate of anthocyanidins in staple crops like potato could be used to breed new anthocyanin-rich cultivars, since their absorption in the digestive tract is very low (Espin et al., 2007). Thus, the aim of this study is to present the changes anthocyanidins undergo during the growth of red- and purple-fleshed potato tubers in field conditions in order to gain understanding about their fate up to the harvest time.

2. Materials and methods

2.1. Plant material

Pigmented potato tubers were grown and harvested at the university research field in Prague, Czech Republic (GPS 50°07'38.4"N 14°22'23.5"E; 290 m above sea level). Four cultivars were used in the study: Blau de la Mancha and Valfi (both purple skinned/purple fleshed); Königspurpur and Red Emma (both red skinned/red fleshed). Pictures of cultivars are given in [Supplementary Material 1](#). Tubers were planted on April 4, 2016 in molded rows containing 100 plants (plant distance 30 × 75 cm). Standard agricultural practices were used, with no irrigation, and regular herbicides and insecticides were applied ([Supplementary Material 1](#)). Meteorological data, sizes of harvested tubers and moisture content are given in [Supplementary Materials 2 and 3](#). After the onset of tubers (June 13, 2016) the tubers were collected in weekly intervals for 15 weeks. On each sampling date, five randomly chosen plants per cultivar were separately harvested for tubers, then washed and weighed. The analytical sample (typically about 70 g, less in the early stages) representing one plant was made from wedges (with skin; cut lengthwise through the middle of the tuber, 3–7 mm thick, 1 piece per tuber) of all tubers harvested from that plant and freeze-dried in the dark (Lyovac GT2, Steris, Germany) for 168 h. The dry wedges were ground (IKA, A11 basic, Germany) and immediately analyzed without any storage.

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