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Fast, cross cultivar determination of total carotenoids in intact carrot tissue by Raman spectroscopy and Partial Least Squares calibration

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ABSTRACT

In order to speed up the breeding of orange carrots for high carotenoid content it is imperative to develop a fast and non-destructive technique. 332 roots from 86 carrot varieties grown in 2014 at the experimental farm in Høje Taastrup (DK) form the basis of this study. All roots were measured by Raman spectroscopy. The carotenoid content of the very same roots was estimated through a wet chemistry method coupled with UV–VIS at 447 nm and 540 nm. For the Raman spectroscopy, measurements were made on a cross section disk approximately 10 cm from the root top at three different positions in the phloem. Since the top of the carrot is intact, it may still be used for growing. The final calibration model shows an uncertainty (RMSECV) of 20.5 ppm, and a R^2 = 0.86. It has thus proven to be well suited for prediction of carotenoids in orange carrots, and especially for ranking them according to the content.

1. Introduction

Carotenoids are among the most applied natural colorants in the food industry (Mortensen, 2006). Extractions of these are done from various red and orange fruits and vegetables, among others carrots, and many other sources (Jaswir, Noviendri, Hasrini, & Octavianti, 2011). However, production of carotenoids is often expensive and environmentally harmful (de Boer, 2014). There are several reasons for this: low concentrations of the colorants in the plant material, productions sites with high production costs, high need for watering and extensive use of pesticides, and/or high expenses for transportation due to large distance between growers of the raw material and the industry making the natural colorants. It is therefore of interest with more efficient and environmental friendly production methods. One way of solving this problem is to grow high yield fruits and/or vegetables closer to the production site of natural colorants (Bruinsma et al., 2013). This will reduce the transportation costs (economically as well as CO_2 footprint).

The biosynthesis of carotenoids in plants are well understood (Jourdan et al., 2015; Just et al., 2007; Tanaka, Sasaki, & Ohmiya, 2008). This also goes for carrot which is an important crop in relation to carotenoids due to high concentration (28 mg/100 gram) accumulated in the tissue (Surles, Weng, Simon, & Tanumihardjo, 2004). The most abundant carotenoid in orange carrots is β-carotene (45–80%), smaller fractions are found of α-carotene and lutein (de Oliveira, Castro, Edwards, & de Oliveira, 2010) (for chemical structure see Fig. 1). All three have color nuances from yellow to orange, dependent on concentration; however, lutein is often found to be the dominant carotenoid in yellow cultivars, but is most often also present in orange cultivars in lower concentrations (Baranski, Baranska, & Schulz, 2005; de Oliveira et al., 2010). Other carrot cultivars are found with purple colored tissue. The color in

Abbreviations: PCA, Principal Component Analysis; PLS, Partial Least Squares; I-PLS, Interval Partial Least Squares; VIP, Variable Importance in Projection; THF, Tetra Hydro Furan; RMSECV, Root Mean Squared Error of Cross Validation; LV, Latent variable(s).

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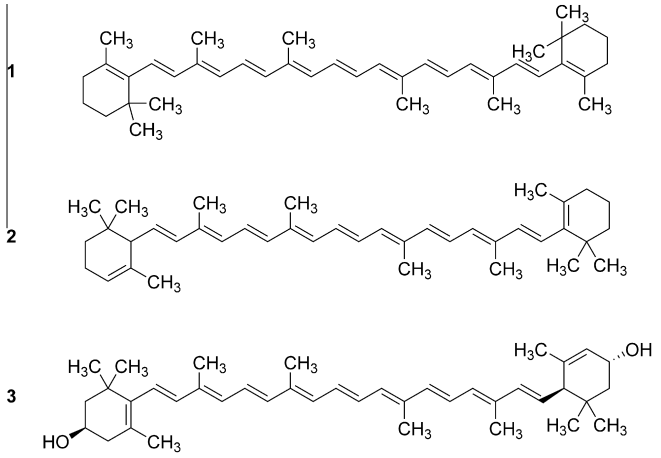

Fig. 1. Chemical structure of the tetraterpenoids β-carotene (1), α-carotene (2) and lutein (3).

these cultivars is primarily due to anthocyanins, though they will often also contain some carotenoids (Kammerer, Carle, & Schieber, 2004; Surles et al., 2004). Anthocyanin are chemically very different from the carotenoids, they are water soluble whereas the carotenoids are fat soluble.

The fact that the carotenoids are fat soluble cause traditional quantification methods for carotenoids to be laborious, time consuming and involves use of hazardous chemicals, for example Tetra Hydro Furan (THF) (Machmudah & Goto, 2013). Current methods for the estimation of carotenoid in plant tissue are cumbersome, with the majority of the papers focusing on HPLC methods (Konings & Roomans, 1997; Maurer, Mein, Chaudhuri, & Constant, 2014; Müller, 1997; Pacheco et al., 2014), and capillary electrochromatography (Herrero-Martínez, Eeltink, Schoenmakers, Kok, & Ramis-Ramos, 2006). Alternative and easier methods for determination of carotenoids in carrots have therefor been requested. NIR spectroscopy and Raman spectroscopy has been suggested, and especially Raman spectroscopy has shown promising results (Baranska, Baranski, Grzebelus, & Romana, 2011; Baranska, Schutz, & Schulz, 2006; Killeen et al., 2013; Quilitzsch, Baranska, Schulz, & Hoberg, 2005; Schulz, Baranska, & Baranski, 2005; Withnall, Chowdhry, Silver, Edwards, & de Oliveira, 2003). Due to in phase stretching of the double and single carbon – carbon bindings in the polyene chain and the rocking of the CH_3 groups attached to the polyene chain, carotenoids have strongly enhanced regions in the resonance Raman spectrum (Gill, Kilponen, & Rimai, 1970; Withnall et al., 2003). Raman spectroscopy has previously been used for qualitative determination of carotenoids in plant materials including carrots (Baranska, Baranski, Schulz, & Nothnagel, 2006; Quilitzsch et al., 2005; Schulz et al., 2005; Withnall et al., 2003). Killeen et al. (2013) showed a good correlation between carrot powder and the carotenoid content measured by Raman. However, up till now no quantitative method based on intact plant tissue using Raman spectroscopy has been published.

The current study is a part of a breeding program that has been initiated with the goal to develop high pigment carrots of orange varieties adapted to Danish climate conditions and which at the same time can exploit the more cost efficient and environmentally friendly farming system (Danish Agriculture, 2014).

In order to select cultivars and landraces for the breeding program screening large collection of carrot cultivars and populations for the concentration of carotenoids. In this paper, we will present a fast method for determination of the total carotenoid level in intact carrot tissue. By use of Raman spectroscopy and multivariate regression methods, we have developed calibration models for total carotenoid valid across a range of different cultivars. The method is (semi) non-destructive because carotenoid content can be generated from a cross section of the intact root and new plant can be grown from the remaining root-top.

2. Materials and methods

2.1. Plant material

A diverse collection of 86 cultivars and lines of carrots (*Daucus carota* L.) were grown in the fields of the experimental farm Højbakkegård, Taastrup (55.67°N; 12.30°E), Denmark during 2014. The carrots were planted May 27 2014. In total 332 roots, 1–5 roots of each line were harvested in September 2014, and stored in the dark at 5 °C until the spectra were acquired the following 3 weeks.

2.2. Reference values obtained by UV–Visible Spectrophotometry

The bottom part of the carrot was used for reference determination, and a slice at the top was used for Raman measurement. The slice was made approx. 10 cm from the top, ensuring the possibility for further regrowth and vernalization. The washed carrot was homogenized in a food processor at room temperature. Approximately 50 g plant material was mixed with sulfuric acid (3% in water) in a 1:1 ratio and blended in a Warring blender for 2 min at full speed. Of the blended material 4 g was mixed with 7 g of tetrahydrofuran (THF), left for one hour and then centrifuged for 15 min. The supernatant was diluted in ethanol ($0.3 < Abs\ max < 1.2$) and UV–VIS spectra were acquired on a HP 8453 UV–Visible Spectrophotometer from 350 to 700 nm, 2 nm resolution using a slit width of 1 nm and a 1 cm optical path length. The carotenoid concentrations were calculated by use of Lambert Beers (Lothian, 1963) law using the absorbance at 447 nm and 540 nm. Extinction coefficients for β-carotene were used commonly for all carotenoids; $2507\ M^{-1}\ cm^{-1}$ (447 nm) and $20\ M^{-1}\ cm^{-1}$ (540 nm). These extinction coefficients and absorbance wavelengths have been optimized by Chr. Hansen from the method described by Craft and Soares (1992). The used extinction coefficient for 447 nm is in agreement with earlier research (Cortés, Esteve, Frígola, & Torregrosa, 2004; Maurer et al., 2014). The uncertainty of the reference analysis is approximately 5% relative to the estimated amount (data not shown).

2.3. Raman spectroscopy

Raman spectra were acquired on the very same carrots using a RamanRxn1 instrument (Kaiser Optical Systems Inc, MI, USA) with a 200 mW 785 nm near-infrared diode laser (Invictus, Kaiser Optical Systems Inc., MI, USA) and a single holographic grating and a Peltier-cooled CCD detector operating at −40 °C. We used a probe with 3 mm spot size. The spectra were acquired as 2 accumulated scans of 10 s exposure time. As the highest concentration of β-carotene has been shown to be located in the secondary phloem (Baranska et al., 2006), the Raman spectra were measured here in order to get the highest signal-to-noise ratio. Furthermore, to get a representative spectrum, three spectra were recorded for each carrot on different spots in the secondary phloem. The carrot disks used for the measurement were from a cross section of the middle of the root, however, not closer than 10 cm from the top. The average spectrum of the three measurements from each root was considered a representative spectrum from one carrot.