Food Chemistry 212 (2016) 495–502

Contents lists available at ScienceDirect

Food Chemistry

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

Quantifying biochemical quality parameters in carrots (*Daucus carota* L.) – FT-Raman spectroscopy as efficient tool for rapid metabolite profiling

Andrea Krähmer [a,*], Christoph Böttcher [a], Andrea Rode [b], Thomas Nothnagel [b], Hartwig Schulz [a]

[a] Julius Kühn-Institut, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Königin-Luise-Strasse 19, D-14195 Berlin, Germany
[b] Julius Kühn-Institut, Institute for Breeding Research on Horticultural Crops, Erwin-Baur-Strasse 27, D-06484 Quedlinburg, Germany

ARTICLE INFO

Article history:
Received 17 February 2016
Received in revised form 25 May 2016
Accepted 30 May 2016
Available online 31 May 2016

Chemical compounds studied in this article:
α-Carotene (PubChem CID: 4369188)
β-Carotene (PubChem CID: 5280489)
Lutein (PubChem CID: 6433159)
Falcarinol (PubChem CID: 5281149)
Falcarindiol (PubChem CID: 5281148)
3-*O*-Acetylfalcarindiol (PubChem CID: 101256943)
Fructose (PubChem CID: 5984)
Glucose (PubChem CID: 5793)
Sucrose (PubChem CID: 5988)
Laserine (PubChem CID: 15560087)
2-Epilaserine (PubChem CID: 92467577)

Keywords:
FT-Raman spectroscopy
Carotenoids
Polyacetylenes
Carbohydrates
Phenylpropanoids
Laserines
Quantification
Daucus carota

ABSTRACT

Application of FT-Raman spectroscopy for simultaneous quantification of carotenoids, carbohydrates, polyacetylenes and phenylpropanoids with high bioactive potential was investigated in storage roots of *Daucus carota*. Within single FT-Raman experiment carbohydrates, carotenoids, and polyacetylenes could be reliably quantified with high coefficients of determination of $R^2 > 0.91$. The most abundant individual representatives of each compound class could be quantified with comparably high quality resulting in $R^2 = 0.97$ and 0.96 for α-carotene and β-carotene, in $R^2 = 0.90$ for falcarindiol (FaDOH), $R^2 = 0.99$, 0.98 and 0.96 for fructose, glucose and sucrose. In contrast, application of FT-Raman spectroscopy for quantification of two laserine-type phenylpropanoids was investigated but failed due to low concentration and Raman response. Furthermore, evaluation of metabolic profiles by principle component analysis (PCA) revealed metabolic variety of carrot root composition depending on root color and botanical relationship.

1. Introduction

Besides tomato and onion, carrot (*Daucus carota* L.) is one of the most cultivated horticultural crops worldwide. Depending on the cultivar, carrot roots display a large variation in root color and shape and accumulate as storage organ a number of different nutrients like mono- or disaccharides (fructose, glucose and sucrose) and carotenoids as well as highly bioactive phytochemicals. Among them, bis-acetylenic oxylipins (polyacetylenes) were reported to have a multitude of bioactivities including bitter taste, allergenic, antibacterial, antimycobacterial and antifungal activities (Dawid, Dunemann, Schwab, Nothnagel, & Hofmann, 2015).

Abbreviations: FaDOH, falcarindiol; FaDOH-Ac, 3-*O*-acetylfalcarindiol; PCA, principal component analysis; FT, Fourier-transform; PLS, partial least squares; DM, dry matter; RMSEP, root mean square error of prediction; RPD, ratio of performance to deviation; LV, latent variables; UHPLC/DAD/ESI-QTOFMS, ultra high-performance liquid chromatography coupled with diode array detection and electrospray ionization-quadrupole time-of-flight mass spectrometry; HPLC/RID, high-performance liquid chromatography coupled with refractive index detection.

* Corresponding author.
E-mail addresses: Andrea.Kraehmer@julius-kuehn.de (A. Krähmer), Christoph.Boettcher@julius-kuehn.de (C. Böttcher), andrearode@yahoo.de (A. Rode), Thomas.Nothnagel@julius-kuehn.de (T. Nothnagel), Hartwig.Schulz@julius-kuehn.de (H. Schulz).

http://dx.doi.org/10.1016/j.foodchem.2016.05.176
0308-8146/

In addition, polyacetylenes are discussed as potential health-promoting and therapeutic agents due to their anti-tumor and anti-inflammatory properties (Garrod, Lewis, & Coxon, 1978; Olsson & Svensson, 1996; Zaini, Brandt, Clench, & Le Maitre, 2012; Zidorn et al., 2005). Recent studies identified new phenylpropanoids in carrots as bitter taste compounds, comprising laserine and 2-epilaserine and their oxides (Schmiech, Uemura, & Hofmann, 2008; Yang, Yan, & Lu, 2008). These laserine derivatives occur in several species of the Apiaceae family and are discussed for their high cytotoxic potential (Dall'Acqua et al., 2010; Yang et al., 2008). Due to their high concentration carotenoids are named according to their occurrence in carrots. Besides α- and β-carotene, also other tetraterpenoids like lutein and lycopene, are described to appear in large quantitative differences depending on the cultivar (Harper & Zscheile, 1945; Surles, Weng, Simon, & Tanumihardjo, 2004). Fig. 1 provides the structures of prominent polyacetylenes, laserines and carotenoids found in carrot roots. Hence, to characterize the nutritional value of carrot roots, a chemically diverse set of compounds has to be analyzed.

Different chromatographic methods have been developed for separation and quantification of carbohydrates, carotenoids, polyacetylenes and phenylpropanoids in carrots but all of them require extraction prior to analyses followed by individual analytical protocols making a chromatography-based quantification approach time consuming and expensive (Christensen & Kreutzmann, 2007; Magwaza & Opara, 2015; Oliver & Palou, 2000; Schmiech et al., 2008; Yang et al., 2008).

In the recent years, Fourier-transform (FT-) Raman spectroscopy has been widely applied for carotenoid and polyacetylene analyses in fresh and dried carrot root material due to the high Raman scattering effect of the conjugated electron system in carotenoids and the unique Raman signals from C—C triple bonds of polyacetylenes set in the region of 2100–2300 cm^{-1} of the FT-Raman spectra (Baranska, Baranski, Grzebelus, & Roman, 2011; Baranska, Baranski, Schulz, & Nothnagel, 2006; Baranska, Roman, Dobrowolski, Schulz, & Baranski, 2013; Baranska & Schulz, 2005; Gill, Kilponen, & Rimai, 1970; Roman, Baranski, & Baranska, 2011; Roman, Dobrowolski, Baranska, & Baranski, 2011; Withnall, Chowdhry, Silver, Edwards, & de Oliveira, 2003). Simultaneous detection of carbohydrates, carotenoids and polyacetylenes by FT-Raman spectroscopy could further be demonstrated (Baranska, Schulz, Baranski, Nothnagel, & Christensen, 2005).

Nevertheless, to our knowledge only one very recent work investigated FT-Raman spectroscopy for quantification of carotenoids and polyacetylenes in freeze dried carrot roots (Killeen et al., 2013). Based on UV–vis spectrophotometry reference data Killeen and coworkers could successfully apply FT-Raman for quantification of the overall content of carotenoids directly in freeze-dried carrot root powder ($R^2 = 0.85$). In contrast, this approach failed for polyacetylene quantification by correlation with gas-chromatography data resulting in $R^2 = 0.57$ (Killeen et al., 2013). Although this model showed only weak quantitative predictive power, a certain relation of FT-Raman spectral features and polyacetylene concentration could be observed.

Therefore, the aim of this study was the application of FT-Raman spectroscopy for fast and holistic quantification of carotenoids, polyacetylenes, carbohydrates and laserin-type phenylpropanoids directly on carrot root material and within single experiments. Besides the prediction of overall concentration of the various compound classes also quantification of individual compounds should be examined for comparison of cultivars on a significant metabolic level. Principal component analysis (PCA) should be investigated for non-targeted screening of different carrot cultivars and wild relatives.

2. Materials and methods

2.1. Chemicals

Organic solvents used for extraction and analysis were of gradient grade quality or higher and purchased either from Th. Geyer (Berlin, Germany) or from Sigma-Aldrich (Karlsruhe, Germany). Water was purified using an Arium 611 water purification system from Sartorius (Göttingen, Germany). LC–MS grade eluent additives (formic acid and ammonium acetate), α-carotene, β-carotene, canthaxanthin and D-(+)-glucose were obtained from Sigma-Aldrich. Lutein, D-(−)-fructose, D-(+)-sucrose, D-(+)-maltose monohydrate and TRIS-HCl were purchased from Roth (Karlsruhe, Germany). Standards of polyacetylenes and laserines were extracted from carrot roots and purified by chromatographic techniques.

2.2. Isolation of polyacetylenes and laserines as reference compounds from carrot roots

Freeze-dried and ground carrot root material (53 g from several orange cultivars) was extracted twice with CH_2Cl_2 (500 mL) at 20 °C for 15 min using an ultrasonic bath. The combined and filtered extract was concentrated under reduced pressure at 30 °C. The resulting residue (1.27 g) was dissolved in CH_2Cl_2 (3 mL) and fractionated by column chromatography (20 × 400 mm) on 50 g

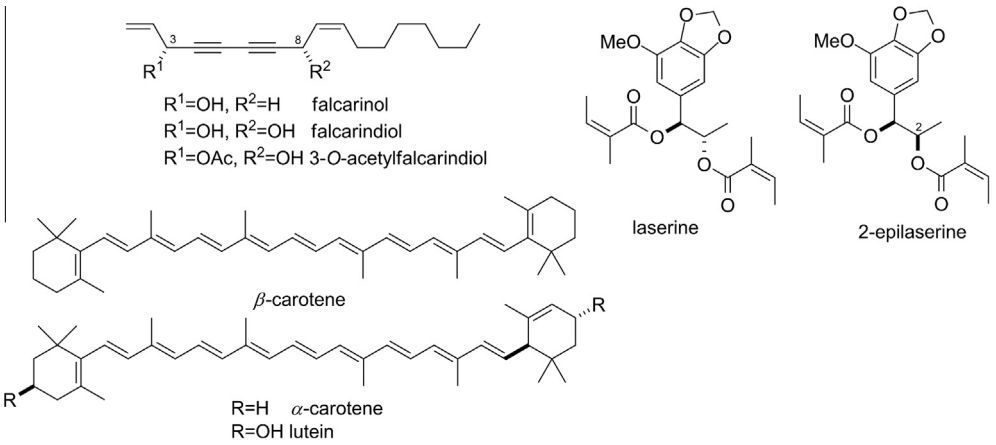

Fig. 1. Molecular structures of polyacetylenes, carotenoids and laserines from carrot roots.