Spatial tissue distribution of polyacetylenes in carrot root

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The presented results show the usefulness of Raman spectroscopy in the investigation of polyacetylenes in carrot root. The components are measured directly in the plant tissue without any preliminary sample preparation. Compared with the strong polyacetylene signals the spectral impact of the surrounding biological matrix is weak, except for carotenoids, and therefore it does not contribute significantly to the obtained results. Three different Raman mapping techniques applied here have revealed essential information about the investigated compounds. Using point acquisition several spectra have been measured to demonstrate the complex composition of the polyacetylene fraction in carrot root. The molecular structures of falcarniol, falcarnidiol and falcarnidiol 3-acetate are similar but their Raman spectra exhibit differences demonstrated by the shift of their $-\text{C}^\text{\ddagger}\text{C}-$ mode. Line mapping performed along the diameter of transversely cut carrot roots has been used to investigate the relative concentration of polyacetylenes and carotenoids. An area map provides detailed information regarding the distribution of both components. It has been found that high accumulation of polyacetylenes is located in the outer section of the root, namely the pericyclic parenchyma, and in the phloem part close to the secondary cambium. The highest concentration of carotenoids is seen in the immediate vicinity to polyacetylene conglomerates.

Introduction

For several decades the origin of bitter off-taste of carrot roots has been the subject of intensive investigation by many groups of scientists. Various reports pointed out that 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin, commonly known as 6-methoxymellein (6-MM) could be responsible for the bitter taste in selected carrots.\(^{1,2}\) On the other hand the concentration of this compound in various carrot batches was detected below the bitter taste threshold estimated as 100 mg kg\(^{-1}\) by Yoshino \textit{et al.} \(^{3}\) and as 20 mg kg\(^{-1}\) by Czepa \textit{et al.} \(^{4}\) thus suggesting that this isocoumarin might have only little effect on carrot bitterness.\(^{4}\) Additionally, some phenolic compounds, such as 5,7-dihydroxy-2-methylchromone and 5-hydroxy-7-methoxy-2-methylchromone (eugenin) were reported to be produced under ethylene atmosphere and to develop bitter taste.\(^{5}\) In contradiction, other investigation did not confirm the correlation between increasing eugenin content and bitter taste in carrots.\(^{6}\) Ethylene can also promote the formation of some terpenes, such as $\gamma$-terpinene, $\alpha$-pinene, limonene and $p$-cymene, whose increased content was suggested to correlate with high sensorial scores for bitterness.\(^{7}\) Beside this investigation, 2,4,5-trimethoxybenzaldehyde (gazarin) was identified as a bitter principle of carrot seeds,\(^{8,9}\) but the contribution of this compound to the bitter off-taste of carrot tissue has been excluded due to its low concentration (<0.1 mg kg\(^{-1}\)), which is below the bitter taste threshold (0.35 mg kg\(^{-1}\)) of this compound.\(^{4}\) As can be seen, the presented data are very contradictory, and no clear correlation is seen between the individual compounds detected in carrots and the sensory evaluation of the bitter taste.

However, it has been quite recently reported that some polyacetylenes present in carrot roots contribute strongly to their bitter off-taste.\(^{4,10}\) Among these, some bisacetylenic oxylipins, (Z)-heptadeca-1,9-dien-4,6-diyn-3-ol (falcarniol), (Z)-heptadeca-1,9-dien-4,6-diyn-3,8-diol (falcarnidiol) and (Z)-3-acetoxy-heptadeca-1,9-dien-4,6-diyn-8-ol (falcarnidiol 3-acetate) have been identified (see Fig. 1).

The correlation between the concentration of these polyacetylenes and their individual bitter detection threshold clearly indicated falcarnidiol as the main compound responsible for the bitter off-taste of fresh and stored carrots.\(^{10}\) The presence of falcarnidiol in carrot root as well as its antifungal properties have already been reported before,\(^{11-16}\) but the role as the key component in the bitter taste of carrot has been discovered quite recently.\(^{4,10}\)

Fig. 1 The chemical structure of falcarniol, falcarnidiol and falcarnidiol 3-acetate.
Furthermore, polyacetylenes, in particular falcarinol, isolated from carrots have been reported as having a beneficial effect on human health.\textsuperscript{17,18} In numerous studies it has been found that high intake of carotenoid-rich vegetables decrease the risk of cancer.\textsuperscript{19,20} However, it has been shown that β-carotene supplementation has no protective effect against cancer development\textsuperscript{21,22} whereas falcarinol, the most bioactive of the carrot polyacetylenes, has a pronounced cytotoxic activity against human tumour cell in vitro\textsuperscript{23,24} as well as in vivo anticancer effect in rats.\textsuperscript{25} Also, ginseng roots have antimutagenic properties and stimulatory effects on the immune system during bacterial infection probably due to the presence of falcarinol and related polyacetylenes.\textsuperscript{4} The antifungal activities of falcarinol and falcarindiol were reported as being important in the resistance of the plant to these infections.\textsuperscript{11} In carrot they restrict the development of lesions caused by storage pathogens such as Mycocentrospora acerina.\textsuperscript{12,16}

On the other hand, falcarinol has also some negative effects, e.g. skin irritation, and it is toxic in relatively high concentration.\textsuperscript{26,27} Nevertheless, it was suggested that falcarinol may have beneficial effects on human health (at low concentration) and may also contribute significantly to the health-promoting properties of carrot.\textsuperscript{17,18}

However, the investigation of polyacetylenes is hampered by the fact that these compounds are unstable, being sensitive to heat and light.\textsuperscript{4} The most popular method used for their analysis is high-performance liquid chromatography (HPLC) which requires the isolation of the compound involving solvent extraction. Furthermore, such a procedure does not allow the determination of the distribution of falcarinol and other polyacetylenes in cellular dimensions. These limitations can be overcome using Raman spectroscopy which has already been successfully applied to the analysis of polyacetylenes in several plants.\textsuperscript{28,29} In particular, the Raman mapping technique gives deeper knowledge of localisation of secondary metabolites in various intact plant tissues.\textsuperscript{29–32}

In this paper, NIR-FT-Raman spectroscopy is used to analyse polyacetylenes and carotenoids in situ in the intact carrot roots. Furthermore, their individual distribution in the analysed plant material is measured by micro-Raman techniques and the obtained results are discussed.

**Experimental**

Raman spectra were recorded using a Bruker NIR-FT-Raman spectrometer (model RFS 100) equipped with a Nd:YAG laser, emitting at 1064 nm, and a germanium detector cooled with liquid nitrogen. The instrument was equipped with an XY stage, a mirror objective and a prism slide for redirection of the laser beam. Compared with the standard vertical sampling arrangement, the samples were mounted horizontally.

Spectral measurements were taken from carrot roots cultivated in the experimental garden of the BAZ. Additionally, falcarinol isolated from carrot roots and dissolved in ethanol was measured. All spectra were performed with a spectral resolution of 4 cm\(^{-1}\) in the range from 100–4000 cm\(^{-1}\). Single measurements of carrot roots were obtained with 128 scans and an unfocused laser beam of 200 mW, whereas for the falcarinol standard solution 64 scans and a laser power of 100 mW were used for spectral analysis. Line measurements along the diameter of transversely cut carrot roots (20.4 mm) were obtained point by point moving the XY stage. Eight scans were collected at each measured point, every 100 μm (205 points), with an unfocused laser beam of 200 mW. 2-D Raman maps of flat samples of the transversely cut carrot roots were obtained point by point; the x and y directions of the accessory were controlled by the spectrometer software. A quarter of carrot root was mapped at an area of 10.5 × 12.0 mm with a spatial resolution of 250 μm. The samples were irradiated with a focused laser beam of 200 mW of a diameter about 0.1 mm; eight scans were collected at each measured point. Spectra or 2-D surface areas of the mapped samples were processed by the Bruker Opus/map software package.

**Results and discussion**

In spite of the fact that the concentration of polyacetylenes in plants, depending on the individual species is in the order of 0.01–1.00 g 100 g\(^{-1}\) fresh matter, they can be successfully detected by using NIR-FT-Raman spectroscopy.\textsuperscript{28} The obtained spectra show strong and polarized bands, due to the triple bonds in the molecule, in the region of about 2200 cm\(^{-1}\). As has been discussed before,\textsuperscript{26} the number of triple bonds as well as substituents influence the frequency of the polyacetylene –C=C– stretching modes. Thus, the spectral position of –C=–C– vibrations and pattern of Raman bands usually provide enough information to recognise the type of substitution and to support the identification of polyacetylenes.\textsuperscript{53}

As can be seen in Fig. 2, the Raman spectrum taken from some spots of the carrot root reveals a strong signal at about 2255 cm\(^{-1}\). It has been reported before that three polyacetylenes can be detected in this vegetable.\textsuperscript{4,10–13} All these compounds possess a similar molecular structure with two adjacent triple bonds substituted with one –OH group (falcarinol), two –OH groups (falcarindiol) or –OH and –OCOCH\(_3\) groups (falcarindiol 3-acetate), respectively (see Fig. 1). Generally, for compounds containing the –C≡C–C– grouping, the vibrational modes are described as asymmetric and symmetric –C≡C–C≡C– stretching, IR and Raman active,
respectively. The characteristic, strong and polarized, Raman band (symmetric stretch) of the R–C≡C–C≡C–R’ structure should be seen at 2257–2251 cm⁻¹.33 The exact coincidence of this narrow wavenumber range and the registered signal from the carrot confirms that the band to be seen in the Raman spectrum at about 2255 cm⁻¹ comes from the reported previously polyacetylenes.

In addition to the polyacetylene band three carotenoid signals, mainly related to β- and α-carotene, are seen in Fig. 2. A strong enhancement of carotenoid bands is observed in the NIR-Raman spectrum due to the known pre-resonance effect.34 Bands at 1520 and 1156 cm⁻¹ can be assigned to in-plane C=C (νl) and C–C (νs) stretching vibrations of the polyene chain of the carotenoids, respectively.32 Additionally, in-plane rocking mode of CH₃ groups attached to the polyene chain and coupled with C–C bonds is seen as a peak of medium intensity at 1006 cm⁻¹.

A closer inspection of the carrot polyacetylene band reveals its complex structure as well as a slight shift in the band position depending on the location of carrot root measurement (see Fig. 3B–D). Usually, one or two overlapping bands can be seen in this range, with one maximum at 2258 or 2252 cm⁻¹. The Raman spectrum performed on pure falcarinol isolated from carrot root and dissolved in ethanolic solution exhibits a strong signal at 2258 cm⁻¹ (see Fig. 3A). This indicates that the band seen in the carrot root spectrum at 2258 cm⁻¹ should be assigned to falcarinol. The signal observed at 2252 cm⁻¹ can be attributed to falcarindiol, which is also present in carrot root in comparatively high amounts (among the three polyacetylenes discussed here falcarindiol 3-acetate occurs in lowest amounts).10 From the literature it is known that the attachment of oxygen to the η-position of acetylenes has only little effect on –C≡C– frequency.33 so the observed Raman shift of 6 cm⁻¹ between both discussed polyacetylene signals can be explained by substitution of an additional -OH group. Furthermore, we have already identified the position of the falcarindiol band at 2252 cm⁻¹ in the Raman spectrum of *Angelica dahurica* root, where this polyacetylene is responsible for the antibiotic activity of this Chinese drug.35

The spectra presented in Fig. 2 and 3 were measured at different places of the same sample and they are randomly related to each other. As a next step of our investigation we used a line mapping which defines a series of spectra to be obtained in one dimension. We have performed measurements along the diameter of a transversely cut carrot root. Several line measurements were executed on the same carrot root as well as various cultivars. Exemplary results are presented in Fig. 4 as a plot showing the intensity of the –C≡C– stretching mode of polyacetylenes at approximately 2255 cm⁻¹ along the measured line and the intensity of –C≡C– stretching mode of carotenoids at 1520 cm⁻¹. Raman intensity is proportional to the concentration of the analyte molecule so the plots seen in Fig. 4 demonstrate the distribution of polyacetylenes and carotenoids along the diameter of the analysed carrot root. Moreover, the relative concentration of both compounds can be discussed.

As can be seen in Fig. 4, polyacetylenes are not uniformly distributed over the whole carrot root, but they are concentrated in specific parts of it. Their high accumulation is located in the outer section of the root, the so-called pericyclic parenchyma, and in the phloem part close to the secondary cambium. Contrary to that, the xylem parenchyma contains only small amounts of polyacetylenes. These results are in agreement with previous reports, which were based on UV spectroscopy, microscopic investigation15,16 and chromatography measurement.4,10 It has been observed that the gradient of resistance against *Mycocentrospora acerina*, a long-term storage disease of carrot, vary from a high level at the periphery to a very low level in the centre.16 In other words, the pericyclic parenchyma was shown to be more resistant than the xylem parenchyma, with the phloem parenchyma occupying an intermediate position.15 This observation was correlated with the distribution of falcarindiol in carrot roots. During microscopic observations performed at sections of carrot roots, several conglomerations of extracellular oily droplets were noticed in periderm/pericyclic parenchyma tissue and it seemed that a channel system is formed extending down the

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![Fig. 3](image1.png)  
**Fig. 3** FT-Raman spectra of falcarinol isolated from carrot root, dissolved in ethanolic solution (A) and three spots of the carrot root containing polyacetylenes (B, C, D) presented in the range between 2290 and 2210 cm⁻¹.  

![Fig. 4](image2.png)  
**Fig. 4** FT-Raman measurements of polyacetylenes and carotenoids in the carrot root. The plots show the intensity of the C≡C peak of polyacetylenes at approximately 2255 cm⁻¹ and C≡C mode of carotenoids at 1520 cm⁻¹ for 205 positions every 100 μm along a diameter of the transversely cut carrot root.
length of the root. The obtained UV spectrum as well as thin layer chromatography (TLC) technique confirmed that in the oil droplets falcarnidiol is present. Traces of this polyacetylene were also found in oil droplets of phloem parenchyma tissue, but they were smaller and less frequent than those found in periderm/pericyclic parenchyma. Czepa et al. investigated the concentration of falcarniol, falcarnidiol and falcarnidiol 3-acetate in upper (basal) and lower (apical) parts of carrot roots as well as in the xylem and phloem. They have found the highest concentration of falcarnidiol in phloem and the upper end (approx. 33 mg kg\(^{-1}\) fresh wt) whereas in xylem tissue and the lower end this polyacetylene was occurring only in amounts below 20 mg kg\(^{-1}\) fresh weight. Anyway, both values are above the bitter taste threshold estimated for falcarnidiol at 10 mg kg\(^{-1}\). The other compounds, falcarniol and falcarnidiol 3-acetate, did not show distinctive differences in concentration between phloem and xylem. Czepa et al. have also noticed that peeled carrot contains less falcarnidiol so the peridermal part of the root is rich in this compound.

Comparing polyacetylene and carotene concentrations along the carrot diameter some correlations can be found (see Fig. 4). The accumulation of polyacetylenes observed in the phloem section close to the secondary cambium relates to the high concentration of carotenes, but the maximum concentration of both components is not identified at the same areas. This observation was confirmed by several measurements—always maximum signals of polyacetylenes were detected in the phloem in immediate vicinity of highly concentrated carotenoids. It seems that the localisation of the polyacetylenes can be related to the presence of vascular bundles in the carrot root; higher density of them is observed in the pericyclic part as well as near the secondary cambium. These channels could be responsible for transport and accumulation of polyacetylenes.

The plot showing the localisation of polyacetylenes is not symmetrical and shows some differences between the measurements performed along different lines through the same transversely cut root. It seems that measurements performed at the vascular bundles occasionally distributed in the phloem section show higher concentration of polyacetylenes.

Fig. 5 presents more details of a non-uniform distribution of polyacetylenes in the carrot root. The results of Raman mapping performed at a quarter of a transversely cut root (10.5 \times 12.0\ mm) coloured according to the band intensity at 2255 (A) and 1520 cm\(^{-1}\) (B) shows the relative content of polyacetylenes (A) and carotenes (B) in the measured section, respectively. It can be seen there that polyacetylenes are concentrated in specific areas of the carrot root, namely the pericyclic parenchyma and the phloem part closely to the secondary cambium, but even there they are not distributed uniformly. Red spots seen in Fig. 5A demonstrate polyacetylene agglomerations whereas in blue areas the level of these compounds is below the detection limit. The maxima of polyacetylene and carotene signals are close together although not at the same place.

Summary

The presented results illustrate that NIR-FT-Raman spectroscopy, and particularly Raman mapping, is a powerful tool that in addition to other analytical techniques can provide very informative data of polyacetylene distribution in plant material. These compounds can be detected in situ in fresh plant samples non-destructively. Furthermore, the distribution of carotenoids can be measured at the same time so that the relative concentration of both compounds can be compared.

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Fig. 5 Raman maps obtained from a quarter of a transversely cut carrot root (10.5 \times 12.0\ mm) coloured according to the band intensity at 2255 (A) and 1520 cm\(^{-1}\) (B) related to the content of polyacetylenes (A) and carotenes (B) in the carrot root, respectively.
References