

## In Situ Simultaneous Analysis of Polyacetylenes, Carotenoids and Polysaccharides in Carrot Roots

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This paper presents an approach to simultaneously analyze polyacetylenes, carotenoids, and polysaccharides in carrot (*Daucus carota* L.) roots by means of Raman spectroscopy. The components were measured in situ in the plant tissue without any preliminary sample preparation. The analysis is based on the intensive and characteristic key bands observed in the Raman spectrum of carrot root. The molecular structures of the main carrot polyacetylenes, falcarinol and falcarindiol, are similar, but their Raman spectra exhibit specific differences demonstrated by the shift of their  $\text{—C}\equiv\text{C—}$  mode from 2258 to 2252  $\text{cm}^{-1}$ , respectively. Carotenoids can be identified by  $\text{—C=C—}$  stretching vibrations (about 1520 and 1155  $\text{cm}^{-1}$ ) of the conjugated system of their polyene chain, whereas the characteristic Raman band at 478  $\text{cm}^{-1}$  indicates the skeletal vibration mode of starch molecule. The other polysaccharide, pectin, can be identified by the characteristic band at 854  $\text{cm}^{-1}$ , which is due to the  $\text{—C—O—C—}$  skeletal mode of  $\alpha$ -anomer carbohydrates. The Raman mapping technique applied here has revealed detailed information regarding the relative distribution of polyacetylenes, carotenoids, starch, and pectin in the investigated plant tissues. The distribution of these components varies among various carrot cultivars, and especially a significant difference can be seen between cultivated carrot and the wild relative *D. carota* ssp. *maritimus*.

**KEYWORDS:** *Daucus carota*; NIR-FT-Raman; mapping; falcarinol; falcarindiol; starch; pectin; nondestructive analysis; carotene

### INTRODUCTION

Carrot storage roots normally contain three major polyacetylenes, namely, (*Z*)-heptadeca-1,9-diene-4,6-diyn-3-ol (falcarinol), (*Z*)-heptadeca-1,9-diene-4,6-diyn-3,8-diol (falcarindiol), and (*Z*)-3-acetoxyheptadeca-1,9-diene-4,6-diyn-8-ol (falcarindiol 3-acetate) (**Figure 1**); however, most investigations have concentrated on falcarinol and falcarindiol (*1–7*). Polyacetylenes have been identified as the dominant cause of carrot bitter taste (*1, 2*). The correlation between their concentration and individual bitter detection threshold clearly indicated falcarindiol as the

main compound responsible for the bitter off-taste of fresh and stored carrots (*2*). Furthermore, polyacetylenes isolated from carrots have been announced as having a beneficial effect on human health (*3–7*). Falcarinol has been pointed out as the most bioactive polyacetylene present in carrot, showing a pronounced cytotoxic activity against human tumor cells (*3, 4, 7*). Falcarinol in low concentrations (35  $\mu\text{g/g}$  of freeze-dried carrot root) has an effect on the development of colon cancer in rats (*6*) and may contribute significantly to the health-promoting properties of carrot, besides the well-known carotenoids (*3–6*). Finally, falcarinol and falcarindiol have antifungal activity (*8–11*) and have been investigated as important substances in plant disease resistance. In carrot they restrict the development of lesions caused by storage pathogens such as *Mycocentrospora acerina* (*9, 11*). Czepa et al. (*2*) investigated the concentration of carrot polyacetylenes in upper (basal) and lower (apical) parts of carrot roots as well as in the xylem and phloem. Highest concentrations of falcarindiol have been detected in the phloem and in the upper

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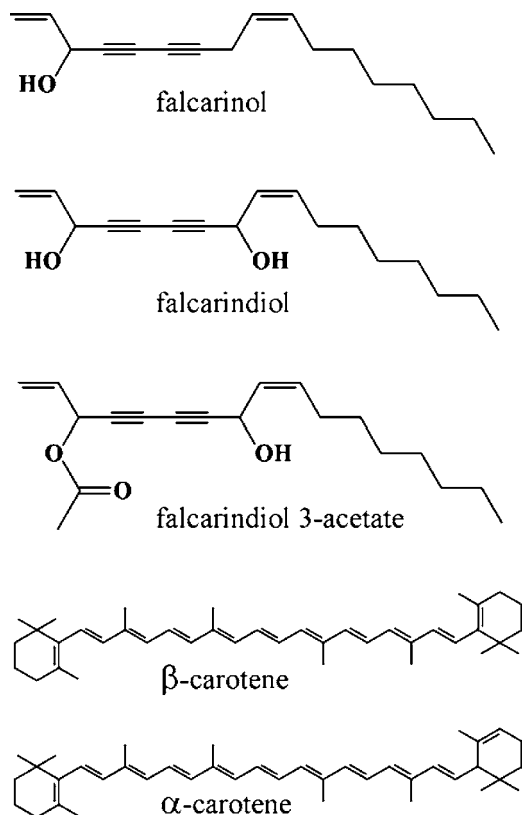
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**Figure 1.** Chemical structures of falcarinol, falcarindiol, falcarindiol 3-acetate,  $\alpha$ -carotene, and  $\beta$ -carotene.

part of the root ( $\sim 33$  mg/kg of fresh wt) and a lower amount ( $< 20$  mg/kg of fresh wt) in xylem tissue and the lower part. Distribution of falcarinol has been found to be more homogeneous in the whole root, with average amounts of 25 mg/kg of fresh wt. Falcarindiol 3-acetate has been found to be more concentrated in the upper part of carrot root (15 mg/kg of fresh wt) than in the lower part (8 mg/kg of fresh wt), and no significant difference could be seen between phloem and xylem tissue (2).

Most studies on the sugars present in carrot roots concern sucrose, glucose, and fructose; only a few publications deal with starch, and moreover they refer only to orange cultivars. The amount of total starch in eight different cultivars was reported to be very low in comparison to other sugars, namely, an average of 9 g/kg of dry matter, whereas the contents of sucrose, glucose, and fructose were found to be 353, 120, and 113 g/kg of dry matter in fresh carrot, respectively (12). In stored carrot the content of starch was reduced to 3 g/kg, and after processing (boiling) it was not detected at all. At the same time the loss of other sugars was not significant. Sturm et al. (13, 14) have ascertained that starch accumulation in roots is transient and requires a constant supply of photoassimilates. They postulated that the bulk of sucrose is transported into the roots during the day and to a large extent converted into starch. During the night, starch may become degraded and sucrose resynthesized.

The other polysaccharide present in carrot root is pectin, which is one of the major carbohydrate constituents of plant cell walls (15). The structural changes of pectin during carrot processing (boiling) have crucial influence on the final taste and consistency of this vegetable (16). Carrots can be prevented from oversoftening during cooking or canning, by pretreating at 50–55 °C for  $\sim 30$  min. This treatment is considered to stimulate the activity of cell wall enzymes such as pectin-methyl esterase (PME); PME de-esterifies pectic polysaccharides,

enhancing their ability to cross-link with calcium and reducing the degree to which they depolymerize during high-temperature processing (16).

Carrot roots are a rich source of carotenoids; in orange carrots  $\beta$ -carotene and  $\alpha$ -carotene are the predominant ones (Figure 1). Animals and human beings are incapable of carotenoid biosynthesis but can modify some of them when absorbed from plant food as, for example,  $\alpha$ - and  $\beta$ -carotene, which can be converted to retinol (vitamin A). The content and rate of carotenes vary with cultivar, production area, and other environmental factors, but are mainly influenced by genotype (17).

In this study, we simultaneously investigated polyacetylenes, carotenoids, starch, and pectin in carrot roots by using NIR-FT-Raman spectroscopy. All of these compounds are determined in situ in the intact carrot root at cellular dimensions. The Raman mapping technique provides detailed information about the relative distribution of these components in the analyzed plant tissue.

## MATERIALS AND METHODS

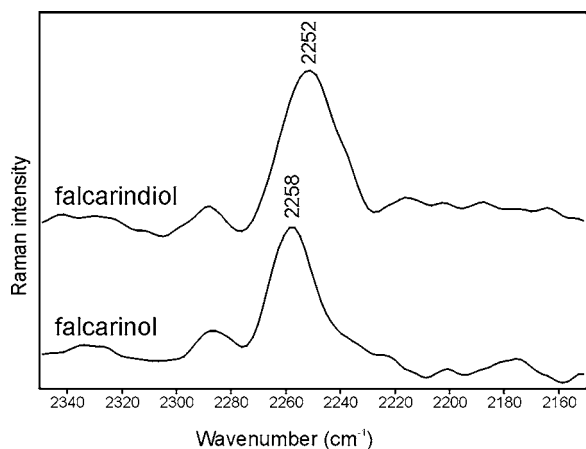
**Plant Material.** Cultivated carrots [*Daucus carota* L. ssp. *sativus* (Hoffm.)] and wild relatives *D. carota* ssp. *maritimus* (Lam.) Batt., *D. carota* ssp. *gummifer* Hook. f., *D. carota* ssp. *commutatus* (Paol.) Thell., and *Daucus halophilus* Brot. were grown in the experimental garden of the BAZ. About 3 mm thick disks of the obtained storage roots were transversely cut and used for analyses without any further preparation.

**Standards.** Falcarinol and falcarindiol were isolated from carrot roots by column chromatography and preparative high-performance liquid chromatography according to the method described by Kidmose et al. (5). Both polyacetylenes were identified by mass spectrometry (EI, 70 eV) and by one-dimensional and two-dimensional NMR ( $^1\text{H}$  and  $^{13}\text{C}$  NMR and  $^1\text{H}$ – $^1\text{H}$  and  $^1\text{H}$ – $^{13}\text{C}$  correlation spectroscopy) as previously described (5), and the data obtained were found to be in accordance with literature values (1). Falcarinol and falcarindiol dissolved in ethanol (3) and starch isolated from potato (Sigma-Aldrich, Germany) were used as standards.

**Histochemical Analysis.** Histochemical staining of starch was done by immersion of hand-sectioned root disks in Lugol's aqueous solution (10% potassium iodide, 5% iodine); starch was stained dark purple-black. Toluidine blue (0.05%) aqueous solution was used for identification of lignified cell walls that stained light blue.

**Raman Spectroscopy.** 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. Carrot root disks were mounted between two glass slides to avoid their movement and deformation during the measurement.

Single measurements from carrot roots and starch standard were obtained with 128 scans and an unfocused laser beam of 200 mW, whereas for falcarinol and falcarindiol solutions, 64 scans and a laser power of 100 mW were used for spectroscopic analysis. All spectra were obtained with a spectral resolution of  $4\text{ cm}^{-1}$  in the wavenumber range from 100 to  $4000\text{ cm}^{-1}$ . Two-dimensional Raman maps were obtained point by point by moving the  $xy$  stage;  $x$  and  $y$  directions of the accessory were automatically controlled by the spectrometer software. All parameters used for micro-Raman measurements, such as mapping area, step size (increment), laser power, and number of scans for each measured point, are given in the figure captions. The samples were irradiated with a focused laser beam of  $\sim 0.1$  mm in diameter. The spectra collected from the mapped areas were baseline corrected and processed by the Bruker Opus/map software package v. 4.3. The maps were obtained by integration of specific signal characteristics for the individual analyte and colored according to the Raman intensity.



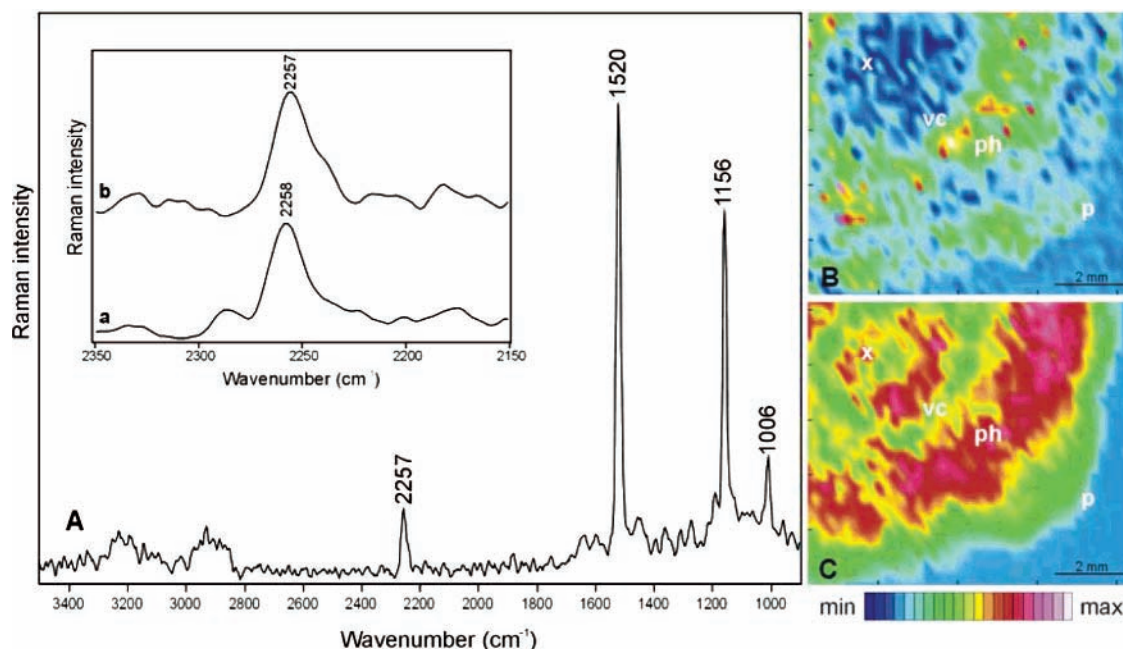
**Figure 2.** NIR-FT-Raman spectra of falcarinol and falcarindiol isolated from carrot (dissolved in ethanol solution).

## RESULTS AND DISCUSSION

**Raman Spectra of Polyacetylenes in Carrot.** Two main polyacetylenes can be detected in carrot root (1–7). Both compounds possess a similar molecular structure with two adjacent triple bonds substituted with one  $-\text{OH}$  group (falcarinol) and two  $-\text{OH}$  groups (falcarindiol), respectively (Figure 1). The frequency of the polyacetylene  $-\text{C}\equiv\text{C}-$  stretching modes depends not only on the number of triple bonds but also on the type of substituents (18). Thus, the spectroscopic position of  $-\text{C}\equiv\text{C}-$  vibrations and the pattern of Raman bands usually provide enough information to recognize the type of substitution and to identify polyacetylenes (19). Generally, for compounds containing a  $-\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-$  grouping, the characteristic, strong and polarized, symmetric stretch of the  $\text{R}-\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-\text{R}'$  structure should be seen at  $2257\text{--}2251\text{ cm}^{-1}$  in the Raman spectrum (20).

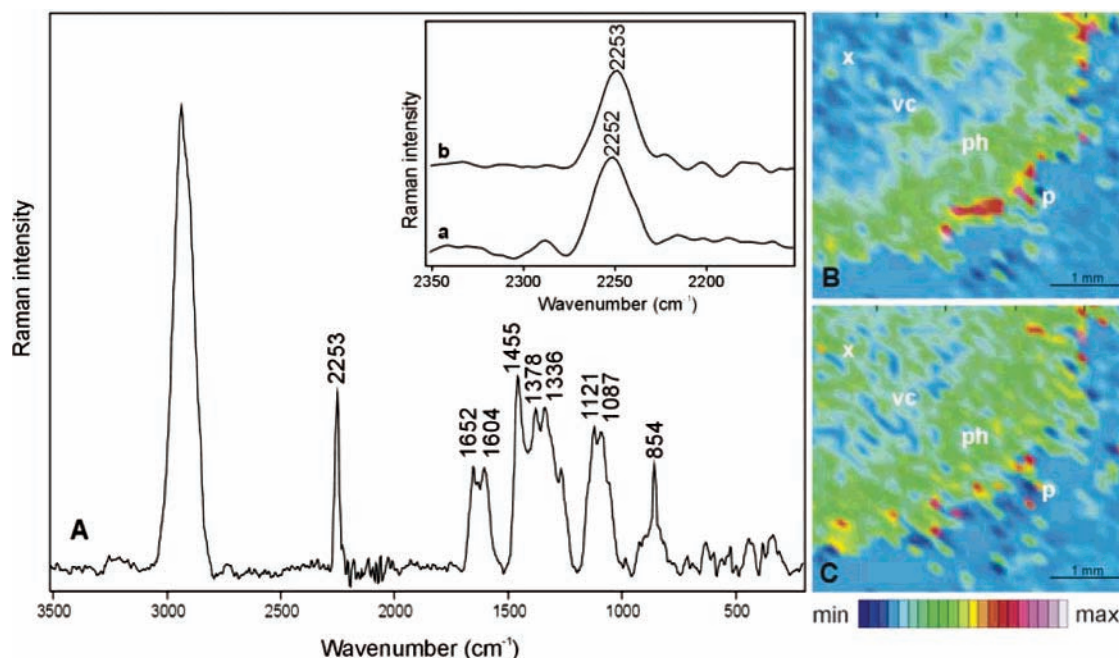
In Figure 2 Raman spectra of falcarinol and falcarindiol isolated from carrot roots and dissolved in ethanolic solution are presented. The symmetric stretch of the  $-\text{C}\equiv\text{C}-\text{C}\equiv\text{C}-$  vibration is observed at  $2258\text{ cm}^{-1}$  for falcarinol and at  $2252\text{ cm}^{-1}$  for falcarindiol. We have already identified tentatively the position of the falcarindiol band at  $2252\text{ cm}^{-1}$  in the Raman spectra of *Angelica dahurica* (Hoffm.) root (21) as well as in carrot root (18), but here we present the confirmation of this assignment based on the spectrum of a pure standard isolated from carrots. The shift of the triple-bond vibration registered for both polyacetylenes is only  $6\text{ cm}^{-1}$ , but it is sufficient to discriminate between both compounds.

**Polyacetylenes and Carotenoids in Cultivated Orange Carrot.** Recently, we reported that the spectra measured at different spots of orange carrot roots usually revealed a complex structure of a  $-\text{C}\equiv\text{C}-$  band and a slight shift in the band position depending on the location of the measurement (19). Also in the current study, the spectra taken from roots of the high-carotene carrot (*D. carota* ssp. *sativus* Hoffm.) line HCM (high carotene mass) (22) usually show a few overlapping bands in the range between  $2260$  and  $2250\text{ cm}^{-1}$ . However, in phloem tissue close to the vascular cambium the maximum intensity of the polyacetylene signal is observed at  $\sim 2257\text{ cm}^{-1}$  (Figure 3A). A closer inspection of this band (see the upper corner of Figure 3A) indicates that falcarinol is the main polyacetylene present in this area. As can be seen in Figure 3B, the accumulation of polyacetylenes is located in the outer section of the root, that is, in pericyclic parenchyma tissue close to the periderm, and, to a higher extent, in secondary phloem tissue close to the vascular cambium. Contrary to that, xylem parenchyma contains only a small amount of polyacetylenes. The localization of the polyacetylenes can be attributed to the presence of vascular bundles in a young secondary phloem as well as pericycle oil channels in the vicinity of the periderm.



**Figure 3.** (A) Raman spectrum taken from the root of a cultivated orange carrot (*D. carota* ssp. *sativus*) line HCM in the spot with high polyacetylene content; in the upper corner a closer inspection of the polyacetylene  $-\text{C}\equiv\text{C}-$  band observed in falcarinol (a) and in carrot root (b). (B, C) Raman maps obtained from a quarter of a transversely cut root of HCM colored according to the intensity of the polyacetylene  $-\text{C}\equiv\text{C}-$  band in the range of  $2238\text{--}2268\text{ cm}^{-1}$  (B) and to the intensity of the carotene  $-\text{C}=\text{C}-$  band in the range of  $1515\text{--}1522\text{ cm}^{-1}$  (C); p, periderm; ph, secondary phloem; vc, vascular cambium; x, secondary xylem (mapping parameters: area =  $8300 \times 5300\ \mu\text{m}$ , increment =  $200\ \mu\text{m}$ , laser power =  $200\text{ mW}$ , no. of scans per point = 6).





**Figure 4.** (A) Raman spectrum taken from the carrot root of *D. carota* ssp. *maritimus* in the spot with high polyacetylene content; in the upper corner a closer inspection of the polyacetylene  $\text{—C}\equiv\text{C—}$  band observed in falcarindiol (a) and in carrot root (b). (B, C) Raman maps obtained from a quarter of a transversely cut root of *D. carota* ssp. *maritimus* colored according to the intensity of the polyacetylene  $\text{—C}\equiv\text{C—}$  band in the range of  $2238\text{—}2268\text{ cm}^{-1}$  (B) and to the intensity of pectin the  $\text{—C—O—C—}$  vibration in the range of  $844\text{—}864\text{ cm}^{-1}$  (C); p, periderm; ph, secondary phloem; vc, vascular cambium; x, secondary xylem (mapping parameters: area =  $5300 \times 5900\ \mu\text{m}$ , increment =  $100\ \mu\text{m}$ , laser power =  $150\ \text{mW}$ , no. of scans per point = 4).

These channels could be responsible for the transport and accumulation of polyacetylenes (9).

In addition to the polyacetylene band, three carotenoid signals, mainly related to  $\alpha$ - and  $\beta$ -carotene, can be seen in **Figure 3A**. Bands at  $1520$  and  $1156\text{ cm}^{-1}$  can be assigned to in-phase  $\text{—C=C—}$  ( $\nu_1$ ) and  $\text{—C—C—}$  ( $\nu_2$ ) stretching vibrations of the carotenoid polyene chain, whereas the signal at  $1006\text{ cm}^{-1}$  is due to in-plane rocking modes of  $\text{CH}_3$  groups attached to the main chain and coupled with  $\text{—C—C—}$  bonds (23–25). Carotenoids are present in carrot root in amounts of several milligrams per kilogram, but a strong enhancement of their bands is observed in the NIR-Raman spectrum due to the known preresonance effect (26).

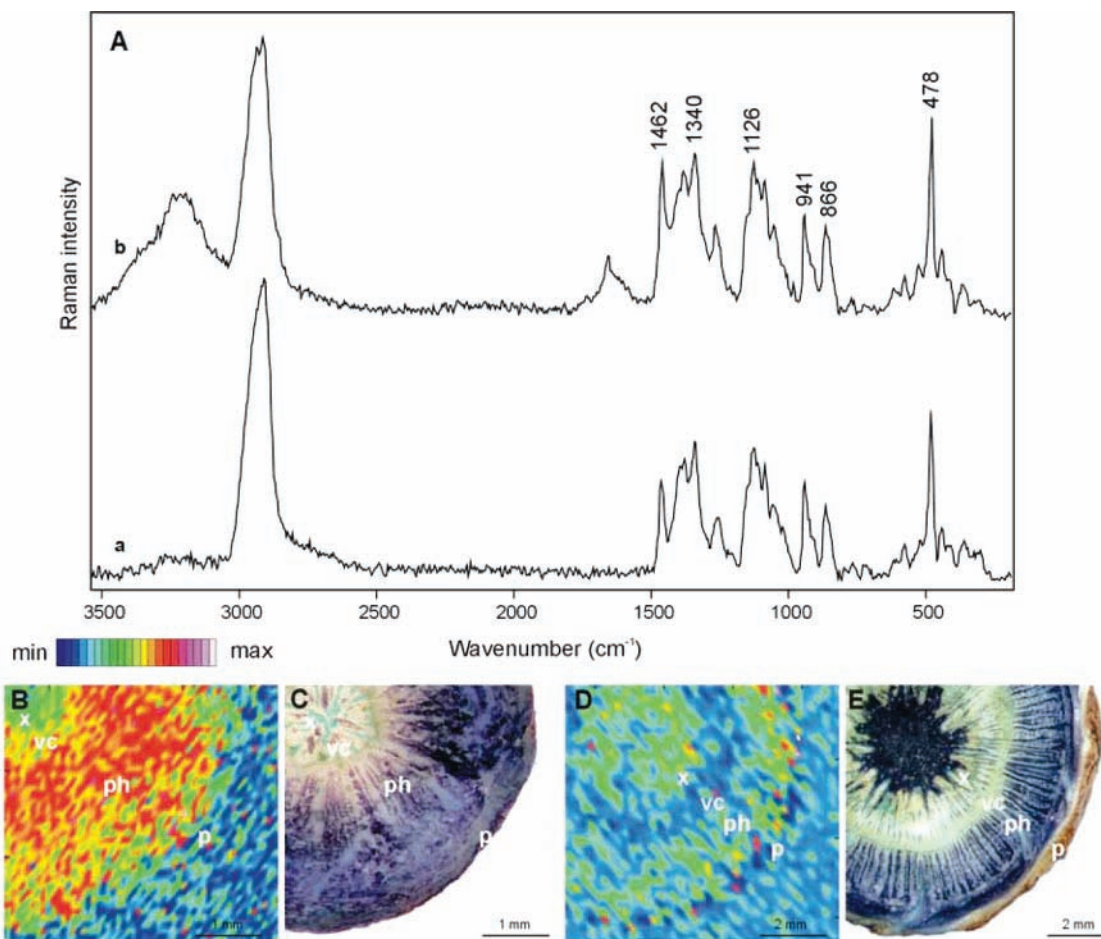
The presence of strong carotenoid signals in the Raman spectrum of carrot root gives a good precondition to apply mapping techniques to investigate simultaneously the distribution of carotenoids (23, 27) and polyacetylenes. Thus, in correspondence to the polyacetylene map (**Figure 3B**) the Raman map of carotenoids in the same HCM root disk is presented in **Figure 3C**. As can be seen here, carotenoids are not uniformly distributed over the root section. The highest accumulation is found in the phloem and xylem parenchyma, whereas pericyclic parenchyma and tissue along the vascular cambium exhibit lower amounts of these compounds. The carotenoid distribution in the phloem was essentially the same for all analyzed orange cultivars. However, a high concentration of carotenoids detected in the xylem has been observed only for carrots with elevated total carotene content, that is, lines HCM and Beta III. A comparison of the polyacetylene and carotene distribution in carrot root allows some conclusions to be drawn (see **Figure 3B,C**). The accumulation of polyacetylenes observed in a young secondary phloem close to the vascular cambium relates to the high concentration of carotenes, but the maximum concentration of both components is not identified at the same areas. Carrots containing high amounts

of carotenoids possess also high levels of polyacetylenes; especially roots of line HCM showed a significantly elevated concentration of polyacetylenes. HCM roots may reach a carotene content of even  $500\text{ mg/kg}$  (22), whereas the other orange cultivars possess usually amounts of  $80\text{—}140\text{ mg/kg}$  (28). We have also found that yellow carrot roots contain lower levels of polyacetylenes than orange ones, which is in agreement with the above statement.

**Polyacetylenes and Pectin in Wild Carrot.** The single spectrum measured in the root of wild carrot *D. carota* ssp. *maritimus* (Lam.) Batt. in a polyacetylene-rich area exhibits a strong  $\text{—C}\equiv\text{C—}$  stretching vibration mode at  $2253\text{ cm}^{-1}$  (**Figure 4A**). A closer inspection of this signal (see the upper corner of **Figure 4A**) indicates that falcarindiol is the main polyacetylene in this carrot. Furthermore, the observed polyacetylene band is symmetric, and the position as well as its structure does not depend on the location of the measurement.

The Raman map presenting the distribution of polyacetylenes for a transversely cut root of *D. carota* ssp. *maritimus* is shown in **Figure 4B**. The whole phloem tissue is rich in polyacetylenes, but the maxima can also be observed near the pericyclic parenchyma. Thus, the polyacetylene distribution is different in comparison with orange carrot root. The polyacetylene accumulation in this wild carrot is observed in similar root tissue as the area presenting a high carotenoid level in the phloem of orange carrot. Analogous distribution of polyacetylenes has been also observed in roots of other carrot wild species, *D. carota* ssp. *gummifer*, *D. carota* ssp. *commutatus*, and *D. halophilus*.

The significant amounts of falcarindiol found in *Daucus* roots can be related to a known resistance of carrot wild relatives to some diseases, for example, root knot nematodes (29). However, on the basis of literature reports, we suspect that this compound has a major influence on the bitter taste of carrot. Contrary to that, falcarinol, present in high amounts in orange cultivated carrot, has only little effect on its bitter taste and additionally



**Figure 5.** (A) Raman spectra taken from pure potato starch (a) and from a root of white carrot (*D. carota* ssp. *sativus* Niiza Ettou Gosun) in the spot with high starch content (b). (B, D) Raman maps obtained from a quarter of a transversely cut root of Niiza Ettou Gosun (B) and *D. carota* ssp. *maritimus* (D) colored according to the band intensity in the range of 460–510  $\text{cm}^{-1}$  related to the content of starch; (C, E) sections of the same carrots (C and E, respectively) stained with Lugol's solution; p, periderm; ph, secondary phloem; vc, vascular cambium; x, secondary xylem [mapping parameters: (B) area =  $3000 \times 4100 \mu\text{m}$ , increment =  $200 \mu\text{m}$ , laser power = 75 mW, no. of scans per point = 4; (D) area =  $8100 \times 6700 \mu\text{m}$ , increment =  $100 \mu\text{m}$ , laser power = 200 mW, no. of scans per point = 4].

is able to play an important role in the health-promoting properties of carrots (3–6). This can be seen as a consequence of the selection carried out during the breeding of cultivated forms, which aimed to obtain carrots of better taste (high sugar content, low bitterness). As a result of the selection, the level of falcarindiol might decrease.

In addition to the polyacetylene band, other strong signals can be seen in **Figure 4A**, which are attributed to the pectin present in *D. carota* ssp. *maritimus* root. Pectin is a linear polysaccharide containing D-galacturonic acid as the principal constituent; the D-galacturonic acid units are linked by  $\alpha$ -(1 $\rightarrow$ 4) glycosidic bonds. The most characteristic bands in the spectrum presented in **Figure 4A** can be identified at 854  $\text{cm}^{-1}$  (C–O–C skeletal mode of  $\alpha$ -anomer carbohydrate), a doublet at 1087 and 1121  $\text{cm}^{-1}$  (C–O– stretch of carbohydrates), at 1336 and 1378  $\text{cm}^{-1}$  (C–H deformation), at 1455  $\text{cm}^{-1}$  (O–CH<sub>3</sub> stretch), and at  $\sim 3000 \text{cm}^{-1}$  (C–H stretch) (30, 31). Signals at 1604 and 1652  $\text{cm}^{-1}$  can be assigned to aromatic ring vibrations caused by some components of the plant matrix, for example, lignin. The polygalacturonic acid can be partly esterified with methyl groups; the ratio of esterified galacturonic acid groups to total galacturonic acid groups is termed the “degree of esterification” (DE) and has a vital influence on the properties of pectin, especially the solubility and the gel-forming characteristics (31). The intensity of the ester carbonyl C=O stretch mode can be seen in the vibrational spectrum

of pectin at  $\sim 1740 \text{cm}^{-1}$  (30–32); therefore, we can recommend the use of this band as an indicator for the DE value. In the spectrum presented in **Figure 4A**, the discussed band can be hardly seen; thus, we suppose that in the investigated carrot the amount of esterified galacturonic acid groups is very low.

The best Raman indicator band for pectin is seen in the fingerprint region at 854  $\text{cm}^{-1}$ , which can be used for mapping purposes. **Figure 4C** displays a Raman map colored according to the intensity of this band showing the distribution of pectin in the investigated carrot root. Pectin can be found in all root tissues because it is one of the major carbohydrate constituents of plant cell walls; however, its lower amount can be seen along the vascular cambium, in an active region of cell proliferation.

**Starch Distribution.** The Raman spectrum measured in a root of white cultivated carrot (*D. carota* ssp. *sativus* Niiza Ettou Gosun) shows the presence of starch (**Figure 5A**, spectrum b). The most characteristic and intense Raman band at 478  $\text{cm}^{-1}$  can be assigned to the skeletal mode of starch vibration (30, 33). Other bands seen in the range between 800 and 1500  $\text{cm}^{-1}$  resemble a typical signal scheme characteristic for polysaccharides. However, the exact position and ratio of the bands observed in spectrum b taken from the carrot are characteristic for starch polysaccharides. Confirmation of this fact is obtained from spectrum a, which has been measured from pure starch, isolated from potato. Additional signals observed in spectrum b at about 1600  $\text{cm}^{-1}$  and above 3000  $\text{cm}^{-1}$  are related to

contributions of the plant matrix, for example, water and lignin. The quality of the spectrum taken from a root region rich in starch is comparable with the one taken from isolated potato starch and indicates that the high crystallinity of the intact granules is well reflected in the spectrum measured in the carrot.

The presence of a characteristic starch signal at  $478\text{ cm}^{-1}$  in the Raman spectrum of carrot root gives a good precondition to use the mapping technique to investigate the distribution of starch in the carrot. As can be seen in **Figure 5B**, starch is not uniformly distributed over a cross section of the root. This polysaccharide can be seen mainly in the whole root, with a significant maximum in the phloem tissue, which corresponds well with the localization of starch revealed by a histochemical staining in Lugol's solution (**Figure 5C**). The staining shows that starch accumulation can be found in a starlike pattern in a cross section of the root, originating at the cambial ring and extending toward the periderm. This pattern coincides with the localization of phloem rays. The results are in agreement with previous studies concerning orange carrot (13, 14), although in white carrot the accumulation is much more prominent.

The distribution of starch has been also studied in *D. carota* ssp. *maritimus* (wild carrot) root. The Raman map presented in **Figure 5D** has been obtained on the basis of the intensity of the starch marker band at  $478\text{ cm}^{-1}$ . A high accumulation of this polysaccharide is located in the parenchymatic center of the root, phloem, and peripheral parenchyma. In contrast, xylem tissue contains a very low amount of starch, which was confirmed by a faint coloration in histochemical staining (**Figure 5E**). The observed difference between analyzed samples in starch accumulation is due to the anatomy of the central part of the root, which is usually much smaller in cultivated carrots. A high starch level found in white carrots may be a valuable novel source of this carbohydrate. Among root umbellifer crops, a commercially valuable amount of starch has been identified only in Peruvian carrot (*Arracacia xanthorrhiza* Bancr.), which is used in Central and South America (34).

The results presented confirm that NIR-FT-Raman spectroscopy and particularly Raman mapping are powerful tools which can provide very informative data for the localization of polyacetylenes, carotenoids, pectin, and starch in plant material. These components can be detected directly in situ in fresh plant samples without the necessity to apply preliminary preparation techniques. Furthermore, the distribution of the mentioned plant components can be measured at the same time, so the relative concentration of these substances in various tissues can be compared. The possible correlation between the concentration of carotenoids and polyacetylenes is of general interest for human health, but detailed investigation should be performed in further studies.

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