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Comparison of different clean-up methods for simultaneous HPLC determination of carotenoids and polyacetylenes in carrot roots

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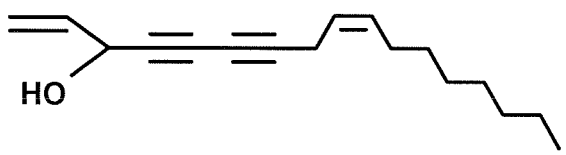
Summary

Carrot roots and leaves contain various polyacetylenes such as falcarinol, falcarindiol and falcarindiol acetate with putative beneficial effects on human health, however, these polyacetylenes are also made responsible for the occasional unpleasant bitter taste of carrot juice. Therefore, the aim of this study was to develop an accelerated sample preparation method for simultaneous extraction of carotenoids and polyacetylenes followed by fast and reliable routine HPLC-DAD and HPLC-MS quantification. The individual recoveries of the analytes obtained by ASE-HPLC were compared to those obtained by Soxhlet extraction and solid phase extraction methods, respectively. It has been found that ASE show significantly better extraction efficiency in comparison to the other clean-up procedures. Furthermore, identical ASE parameters can be used for the clean-up of carotenoids and polyacetylenes. In order to further speed up sample preparation a subsequent concentration step using a rotary vacuum concentrator (Christ, Osterode, Germany) was performed. The novel extraction procedure was found to be a very useful tool especially applicable for routine analysis, e.g. in plant breeding and evaluation of genetic resources or quality control in food industry. The combination of ASE and rotary vacuum concentration allows high-throughput analysis also ensuring high sensitivity and reproducibility by using HPLC-DAD and HPLC-MS methods for subsequent identification and quantification of the individual analytes.

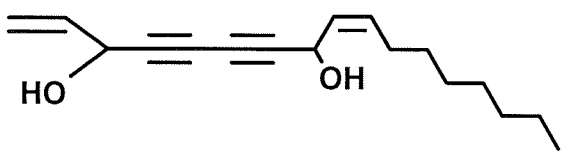
1 Introduction

Carrot roots and leaves may contain various polyacetylenes with falcarinol [(Z)-heptadeca-1,9-dien-4,6-diin-3-ol] (FaOH), falcarindiol [(Z)-heptadeca-1,9-dien-4,6-diin-3,8-diol] (FaDOH) and falcarindiol-3-acetate [(Z)-3-acetoxy-heptadeca-1,9-dien-4,6-diin-8-ol] (Fig. 1) being the most prominent representatives (LUND and MARION, 1990).

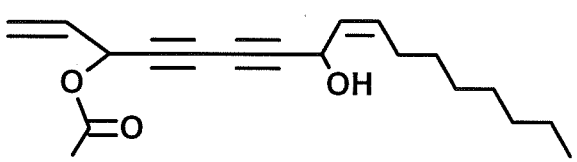
Several beneficial effects on human health have been described, however occasionally bitter taste of carrot juices has been observed (HANSEN et al., 2003; CZEPA and HOFMANN, 2004). Recently, FT-Raman spectroscopy has been successfully applied to localize polyacetylenes and carotenoids in the tissues of different carrot cultivars (BARANSKA and SCHULZ, 2005; BARANSKA et al., 2006). First attempts have been made to use accelerated solvent extraction (ASE) for the extraction of natural food colorants (BREITHAUPT, 2004). Various methods have been also described for determination of carotenoids and polyacetylenes in carrot roots by means of HPLC-DAD and HPLC-MS which are widespread used in food industry and plant breeding. For the latter purpose fast analysis of carotenoids and polyacetylenes with high reproducibility at high sample throughput is a prerequisite. However, the published methods do not allow simultaneous extraction and analysis of carotenoids and polyacetylenes within a very short time (FIKSELOVA et al., 2008; KREUTZMANN et al., 2008). Therefore, acceleration of sample clean-up is a valuable measure to eliminate the analytical bottleneck (RICHTER et al., 1996).



(3R)-Z-heptadeca-1,9-dien-4,6-diin-3-ol (falcarinol)



(3R, 8S)-Z-heptadeca-1,9-dien-4,6-diin-3,8-diol (falcarindiol)



Z-3-acetoxy-heptadeca-1,9-dien-4,6-diin-8-ol (falcarindiol-3-acetate)

Fig. 1: Structural formula of falcarindiol (FaDOH), falcarinol (FaOH) and falcarindiol-3-acetate

Furthermore, costs for consumables, solvents, eluents, equipment and manpower need to be considered when large sample numbers have to be analyzed. Until today the extraction of carotenoids are usually performed by solid phase extraction (SPE) (PUTZBACH et al., 2005; IWASE, 2002), whereas polyacetylenes are usually isolated by Soxhlet extraction (MILADI et al., 2008; SCHINKOVITZ and VERHEUL, 2008). Both methods have been found to be unsuitable for rapid screening of carotenoids and polyacetylenes. Therefore, the aim of this study was to establish a simultaneous sample preparation method for carotenoids and polyacetylenes followed by fast HPLC-DAD/HPLC-MS analysis combining high sensitivity and good reproducibility with high sample throughput.

2 Materials and methods

2.1 Materials

Petroleum ether and ethyl acetate were obtained from Roth (Karlsruhe, Germany). Methanol, acetonitrile and n-butanol were from Merck (Darmstadt, Germany). HPLC reference substances were purchased from different sources: α-carotene from LGC Standards (Wesel, Germany), β-carotene and lycopene from Sigma-Aldrich (Taufkirchen, Germany) and lutein from Roth (Karlsruhe, Germany). For accelerated solvent extraction reflex pearls of unleaded lime

sodium glass (Ø 1.7-2.0 mm) from Roth (Karlsruhe, Germany) and ASE frit protection glassfiber/cellulose filters (Ø 19.8 mm) from Dionex (Idstein, Germany) were used. For HPLC-MS analysis HPLC-pure water was applied. Pure polyacetylene standards were purchased from two different sources: falcarindiol from SiChem (Bremen, Germany) and falcarinol from Toroma organics (Saarbrücken, Germany). In order to improve the detection sensitivity a vacuum rotary concentrator RVC 2-18 (Christ, Osterode, Germany) and a sample concentrator SE 400 (Dionex, Idstein, Germany) were applied.

2.1.1 Plant material

For the optimization of accelerated solvent extraction different types of wild and cultivated carrots (*Daucus carota* L.) from different gene banks and breeders were used. Carrots were cultivated in the greenhouses of the Julius Kühn-Institut (Federal Research Centre for Cultivated Plants) in Quedlinburg, Germany. To develop optimal extraction conditions for carotenoid determination in carrot roots, orange cv. 'Nerac F1' and red cv. 'Nutrired' carrot cultivars were used. For polyacetylene determination, wild carrot roots were analysed. The simultaneous extraction of carotenoids and polyacetylenes was performed with both carrot types since even cultivated carrots were reported to contain polyacetylenes (CHRISTENSEN and KREUTZMANN, 2007). To retain chemically labile compounds, carrot roots were gently handled in the greenhouse and in the laboratories of the JKI.

2.2 Sample preparation

Harvested carrots were washed, and the foliage was removed. Roots were manually cut into cubes (1 x 1 cm) and immediately stored deep-frozen and hermetically treated at -80 °C for at least 3 hours. Subsequently, the carrot cubes were lyophilized (Christ Gamma 1-16 LSC vers. 2.233) for 72 hours. The freeze-dried samples were milled for 2 minutes in a ball mill (type MM 301, Retsch, Haan, Germany) obtaining a homogeneous powder. Aliquots of 0.5 g of carrot powder were mixed with 14 g reflex glass pearls and filled into 11 mL ASE extraction cells. The bottom layer of the extraction cells was separated from the probe by a glass fiber cellulose ASE frit protection filter (Dionex, Idstein, Germany). Automated accelerated sample extraction was performed using an ASE-200 sample carousel allowing serial extraction of 26 samples. Extraction time for carotenoids and polyacetylenes was set to 15 minutes per sample by applying the ASE specified covered below.

2.3 Extraction parameters

According to Breithaupt (BREITHAUPT, 2004) eight ASE variables were adapted to the extraction of carotenoids and polyacetylenes. The reduction of extraction times and the simultaneous extraction of both compound classes was the main aim of the present study. To optimize the extraction temperature, pressure, preheating time, heating period, static interval, flushing, purging and number of cycles were varied in separate trial runs. For carotenoid extraction optimal ASE-200 settings were as follows: pressure 70 bar; temperature 40 °C; time for preheating 0 min.; heating period 5 min.; static interval 2 min.; flush settings 70 %; purge interval 50 sec.; cycles 3; cell volume 11 mL. Optimal results for polyacetylene extraction were achieved applying the following parameter settings: pressure 50 bar; temperature 50 °C; preheating 2 min.; heating period 5 min.; static interval 2 min.; flush settings 100 %; purge interval 30 sec.; cycles 1; cell volume 11 mL. For simultaneous extraction of carotenoids and polyacetylenes, temperature was limited at 40 °C, since at higher values "plugging" may occur, thus reducing the extraction yield (BREITHAUPT, 2004). Pressure was set to 70 bar avoiding the formation of channels in the probe, which may result in incomplete sample extraction due to insufficient interaction between sample and solvent (BREITHAUPT, 2004). From variation experiments the following ASE parameters were found to be most suitable for simultaneous carotenoid and polyacetylene extraction: preheating 0 min; heating period 5 min; static interval 2 min; flush settings 70 %; purge interval 50 sec; cycles 3; cell volume 11 mL.

2.4 Composition of ASE solvents

Analogous to a previous study (BREITHAUPT, 2004), different solvent compositions for the extraction of carotenoids and polyacetylenes were applied. In the present study n-hexane, ethanol/n-hexane (4:3, v/v) and ethyl acetate/petroleum ether/methanol (1:1:1, v/v/v), respectively, were used for extraction. The ternary system consisting of ethyl acetate, petroleum ether and methanol was found to be most suitable for extraction of both analytes (Fig. 2 and Fig. 3).

2.5 Sample concentration

The extraction of carotenoids and polyacetylenes from 0.5 g of carrot powder yielded samples of approx. 11 mL. The solvents were evaporated using a rotary concentrator (RVC 2-18, Christ, Osterode, Germany) and adjusted to 2.0 mL with the solvent mixture (ethyl acetate, petroleum ether and methanol, 1:1:1, v/v/v). These solutions were filtered through a disposable syringe filter (PET-45/15, Chromafil) prior to carotenoid and polyacetylene analysis performed by HPLC-DAD and HPLC-MS.

2.6 HPLC calibration

For quantification purposes calibration functions of lutein, α-carotene, β-carotene and lycopene were established. The linearity within the individual concentration range presented correlation coefficients of 0.999, 0.998, 0.996 and 0.992 for lutein, α-carotene, β-carotene

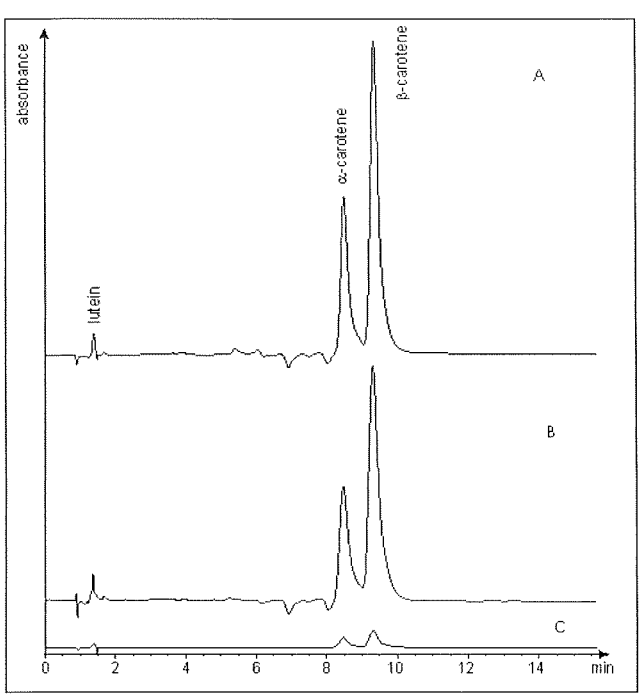


Fig. 2: HPLC analysis of different carotenoids including lutein, α-carotene and β-carotene obtained by ASE from cultivated carrots using different solvent mixtures (A: ethyl acetate/petroleum ether/methanol (1:1:1 v/v/v), B: ethanol/n-hexane (4:3, v/v), C: n-hexane)

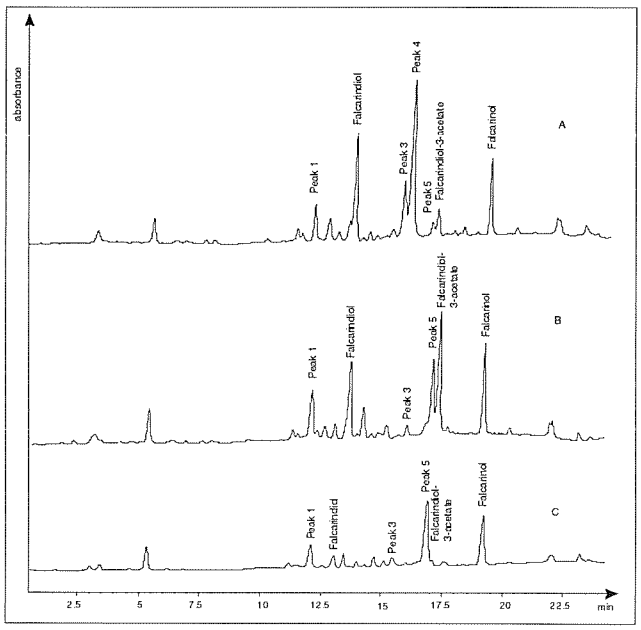


Fig. 3: HPLC analysis of different polyacetylenes including falcarinol, falcarindiol and falcarindiol-3-acetate obtained by ASE from cultivated carrots using different solvent mixtures (A: ethyl acetate/petroleum ether/methanol (1:1:1 v/v/v), B: ethanol/n-hexane (4:3, v/v), C: n-hexane, peaks 1-5: unknown polyacetylenes)

and lycopene, respectively. Isolated pure standard samples of FaOH and FaDOH were used for polyacetylene quantification in carrot roots. For the identification of FaDOH-3-acetate HPLC-MS was used because of the unavailable reference substance. The linearity within the individual concentration ranges showed correlation coefficients of 0.947, 0.987 and 0.962 for FaOH, FaDOH and FaDOH-3-acetate.

2.7 HPLC-DAD and HPLC-MS analyses of carotenoids and polyacetylenes

According to previous studies (SLIMESTAD et al., 2005; VASQUEZ CAICEDO et al., 2006) for identification and quantification of carotenoids a Develosil RP-aqueous C30 (150 x 3 mm; 3 µm) column (Phenomenex, Aschaffenburg, Germany) equipped with a 60 mm guard column (Agilent, Böblingen, Germany) and a protection frit (Ø 4.6 mm) was used. The column temperature was set to 30 °C. The mobile phase consisted of n-butanol (A) and acetonitrile (B). The flow rate was set to 0.95 mL/min. The following linear gradient was used (min/% (A): 0/20; 6/30; 12/20; 16/20; the total run time was 16 min. The injection volume for all samples was 10 µL. Alpha-carotene and lutein were monitored at 488 nm, β-carotene at 455 nm and lycopene at 476 nm. HPLC analyses were performed with an HP1100 system (Agilent, Böblingen, Germany) equipped with a diode array detector (DAD). For data processing Agilent ChemStation software (B.03.02) was applied.

For the analysis of polyacetylenes an HPLC-MS system series 1200 (Agilent, Böblingen, Germany) coupled to an Esquire 3000 ion trap mass spectrometer with an APCI interface (Bruker Daltonik GmbH, Bremen, Germany) was used. A Zorbax Eclipse XDB-C18 column (150 x 3 mm; 3.5 µm) with a guard column (60 mm) and protection frit (Ø 4.6 mm) was applied and kept at 30 °C. For data processing Agilent ChemStation software (B 01.03) and Bruker Esquire Control (version 5.3) were used. All polyacetylenes were monitored at 245 nm. For gradient elution water (A) and methanol (B) were used the following gradient (min/% (A): 0/40; 24/5; 27/5; 29/40; 35/40 (flow rate: 0.5 mL/min; injection volume: 5 µL); the total run time needed was 35 minutes. For mass spectrometric detection the following conditions were applied: APCI source, positive mode; nebulizer (N_2): 25.0 psi; dry gas (N_2): 9.0 L/min; drying temperature: 300 °C; vaporizer temperature: 450 °C; scan: 50-900.

3 Results and discussion

Generally, results obtained for carotenoid and polyacetylene analysis in carrot samples by using accelerated solvent extraction presented high reproducibility and significant reduction of analysis time in comparison to formerly described analytical methods (BREITHAUPT, 2004; PFERSCHY WENZIG et al., 2009). To verify the applicability of the new ASE method the process was compared with conventional extraction methods applying SPE and Soxhlet extraction with methanol/methylene chloride (SPE; 45:55, v/v) and n-hexane (Soxhlet) as solvents (Fig. 4 and Fig. 5). It was found that the ASE method provides better extraction results in comparison to the established Soxhlet and SPE procedures.

Conventional methods for carotenoid (FIKSELOVA et al., 2008; MARX et al., 2000) and polyacetylene (KREUTZMANN et al., 2008; CZEPA and HOFMANN, 2003) analysis have been optimized for carrot juice, purée or sticks. Usually, fresh carrot samples are applied for extraction process which does not guarantee an exhaustive extraction and therefore comparatively high percentages of the analytes may remain in the pomace (JARAMILLO-FLORES et al., 2005). When carrot sticks are used for the extraction process, only small amounts of carotenoids and polyacetylenes could be detected in the extract due to the short interaction time between the solvent and the plant material to be extracted. In contrast varying contents and the spatial distribution of carotenoids (BOOTH, 1951) and polyacetylenes (BARANSKA and SCHULZ, 2005) in carrot roots do not negatively affect analytical results when homogenized and freeze-dried carrot powder is used for extraction. According to former studies (BREITHAUPT, 2004) three solvent systems (n-hexane, ethanol/n-hexane (1:1, v/v) and ethyl acetate/petroleum ether/methanol (1:1:1, v/v/v)) were tested with regard to carotenoid and subsequent polyacetylene extraction rates. In

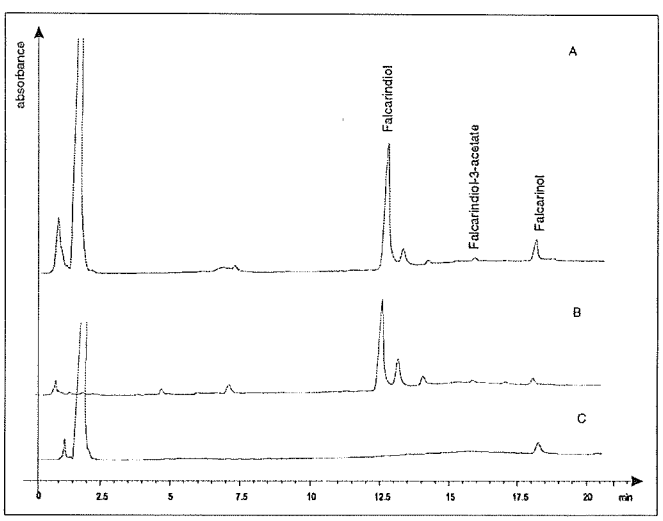


Fig. 4: HPLC analysis of different polyacetylenes including falcarinol, falcarindiol and falcarindiol-3-acetate obtained by ASE, SPE and Soxhlet extraction from cultivated carrots using different solvent mixtures A: (ASE; ethyl acetate/petroleum ether/methanol (1:1:1, v/v/v), SPE (methanol/methylene chloride (45:55, v/v) and Soxhlet (n-hexane); A: ASE; ethyl acetate/petroleum ether/methanol (1:1:1), B: Soxhlet; n-hexane, C: SPE; methanol/methylene chloride (45:55, v/v)

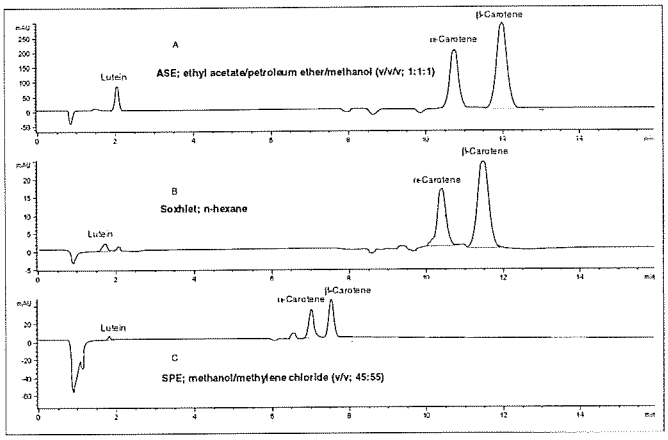


Fig. 5: HPLC analysis of different carotenoids including lutein, α-carotene and β-carotene obtained by ASE, Soxhlet and SPE extraction from cultivated carrots using different solvent mixtures A: ASE; ethyl acetate/petroleum ether/methanol (1:1:1), B: Soxhlet; n-hexane, C: SPE; methanol/methylene chloride (45:55, v/v)

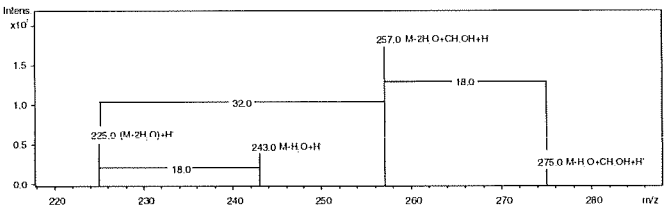


Fig. 6: Fragmentation of pure falcarindiol (1.53 µg dissolved in 1 µL acetonitrile)

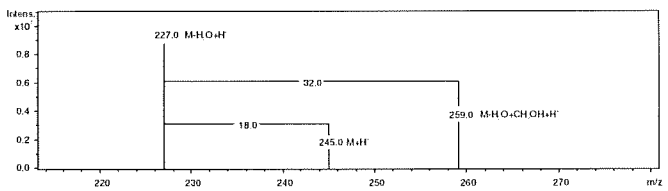


Fig. 7: Fragmentation of pure falcarinol (5.20 µg dissolved in 1 µL acetonitrile)

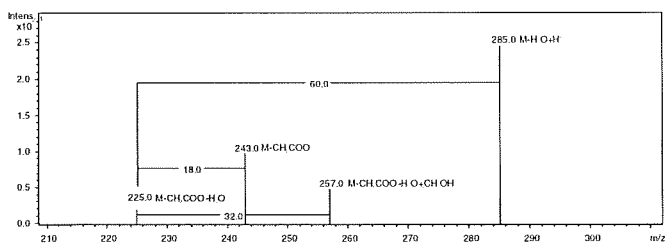


Fig. 8: Fragmentation of pure falcarindiol-3-acetate (2.52 µg dissolved in 1 µL acetonitrile)

this context the solvent mixture consisting of ethyl acetate, petroleum ether and methanol revealed best results for the simultaneous extraction of the analytes (Fig. 2 and Fig. 3). Evaporation of the solvent using a sample concentrator SE 400 and a rotary vacuum concentrator RVC-18 (Christ, Osterode, Germany) guaranteed comparatively high concentration of carotenoids and polyacetylenes. The first approach provided reliable results, but needed high amounts of nitrogen for the concentration process. In contrast rotary vacuum concentration presents a high automation standard and significant lower operation costs by operating without nitrogen. A sample amount of only 0.5 g of freeze-dried carrot powder was necessary for simultaneous extraction of analytes. Based on the experience that water content of carrots is approx. 90 % (SCHERZ and SENSER, 2000) 5 g of fresh carrot root material is sufficient for routine analysis of carotenoids and polyacetylenes. By performing ASE of freeze-dried carrot powder with a solvent mixture of ethyl acetate/petroleum ether/methanol (1:1:1, v/v/v) simultaneous extraction of carotenoids and polyacetylenes within approx. 15 minutes can be achieved. Using the HPLC-DAD method for carotenoid analysis described in this study the determination of lutein, α-carotene and β-carotene within approx. 16 minutes can be done. The reduction of analysis time represents a significant advantage over established methods (SLIMESTAD et al., 2005; VASQUEZ-CAICEDO et al., 2006). Also for polyacetylenes an acceleration of existing HPLC-MS methods (ZIDORN et al., 2005; ZIDORN et al., 2002) could be successfully achieved by reducing analysing time to approx. 35 minutes per sample. Applying the MS conditions mentioned above the following molecule ions, adducts and fragment ions were generated: For FaDOH (M: 260 Da) *m/z* 257 $[M\text{-}2H_2O\text{+}CH_3OH\text{+}H^+]^+$, *m/z* 225 $[M\text{-}2H_2O\text{+}H^+]^+$ and *m/z* 243 $[M\text{-}H_2O\text{+}H^+]^+$ were detected as main ions but also *m/z* 275 $[M\text{-}H_2O\text{+}CH_3OH\text{+}H^+$ +was abundant (Fig. 6).

For FaOH (M: 244) *m/z* 227 $[M\text{-}H_2O\text{+}H^+]^+$ and *m/z* 259 $[M\text{-}H_2O\text{+}H^+\text{+}CH_3OH]^+$ were found to be the main ions while the molecule ion *m/z* 245 $[M\text{+}H^+]^+$ was present with lower abundance (Fig. 7).

For FaDOH-3-acetate (M: 302) *m/z* 285 $[M\text{-}H_2O\text{+}H^+]^+$, *m/z* 243 $[M\text{-}CH_3COO^-$ + and *m/z* 257 $[M\text{-}CH_3COO^-\text{-}H_2O\text{+}CH_3OH]^+$ were registered as main ions; *m/z* 225 $[M\text{-}CH_3COO^-\text{-}H_2O]^+$ was present with lower abundance (Fig. 8).

In comparison to former studies (BREITHAUPT, 2004; PFERSCHY WENZIG et al., 2009; CHRISTENSEN et al., 2006) the newly developed HPLC method demonstrates that very short run times speed up the whole analysis process of carrots significantly.

4 Discussion

The aim of the study was to develop an accelerated sample preparation method with subsequent HPLC-DAD and HPLC-MS analysis for a reliable determination of carotenoids and polyacetylenes in carrot roots applicable in the areas of plant breeding and food industry. Using accelerated solvent extraction followed by rotary vacuum concentration for sample preparation, the analysis time can be significantly reduced without renouncing accuracy and sensitivity. The high grade of automatization does not only save a lot of time and man power but also reduces the usually observed variation caused by manual sample preparation. Another advantage of the new ASE method is that not only carotenoids but also polyacetylenes could be extracted from freeze-dried carrot matrix. In comparison to standard extraction methods like Soxhlet or solid phase extraction (SPE), the extraction yields are significantly higher (Fig. 4 and Fig. 5). For subsequent HPLC-DAD analysis of carotenoids a fast method was adapted for the clear separation and determination of the four target carotenoids (Fig. 9).

The new analytical approach needs only half-time of presently described methods which is especially useful for applications in the fields of food quality control and plant breeding. For determination of the three target polyacetylenes (falcarinol, falcarindiol and falcarindiol-3-acetate) an existing HPLC-MS method was adapted which also takes less time than approved methods and allows a fast determi-

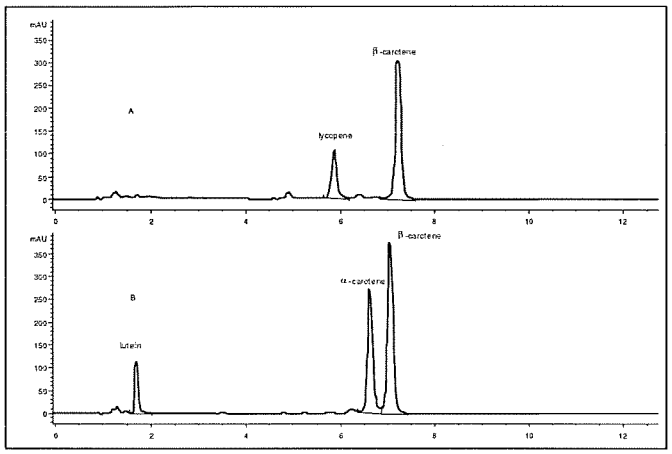


Fig. 9: HPLC-DAD separation of carotenoids detected in two different carrot cultivars (A: 'Nutrired', B: 'Nerac F1')

nation of known and unknown polyacetylenes. The whole extraction process including improved HPLC methods presented in this study does not only allow a fast, cost-saving and reliable extraction of valuable carrot components but also enables high throughput screening experiments in routine studies.

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References

BARANSKA, M., SCHULZ, H., BARANSKI, R., NOTHNAGEL, T., CHRISTENSEN, L.P., 2005: In situ simultaneous analysis of polyacetylenes, carotenoids and polysaccharides in carrot roots. J. Agric. Food Chem. 53, 6565-6571.

BARANSKA, M., SCHULZ, H., 2005: Spatial tissue distribution of polyacetylenes in carrot root. Analyst 130, 855-859.

BARANSKA, M., BARANSKI, R., SCHULZ, H., NOTHNAGEL, T., 2006: Tissue-specific accumulation of carotenoids in carrot roots. Planta 224, 1028-1037.

BOOTH, V.H., 1951: Distribution of carotenoids in different parts of the carrot. J. Sci. Food. Agric. 2, 350-353.

BREITHAUPT, D.E., 2004: Simultaneous HPLC determination of carotenoids used as food coloring additives: applicability of accelerated solvent extraction. Food Chem. 86, 449-456.

CHRISTENSEN, L.P., JENSEN, M., KIDMOSE, U., 2006: Simultaneous determination of ginsenosides and polyacetylenes in American ginseng root (*Panax quinquefolium* L.) by high-performance liquid chromatography. J. Agric. Food Chem. 54, 8995-9003.

CHRISTENSEN, L.P., KREUTZMANN, S., 2007: Determination of polyacetylenes in carrot roots (*Daucus carota* L.) by high-performance liquid chromatography coupled with diode array detection. J. Sep. Sci. 30, 483-490.

CZEPA, A., HOFMANN, T., 2003: Structural and sensory characterization of compounds contributing to the bitter off-taste of carrots (*Daucus carota* L.) and carrot puree. J. Agric. Food Chem. 51, 3865-3873.

CZEPA, A., HOFMANN, T., 2004: Quantitative studies and sensory analyses on the influence of cultivar, spatial tissue distribution, and industrial processing on the bitter off-taste of carrots (*Daucus carrota* L.) and carrot products. J. Agric. Food Chem. 52, 4508-4514.

FIKSELOVA, M., SILHAR, S., MARECEK, J., FRANCAKOVA, H., 2008: Extraction of carrot (*Daucus carota* L.) carotenes under different conditions. Czech J. Food Sci. 26, 268-274.

HANSEN, S.L., PURUP, S., CHRISTENSEN, L.P., 2003: Bioactivity of falcarinol and the influence of processing and storage on its content in carrots (*Daucus carota* L.). J. Sci. Food Agric. 83, 1010-1017.

IWASE, H., 2002: Simultaneous sample preparation for high-performance liquid chromatographic determination of Vitamin A and β-carotene in emulsified nutritional supplements after solid-phase extraction. Anal. Chim. Acta 463, 21-29.

JARAMILLO-FLORES, M.E., LUGO-MARTINEZ, J.J., RAMIREZ-SANJUAN, E., MONTELLANO-ROSALES, H., DORANTES-ALVAREZ, L., HERNANDEZ-SANCHEZ, H., 2005: Effect of sodium chloride, acetic acid, and enzymes on carotene extraction in carrots (*Daucus carota* L.). J. Food Sci. 70, 136-142.

KREUTZMANN, S., CHRISTENSEN, L.P., EDELENBOS, M., 2008: Investigation of bitterness in carrots (*Daucus carota* L.) based on quantitative chemical and sensory analyses. LWT 41, 193-205.

LUND, E.D., WHITE, J.M., 1990: Polyacetylenes in normal and water-stressed "Orlando Gold" carrots (*Daucus carota*). J. Sci. Food Agric. 51, 507-516.

MILADI, S., JARRAYA, R., DAMAK, M., 2008: Lipid composition and antioxidant activities of *Daucus maritimus* Seeds. J. Appl. Sci. 8, 4689-4693.

MARX, M., SCHIEBER, A., CARLE, R., 2000: Quantitative determination of carotene stereoisomers in carrot juices and vitamin supplemented (ATBC) drinks. Food Chem. 70, 403-408.

PFERSCHY-WENZIG, E.M., GETZINGER, V., KUNERT, O., WOELKART, K., ZAHRL, J., BAUER, R., 2009: Determination of falcarinol in carrot (*Daucus carota* L.) genotypes using liquid chromatography/mass spectrometry. Food Chem. 114, 1083-1090.

PUTZBACH, K., KRUCKER, M., ALBERT, K., GRUSAK, M.A., TANG, G., DOLNIKOWSKI, G.G., 2005: Structure determination of partially deuterated carotenoids from intrinsically labeled vegetables by HPLC-MS and H^1 NMR. J. Agric. Food Chem. 53, 671-677.

RICHTER, B.E., JONES, B.A., EZZELL, J., PORTER, N.L., AVDALOVIC, N., POHL, C., 1996: Accelerated solvent extraction: a technique for sample preparation. Anal. Chem. 68, 1033-1039.

SCHERZ, H., SENSER, F., 2000: Food composition and nutrition tables. Medpharm Scientific Publishers, Stuttgart.

SCHINKOVITZ, A., STAVRI, M., GIBBONS, S., BUCAR, F., 2008: Anti-mycobacterial polyacetylenes from *Levisticum officinale*. Phytother. Res. 22, 681-684.

SLIMESTAD, R., VERHEUL, M.J., 2005: Seasonal variations in the level of plant constituents in greenhouse production of cherry tomatoes. J. Agric. Food Chem. 53, 3114-3119.

VASQUEZ-CAICEDO, A.L., HELLER, A., NEIDHART, S., CARLE, R., 2006: Chromoplast morphology and beta-carotene accumulation during post-harvest ripening of mango cv. 'Tommy Atkins'. J. Agric. Food Chem. 54, 5769-5776.

ZIDORN, C., STURM, S., DAWSON, J.W., KLINK, J.W.v., STUPPNER, H., PERRY, N.B., 2002: Chemosystematic investigation of irregular diterpenes in *Anisotome* and related New Zealand Apiaceae. Phytochemistry 59, 293-304.

ZIDORN, C., JÖHRER, K., GANZERA, M., SCHUBERT, B., SIGMUND, E.M., MADER, J., GREIL, R., ELLMERER, E.P., STUPPNER, H., 2005: Polyacetylenes from the apiaceae vegetables carrot, celery, fennel, parsley, and parsnip and their cytotoxic activities. J. Agric. Food Chem. 53, 2518-2523.

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