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Effects of Harvesting Date and Storage on the Amounts of Polyacetylenes in Carrots, *Daucus carota*

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The amounts of three main polyacetylenes in carrots; falcarinol, falcarindiol, and falcarindiol-3-acetate, were determined by HPLC, during three seasons, in carrots harvested several times per season and at different locations in Sweden. The amounts of falcarindiol first decreased from a relatively high level and then increased later in the harvest season. The amounts of falcarindiol-3-acetate showed similar variations, whereas the amounts of falcarinol did not exhibit any significant variation during the harvest season. During storage the amount of polyacetylenes leveled off, increasing in samples initially low and decreasing in samples initially high in polyacetylenes. The amounts of all polyacetylenes varied significantly due to external factors and between stored and fresh samples. This variation opens up possibilities to achieve a chemical composition of polyacetylenes at harvest that minimizes the risk of bitter off-taste and maximizes the positive health effects reported in connection with polyacetylenes in carrots.

KEYWORDS: Carrot; *Daucus carota*; falcarinol; falcarindiol; falcarindiol-3-acetate; polyacetylenes; seasonal variation

INTRODUCTION

Carrot is a popular root crop, not least appreciated for its taste (*1*) and reported positive health-related properties (*2*). However, carrots can sometimes develop bitter off-tastes (*3*) or cause allergic reactions (*4*). Polyacetylenic compounds have been described as being related to both positive (*4*) and negative properties of carrots (*5*). Three common polyacetylenes in carrots are falcarinol, (*Z*)-heptadeca-1,9-diene-4,6-diyn-3-ol; falcarindiol, (*Z*)-heptadeca-1,9-diene-4,6-diyne-3,8-diol; and falcarindiol-3-acetate, (*Z*)-3-acetoxyheptadeca-1,9-diene-4,6-diyn-8-ol (*6*). In this paper these three compounds are referred to as belonging to the group of falcarinol-type polyacetylenes. The general properties of falcarinol-type polyacetylenes have recently been reviewed (*4*, *6*). It is reported that the metabolic pathway starts with the formation of crepenynic and dehydrocrepenynic acid (*6*) and that falcarinol derivatives remain as glycerolipids until the chain-end desaturation occurs in the final stages of biosynthesis (*6*). Falcarinol is reported to be the precursor of both falcarindiol and falcarindiol-3-acetate (*6*). The metabolism of falcarinol-type polyacetylenes in carrots occurs mainly in oil-filled channels or clusters within the periderm/phloem parenchyma tissue running along the length of the whole plant (*7*). The localization of the falcarinol-type polyacetylenes in the carrot root has been attributed to vascular bundles in young secondary phloem and to pericycle oil channels in the vicinity of the periderm (*8*). Falcarinol is more evenly distributed in all parts of the carrot root, whereas falcarindiol and falcarindiol-3-acetate are more abundant in the upper and outer segments (*5*). Falcarindiol is more concentrated in the periderm and the outer part of the phloem than falcarinol and falcarindiol-3-acetate (*7*). In peeled raw carrots the amount of falcarindiol and falcarindiol-3-acetate decreases with increasing root weight, but the amount of falcarinol does not (*9*). The amount of falcarinol-type polyacetylenes in carrots differs depending on cultivar (*9*−*11*), geographic location (*9*), water stress (*12*), storage (*9*, *13*), and industrial processing (*5*), and especially falcarindiol is also correlated with different forms of fungal attack, mainly *Mycocenterospora acerina* during storage (*14*). No reports have been found investigating the variation of falcarinol-type polyacetylenes during the harvest season or from one cultivation year to the next. The aim of this paper was to report how harvesting date, year, and geographic location influence the amounts of falcarinol-type polyacetylenes in carrots. The aim was furthermore to evaluate the influence of storage and cultivar on the amounts of falcarinol-type polyacetylenes and how these parameters interacted with harvesting date, year, and geographic location.

MATERIALS AND METHODS

During 2005, 2006, and 2007 carrot samples were harvested on the same day, three to six times per season, at four different, commercial, organic farms in southern and central Sweden. During 2005 carrots were harvested at sites 1, 2 and 3, during 2006 at sites 2 and 3, and during 2007 at sites 2, 3, and 4 (**Table 1**). At site 2 in 2005 carrots were harvested only on the three first harvest occasions. Due to severe drought no carrots were harvested at site 1 during 2006 and 2007. At site 3 no carrots were left on the last harvest occasion in 2007,and no 'Bolero' carrots were left on the last harvest occasion in 2006.

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Table 1. Geographic Location, Soil Type, Manuring Regimen, Cultivars Used, and Sowing and Harvesting Dates for Each Site and Annual Date for Terminating Storage for All Samples

site	location	soil	manuring	cultivar	sowing date	harvest dates	end of storage date
					2005		
1	59.3° N 17.2° E	heavy clay	cow manure compost	Kämpe	May 9, 2005	August 6, 2005; August 20, 2005; September 2, 2005; September 17, 2005; October 2, 2005; October 16, 2005	March 9, 2006
2	59.2° N 17.4° E	clay	cow manure compost	Kämpe	May 9, 2005	August 6, 2005; August 20, 2005; September 2, 2005	March 9, 2006
3	56.3° N 16.1° E	silty sand	cow manure compost	Kämpe/Bolero	May 9, 2005	August 6, 2005; August 20, 2005; September 2, 2005; September 17, 2005; October 2, 2005; October 16, 2005	March 9, 2006
					2006		
2	59.2° N 17.4° E	clay	cow manure compost	Kämpe	April 30, 2006	August 12, 2006; August 26, 2006; September 10, 2006; September 24, 2006; October 8, 2006	March 9, 2007
3	56.3° N 16.1° E	silty sand	cow manure compost	Kämpe/Bolero	April 30, 2006	August 12, 2006; August 26, 2006; September 10, 2006; September 24, 2006; October 8, 2006 (cv. Kämpe only)	March 9, 2007
					2007		
2	59.2° N 17.4° E	clay		Kämpe	June 1, 2007	August 23, 2007; September 15, 2007; October 20, 2007	June 9, 2008
3	56.3° N 16.1° E	silty sand	cow manure compost	Kämpe/Bolero	April 28, 2007	August 23, 2007; September 15, 2007	June 9, 2008
4	55.6° N 13.3° E	sand rich in humus		Kämpe	May 25, 2007	August 23, 2007; September 15, 2007; October 20, 2007	June 9, 2008

Plant Material. The carrot cultivar 'Kämpe' was grown at all sites each year. At site 3 the cultivar 'Bolero' was also grown, making it possible to evaluate cultivar differences. The cultivar 'Kämpe' is an open-pollinated carrot of the Chantenay type, and 'Bolero' is an F.1 of the Nantes/Berlicum type. Seeds from both cultivars came from Lindbloms Frö, Kivik, Sweden.

Sampling and Postharvest Treatment. The carrot samples were taken from six to seven locations spread over the field. A total of 40−60 carrots were harvested from each field. On four occasions (August 20, 2005, sites 1 and 3; October 20, 2007, sites 2 and 4) two independent samples were collected simultaneously from the same field to investigate the reliability of the sampling procedure. Within 24 h after harvest the carrots were transported in cold-store boxes to the laboratory, where they were divided into two equal parts. One was directly placed into storage (hereafter referred to as stored samples). The other (hereafter referred to as fresh samples) was cut up immediately. Using a knife, 1−1.5 cm was removed from the upper and lower ends of the carrot. The remaining section was divided into four strips, which were thereafter cut into pieces approximately 0.5−1 cm thick. Approximately 60 g of these carrot cubes was frozen and kept at −80 °C until further analysis. Before chemical investigations, the carrot samples were freeze-dried during 5 days and then ground to a powder using a type A10 grinder (Ika-werke Staufen, Germany).

Storage. Each of the stored samples was kept in small perforated plastic bags at approximately 1 °C and 97% relative humidity. The storage period was terminated on March 9, in 2005 and 2006, and on June 9, in 2007, regardless of harvesting date. During preparation and analysis the stored samples were treated in the same way as the fresh samples.

Analysis. Fresh and stored samples were analyzed simultaneously for their falcarinol-type polyacetylene content according to methods from Zidorn et al. (*15*) and Christensen and Kreutzmann (*16*), with modifications. Each carrot sample, 200 mg of powder, was extracted with 7 mL of ethyl acetate (Merck & Co., USA) containing 0.5% 4-chlorobenzophenone (Alfa Aesar Gmbh & Co., Germany) as an internal standard. The extract was shaken in an orbital shaker (Forma Scientific Inc., USA) for 16 h in darkness at 4 °C and then centrifuged at 1680*g* for 5 min using a Universal 30RF centrifuge (Hettich GmbH & Co., Germany). The extract, 4 mL, was evaporated using pure nitrogen gas, and the remaining substance was dissolved in 200 μL of acetone (Merck & Co., USA) and filled into glass vials. The column used at 40 °C for HPLC analysis was a 100 mm × 3 mm, particle size = 3 μm, Luna C18 (2) (Phenomenex, USA) combined with an Agilent 1100 HPLC system equipped with a diode array detector (Agilent Technology, USA). The flow rate was 1 mL/min. Acetonitrile (Merck & Co., USA) and water of 18.2 MΩ·cm quality, produced by a Milli-Q system (Millipore, France) were used as eluents. The binary gradient, expressed in percentage of acetonitrile, was as follows: 0−5 min, steady at 20%; 5−10 min, rising to 52.5%; 10−31 min, steady at 52.5%; 31−55 min, rising to 95%; 55−60 min, steady at 95%; 60−61 min, falling to 20%; 61−65 min, steady at 20%. Data were evaluated by Chemstation A09.03 software (Agilent Technology, USA). The different falcarinol-type polyacetylenes, at the detection wavelength of 205.5 nm, were identified using retention time and data from the UV spectra at 200−320 nm with a 2 nm bandwidth. An example of the obtained chromatograms is given in **Figure 1**. The obtained spectra were compared with those given in the literature (*16*). Due to the lack of a commercial standard of falcarinol-type polyacetylenes, at the start of the analysis the amount of falcarinol-type polyacetylenes was initially expressed as equivalents of the internal standard. A standard of falcarindiol (Atomax Chemicals Co., Shenzhen, China) became available during the summer of 2009. A factor of 1.235 was obtained by dividing the linear constant of 4-chlorobenzophenone at 205 nm with the corresponding constant for falcarindiol at 205 nm. The factor 1.235 was therefore used to express the amounts of falcarinol-type polyacetylenes as micrograms per gram of dry weight (μg/g DW). All reagents used in the analysis were of HPLC grade. For HPLC measurements triplicate analyses have been performed on all samples.

Statistics and Treatment of Data. Calculations and statistical evaluations were made using Excel 2003 (Microsoft Corp., USA) and SPSS 16.0 (SPSS Inc., USA). Differences between subjects were determined using one-way ANOVA together with the Duncan post hoc test at a significance level of $P \leq 0.05$ if not stated otherwise. Due to the unbalanced sampling, different sets of samples were used during the treatment of data. These sets are given in the tables and figures.

RESULTS

When all samples were analyzed, both fresh and stored carrots, falcarindiol was present in the highest amount, followed by

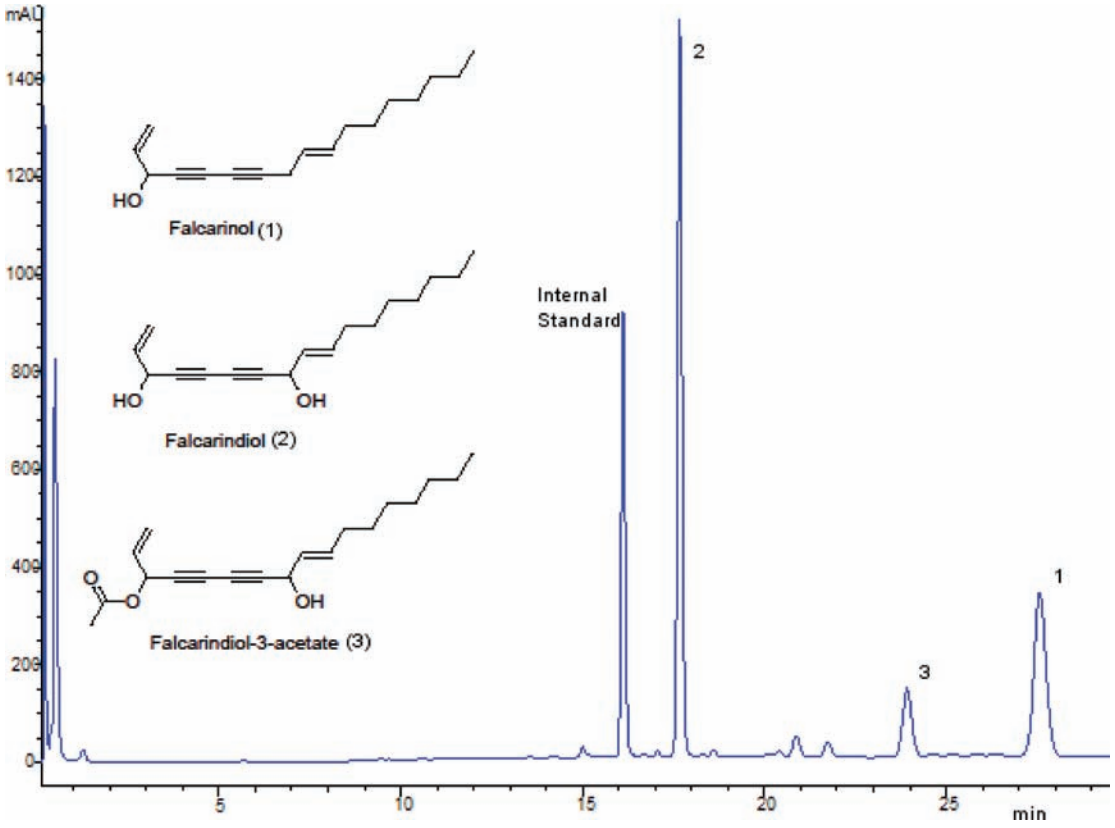

Figure 1. Chemical structures of the falcarinol-type polyacetylenes and example of chromatogram, DAD at 205.5 nm, indicating the peaks of the internal standard and the polyacetylenes: (*Z*)-heptadeca-1,9-diene-4,6-diyn-3-ol (falcarinol, **1**); (*Z*)-heptadeca-1,9-diene-4,6-diyne-3,8-diol (falcarindiol, **2**); (*Z*)-3-acetoxyheptadeca-1,9-diene-4,6-diyn-8-ol (falcarindiol-3-acetate, (**3**).

Table 2. Amounts of Falcarinol-Type Polyacetylenes (μg/g DW $\pm$ SD) in Carrot Samples Harvested on Different Dates Expressed in Days After Sowing[a]

			falcarindiol		falcarinol		falcarindiol-3-acetate	
harvest period, days after sowing	harvest period	*N*	fresh	stored	fresh	stored	fresh	stored
103−104[b]	very early	6	585 ± 375 a	434 ± 149 ab	118 ± 118 a	85 ± 48 b	86 ± 90 a	78 ± 62 a
117−118[b]	early	6	292 ± 207 c	374 ± 158 bc	195 ± 193 a	111 ± 85 b	42 ± 22 bc	57 ± 23 ab
131−133[b]	normal	6	479 ± 86 ab*	289 ± 93 c	199 ± 159 a*	92 ± 47 b	70 ± 34 ab*	41 ± 18 b
146−147[b]	late	6	410 ± 101 bc	504 ± 203 a	226 ± 220 a	230 ± 199 a	30 ± 19 c	72 ± 37 a*
mean of all samples		44	507 ± 273*	414 ± 167	249 ± 183*	165 ± 117	78 ± 64	78 ± 60

[a] Means within a column followed by different letters are significantly different, significance level, $P \leq 0.05$. * indicates the significantly highest mean value of fresh and stored samples. [b] Mean of samples from site 1 (2005), site 2 (2006), and site 3 (2005, 2006).

falcarinol and falcarindiol-3-acetate (**Table 2**). The results indicate that there is considerable variation between the samples (**Tables 2**−**5**). The variation among the triplicates from the same sample was for the most part negligible, and a comparison between the samples harvested independently from the same field at the same harvest occasion revealed only minor variation (data not shown). The major variations between samples were the result of external factors such as harvest date, storage, location, cultivar used, and harvest year (**Tables 2**−**5**).

Harvesting Date. Carrots harvested 103−104 days after sowing have been categorized as very early, those harvested between 117 and 118 days as early, those harvested between 131 and 133 days as normal, and those harvested 146−147 days after sowing as late. In fresh samples there was no indication that the harvesting date influenced the amounts of falcarinol (**Table 2**). The amount of falcarindiol in fresh samples was lower in carrots harvested early compared to those harvested in the other three harvest periods, and fresh carrots harvested very early had higher amounts of falcarindiol than carrots harvested late (**Table 2**). The amount of falcarindiol-3-acetate in the fresh samples was higher in carrots harvested very early compared to those harvested early or late, and fresh carrots harvested during the normal period had higher amounts of falcarindiol-3-acetate compared to those harvested late (**Table 2**). In the stored samples the highest amounts of total falcarinol-type polyacetylenes and falcarinol were found in carrots harvested late compared to all other harvest periods (**Table 2**). The amounts of falcarindiol and falcarindiol-3-acetate in stored samples were higher in carrots harvested very early or late compared to those harvested during the normal period. Stored carrots harvested late also had higher amounts of falcarindiol compared to those harvested early (**Table 2**).

Geographic Location. Carrots harvested at site 1 had higher amounts of all falcarinol-type polyacetylenes in both fresh and stored samples, with the exception of falcarinol in the stored samples, when compared with carrots harvested at site 3 during 2005 (**Table 3**). Carrots harvested in 2005, 2006, and 2007 from site 2 had higher amounts of falcarindiol, falcarinol, and falcarindiol-3-acetate in both fresh and stored samples compared to comparable samples from site 3 (**Table 3**). When carrots from

Table 3. Amounts of Falcarinol-Type Polyacetylenes (μg/g DW $\pm$ SD) in Carrot Samples Harvested Simultaneously at Different Geographic Locations[a]

			falcarindiol		falcarinol		falcarindiol-3-acetate	
year	sites compared	N	fresh	stored	fresh	stored	fresh	stored
2005	1	6	608 $\pm$ 234 a*	447 $\pm$ 193 a	453 $\pm$ 111 a*	244 $\pm$ 183 a	79 $\pm$ 42 a	66 $\pm$ 32 a
	3	6	459 $\pm$ 90 b*	308 $\pm$ 149 b	240 $\pm$ 85 b*	165 $\pm$ 69 a	54 $\pm$ 13 b	44 $\pm$ 25 b
2005−2007	2	10	498 $\pm$ 369 a	531 $\pm$ 154 a	233 $\pm$ 221 a	187 $\pm$ 109 a	79 $\pm$ 69 a	119 $\pm$ 65 a*
	3	10	364 $\pm$ 133 b	405 $\pm$ 134 b	115 $\pm$ 117 b	138 $\pm$ 85 b	47 $\pm$ 17 b	81 $\pm$ 78 b

[a] Means of corresponding samples, cultivar 'Kämpe', harvested on the same date on the sites compared. Means within a column followed by different letters are significantly different, significance level, $P \leq 0.05$. * indicates the significantly highest mean value of fresh and stored samples.

Table 4. Amounts of Falcarinol-Type Polyacetylenes (μg/g DW $\pm$ SD) in the Two Cultivars 'Kämpe' and 'Bolero' Harvested Simultaneously at Site 3[a]

		falcarindiol		falcarinol		falcarindiol-3-acetate	
cultivar	N	fresh	stored	fresh	stored	fresh	stored
Kämpe	11	402 $\pm$ 115 a	364 $\pm$ 145 a	163 $\pm$ 119 a	164 $\pm$ 86 a	48 $\pm$ 16 a	78 $\pm$ 76 a*
Bolero	11	470 $\pm$ 307a*	333 $\pm$ 14 a	151 $\pm$ 113 a*	92 $\pm$ 64 b	65 $\pm$ 74 a	45 $\pm$ 19 b

[a] Mean of corresponding samples harvested on the same date on site 3. Means within a column followed by different letters are significantly different, significance level, $P \leq 0.05$. * indicates the significantly highest mean value of fresh and stored samples.

Table 5. Amounts of Falcarinol-Type Polyacetylenes (μg/g DW $\pm$ SD) in Samples Harvested at Site 2 during 2005, 2006, and 2007 at Corresponding Harvest Date[a]

		falcarindiol		falcarinol		falcarindiol-3-acetate	
seasons compared	N	fresh	stored	fresh	stored	fresh	stored
2005[b]	2	776 $\pm$ 208 a*	545 $\pm$ 28 a	479 $\pm$ 73 a*	198 $\pm$ 51 b	160 $\pm$ 55 a*	73 $\pm$ 8 c
2006[c]	2	154 $\pm$ 59 c	535 $\pm$ 238 a*	17 $\pm$ 5 c	94 $\pm$ 46 c*	20 $\pm$ 15 b	145 $\pm$ 84 b*
2007[d]	2	477 $\pm$ 39b	501 $\pm$ 67 a	113 $\pm$ 50 b	315 $\pm$ 58 a*	54 $\pm$ 16 b	209 $\pm$ 24 a*

[a] Means within a column followed by different letters are significantly different, significance level, $P \leq 0.05$. * indicates the significantly highest mean value of fresh and stored samples. [b] Means of samples harvested at 103 and 117 days after sowing during 2005. [c] Means of samples harvested at 104 and 118 days after sowing during 2006. [d] Means of samples harvested at 83 and 107 days after sowing during 2007.

sites 1 and 2 were compared, those from site 2 had significantly higher amounts of falcarindiol-3-acetate, in both fresh and stored samples (data not shown). In corresponding fresh samples harvested during 2007 the highest amounts of all falcarinol-type polyacetylenes were found in carrots from site 4, whereas among the stored samples the highest amounts of falcarindiol and falcarindiol-3-acetate were found in carrots from site 2 (data not shown). In 2007 carrots harvested from site 3 had lower amounts of falcarinol-type polyacetylenes in comparison with the two other sites, with the exception of the amounts of falcarindiol-3-acetate and falcarinol in the fresh samples (data not shown).

Cultivar. Differences between the two cultivars used on site 3 were found only among the stored samples. 'Kämpe' had higher amounts of falcarinol and falcarindiol-3-acetate compared to the corresponding sample of 'Bolero' (**Table 4**). The fresh samples of 'Bolero' harvested late had a higher amount of falcarindiol than the corresponding samples of 'Kämpe', but by the end of storage late-harvested samples of 'Kämpe' had higher amounts of all falcarinol-type polyacetylenes (data not shown).

Cultivation Year. By comparison of carrots from the two first harvesting periods each year at site 2 it is possible to analyze differences between the harvest years. Fresh samples from 2005 had higher amounts of falcarindiol, falcarindiol-3-acetate, and falcarinol than the corresponding samples harvested during 2006 or 2007 (**Table 5**). Samples harvested during 2007 had higher amounts of falcarindiol and falcarinol compared to samples harvested during 2006 (**Table 5**). Stored samples harvested during 2007 had higher amounts of falcarindiol and falcarinol than the corresponding samples from 2005 or 2006 (**Table 5**). Stored samples harvested during 2005 had higher amounts of falcarinol but lower amounts of falcarindiol-3-acetate than the corresponding samples harvested during 2006 (**Table 5**).

Storage. The mean of all stored samples exhibited lower amounts of falcarindiol and falcarinol compared to the corresponding fresh samples (**Table 2**, indicated by *). When harvested during the normal period, stored samples exhibited lower values of falcarindiol, falcarindiol-3-acetate, and falcarinol but in late-harvested samples there were higher amounts falcarindiol-3-acetate in the stored carrots, compared to fresh (**Table 2**, indicated by *). Stored samples from both sites 1 and 3 had lower amounts of falcarindiol and falcarinol compared to the corresponding fresh samples during 2005 (**Table 3**, indicated by *). Carrots harvested at site 2 had higher amounts of falcarindiol-3-acetate after storage compared to the corresponding fresh samples (**Table 3**, indicated by *). The cultivar 'Bolero' had lower amounts of falcarindiol and falcarinol and 'Kämpe' had higher amount of falcarindiol-3-acetate in the stored samples compared to the corresponding fresh samples (**Table 4**, indicated by *). Carrots grown at site 2 exhibited lower amounts of falcarindiol, falcarinol, and falcarindiol-3-acetate in the stored samples harvested during 2005 but higher amounts of falcarindiol, falcarinol, and falcarindiol-3-acetate in the stored samples harvested during 2006 compared to the corresponding fresh samples (**Table 5**, indicated by *).

DISCUSSION

According to the results presented in this paper the amount of falcarindiol first decreased during the early part of the harvest season and then increased toward the end of the harvest season. This indicates a correlation between the amount of falcarindiol and the carrot root development.

During the harvest season the amounts of most types of substances have been reported to either remain at the same level, as, for example, free amino acids and minerals (*17*), or change in a more linear way like carotenoids (*18*) and sugars (*17*). No reference in the literature on carrots has been found describing a seasonal variation of a substance similar to the variation of falcarindiol reported here. There are some indications that the amounts of hexoses (*19*) and some volatiles (*20*) can exhibit related seasonal variation, but these descriptions are not supported by statistical evaluations. A similar, but inversed, seasonal variation in the amount of starch (*21*) and a reported variation of the respiratory rate in carrots (*22*) correspond well with the variation of falcarindiol described here. According to the results from Korolev et al. (*23*) malate and sucrose in the xylem follow a more linear development during the final phase of carrot root development, whereas the same substances in the phloem during the same phase tend to vary in a way similar to the variation of falcarindiol reported here. As falcarindiol is reported to be mainly concentrated in the phloem (*5*, *7*) this might explain a seasonal variation of this substance that could be correlated to the respiratory substrate available. However, to confirm this, a more detailed study on the annual variation of falcarinol-type polyacetylenes in the different tissues and on the correlation to the respiratory rate in carrots is needed.

Falcarindiol has been reported to contribute to an undesired bitter flavor in carrots (*5*, *24*). However, in this investigation the highest values of falcarindiol were found in carrots harvested very early (103−104 days after sowing). Carrots harvested after a short growing period have previously been reported to be sweet in taste (*25*, *26*). According to Keast and Breslin (*27*) "most taste compounds interact perceptually and in a manner that follows an apparently complex set of rules". The apparent contradiction between the high levels of falcarindiol in early-harvested carrots found in this investigation and the previously reported sweet taste of young carrots can have at least three different plausible explanations: First, the higher proportion in young carrots of fructose (*29*), that is, a sugar with a sweeter taste, may conceal the bitter taste. Second, the presence of other compounds may be necessary to experience bitter taste, compounds that the young carrot may lack or have low amounts of. One such example is terpenes. These compounds have been reported to correlate to bitterness in carrots and to increase when the harvest season is prolonged (*25*). Third, falcarindiol may be in a more inactive form, as far as human perception of bitter taste is concerned, during the early-harvest period. Falcarinol-type polyacetylenes have been found to be conjugated with glycerolipids before the final stages of their synthesis (*6*). If the chemical extraction method used in this study is more effective than the human gustatory system to separate falcarindiol from its surroundings at the final stages of its synthesis, this might contribute to the discrepancy between the chemical and the sensory analysis during the very early harvest period.

The results presented here indicate that the changes in the amount of falcarinol-type polyacetylenes during storage are related to the level of falcarinol-type polyacetylenes in the carrots when harvested. Most samples low in falcarinol-type polyacetylenes at harvest exhibited higher amounts by the end of storage, whereas samples high in falcarinol-type polyacetylenes at harvest exhibited lower amounts by the end of storage, resulting in diminishing differences between the stored samples and eventually moving toward stabilized levels. In this study these levels were approximately falcarindiol, 400 μg/g DW; falcarinol, 160 μg/g DW; and falcarindiol-3-acetate, 80 μg/g DW. Only two papers have been found dealing with the question of how storage influences the levels of falcarinol-type polyacetylenes (*9*, *13*). Although they both report on carrots grown in close proximity, during the same year, treated similarly in most respects and kept for 120 days in the same storage, the results in the two papers are contradictory. Hansen et al. (*13*), investigating only falcarinol, reported a decrease in the amount from 23.5 mg/kg FW in the fresh to 15.3 mg/kg FW in the stored samples, whereas Kidmose et al. (*9*) report an increase in the amounts of all three falcarinol-type polyacetylenes and that the amount of falcarinol changed from 11 mg/kg FW in the fresh to 16.2 mg/kg FW in the stored samples. Assuming 10% dry weight in the carrot samples in this study, the stabilized level for falcarinol corresponds to approximately 16 mg/kg FW. This means that the amount of falcarinol in the fresh samples reported by Hansen et al. (*13*) is above the expected stabilized level, whereas the amount of falcarinol reported by Kidmose et al. (*9*) is below, thus explaining the inconsistencies between the results presented in the two papers. It must, however, be stressed that the stabilized levels in the stored samples assumed in this investigation are based on the cultivar 'Kämpe'. 'Bolero' reacted quite differently during storage. However, in both cultivars the levels of falcarinol-type polyacetylenes stabilized during storage.

The data presented here confirm the earlier findings from Kidmose et al. (*9*) that there is considerable variation in the amounts of falcarinol-type polyacetylenes in carrots grown at different locations (*9*). Due to differences in the cultivation practices and soils, it is, however, not possible to draw any conclusions about the influence of the geographic location of the cultivation site on the levels of falcarinol-type polyacetylenes as has been done previously concerning other substances (*26*).

Significant differences in the levels of falcarinol-type polyacetylenes between cultivars have earlier been described by Kidmose et al. (*9*). The cultivars 'Kämpe' and 'Bolero' compared in this paper differed mainly after storage, indicating that although they were grown and stored side by side, there was also a genetic factor involved in the metabolism of falcarinol-type polyacetylenes.

No previous studies have been found dealing with the variation of falcarinol-type polyacetylenes in carrots from one year to another. According to the results presented here the greatest variation in the amount of falcarinol-type polyacetylenes in carrots was a result of the year of cultivation. Further investigations are required to better understand the factors affecting this considerable variation from one year to the next.

The variation among the triplicates from the same sample that sometimes occurred during analysis was probably to some extent due to an uneven evaporation of ethyl acetate and/or acetone during the extraction procedure. This stresses the importance of performing the extractions and the analysis of the samples at a constant and low temperature.

Falcarinol is frequently mentioned as a compound connected with positive health effects on humans (*13*, *28*) and falcarindiol as a compound that has a negative influence on the eating quality of the carrots (*5*, *24*). Knowledge on how to increase the former and minimize the latter is, however, more or less lacking. From the results presented here it is clear that the metabolism of falcarinol-type polyacetylenes in carrots is strongly influenced by external factors. This indicates the possibility to optimize the levels of falcarinol-type polyacetylenes when carrots are grown. The ratio falcarinol/(falcarinol + falcarindiol) could possibly be used to express the value of falcarinol-type polyacetylenes in carrots for human consumption. A higher ratio would be considered as more valuable. In this study the ratio falcarinol/(falcarinol + falcarindiol) varied from one year to another by a factor of close to 3, among harvest periods by a factor of 1.4, and between geographic locations by a factor of 1.3. There was hardly any difference in the ratio between fresh and stored samples or between the two cultivars used. Applying a preliminary general linear model on the results also indicates that the ratio

falcarinol/(falcarinol + falcarindiol) is influenced by external factors in the following order, starting with the most important: harvest year, harvest date, geographic location, cultivar, and storage. There are also interactions among the external factors. A good quality analysis of the interactions among the external factors requires a more balanced sampling than was used in this study.

If the ratio falcarinol/(falcarinol + falcarindiol) is applied as a measure, it might be concluded that the most suitable time to harvest carrots for optimal taste and health-promoting effects would be during the normal or the late-harvesting period but also that the most important factor is the annual variation. However, this still needs to be evaluated by use of human perception and health studies.

LITERATURE CITED

(1) Hohn, E.; Scharer, H.; Kunsch, U. Carrot flavour − acceptance, sweetness and bitterness. *Agrarforschung* **2003**, *10* (4), 144−149.

(2) Brandt, K.; Christensen, L. P.; Hansen-Möller, J.; Hansen, S. L.; Haraldsdóttir, J.; Jespersen, L.; Purup, S.; Kharazmi, A.; Barkholt, V.; Frökiaer, H.; Kobaek-Larsen, M. Health promoting compounds in vegetables and fruits: a systematic approach for identifying plant components with impact on human health. *Trends Food Sci. Technol.* **2004**, *15*, 384−389.

(3) Dodson, A.; Fukui, H. N.; Ball, C. D.; Carolus, R. L.; Sell, H. M. Occurrence of a bitter principle in carrots. *Science* **1956**, *124*, 984−985.

(4) Christensen, L. P.; Brandt, K. Bioactive polyacetylenes in food plants of the Apiaceae family: occurrence, bioactivity and analysis. *J. Pharm. Biomed. Anal.* **2006**, *41* (3), 683−693.

(5) Czepa, A.; Hofmann, T. Quantitative studies and sensory analyses on the influence of cultivar, spatial tissue distribution, and industrial processing on the bitter off-taste of carrots (*Daucus carota*) and carrot products. *J. Agric. Food Chem.* **2004**, *52* (14), 4508−4514.

(6) Minto, R. E.; Blacklock, B. J. Biosynthesis and function of polyacetylenes and allied products. *Prog. Lipid Res.* **2008**, *47*, 233−306.

(7) Garrod, B.; Lewis, B. G. Location of the antifungal compound falcarindiol in carrot root tissue. *Trans. Br. Mycol. Soc.* **1979**, *72* (3), 515−517.

(8) Baranska, M.; Schulz, H. Spatial tissue distribution of polyacetylenes in carrot root. *Analyst* **2005**, *130* (6), 855−859.

(9) Kidmose, U.; Hansen, S. L.; Christensen, L. P.; Edelenbos, M.; Larsen, E.; Norbaek, R. Effects of genotype, root size, storage, and processing on bioactive compounds in organically grown carrots (*Daucus carota*). *J. Food Sci.* **2004**, *69* (9), S388−S394.

(10) Mercier, J.; Ponnampalam, R.; Berard, L. S.; Arul, J. Polyacetylene content and UV-induced 6-methoxymellein accumulation in carrot cultivars. *J. Sci. Food Agric.* **1993**, *63* (3), 313−317.

(11) Olsson, K.; Svensson, R. The influence of polyacetylenes on the susceptibility of carrots to storage diseases. *J. Phytopathol.* **1996**, *144* (9−10), 441−447.

(12) Lund, D. L.; White, J. M. Polyacetylenes in normal and water-stressed "Orlando-gold" carrots (*Daucus carota*). *J. Sci. Food Agric.* **1990**, *51*, 507−516.

(13) Hansen, S. L.; Purup, S.; Christensen, L. P. Bioactivity of falcarinol and the influence of processing and storage on its content in carrots (*Daucus carota*). *J. Sci. Food Agric.* **2003**, *83*, 1010−1017.

(14) Garrod, B.; Lewis, B. G. Effect of falcarindiol on hyphal growth of *Mycocentrospora acerina*. *Trans. Br. Mycol. Soc.* **1982**, *78* (3), 533−536.

(15) Zidorn, C.; Johrer, K.; Ganzera, M.; Schubert, B.; Sigmund, E. M.; Mader, J.; Greil, R.; Ellmerer, E. P.; Stuppner, H. Polyacetylenes from the Apiaceae vegetables carrot, celery, fennel, parsley, and parsnip and their cytotoxic activities. *J. Agric. Food Chem.* **2005**, *53* (7), 2518−2523.

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

(17) Nilsson, T. Growth and chemical composition of carrots as influenced by the time of sowing and harvest. *J. Agric. Sci.* **1987**, *108*, 459−468.

(18) Fritz, D.; Weichmann, J. Influence of the harvest date of carrots on quality preservations. *Acta Hortic.* **1979**, *93*, 91−100.

(19) Suojala, T. Pre- and postharvest development of carrot yield and quality. Department of Plant Production, University of Helsinki, 2000.

(20) Heatherbell, D. A.; Wrolstad, R. E. Carrot volatiles II. Influence of variety, maturity and storage. *J. Food Sci.* **1971**, *36*, 225−227.

(21) Goris, M. A. Métabolisme glucidique de la racine de carotte cultivée (variéte nantaise demi-loungue) au cours du cycle végétatif de la plante. *Qual. Plant.* **1969**, *18*, 307−330.

(22) Weichmann, J.; Käppel, R. Harvesting dates and storage-ability of carrots. *Acta Hortic.* **1977**, *62*, 191−194.

(23) Korolev, A. V.; Tomos, A. D.; Bowtell, R.; F., F. J. Spatial and temporal distribution of solutes in the developing carrot taproot mesured at single-cell resolution. *J. Exp. Bot.* **2000**, *51* (344), 566−577.

(24) Kreutzmann, S.; Christensen, L. P.; Edelenbos, M. Investigation of bitterness in carrots (*Daucus carota*) based on quantitative chemical and sensory analyses. *LWT−Food Sci. Technol.* **2008**, *41* (2), 193−205.

(25) Rosenfeld, H. J.; Vogt, G.; Aaby, K.; Olsen, E. Interaction of terpenes with sweet taste in carrots (*Daucus carota*). *Acta Hortic.* **2004**, *637*, 377−386.

(26) Rosenfeld, H. J.; Samuelsen, R. T.; Lea, P. Relationship between physical and chemical characteristics of carrots grown in nortern latitudes. *J. Hortic. Sci. Biotechnol.* **1998**, *73*, 265−273.

(27) Keast, R. S. J.; Breslin, P. A. S. An overview of binary taste−taste interactions. *Food Qual. Pref.* **2002**, *14*, 111−124.

(28) Brandt, K.; Christensen, L. P. Vegetables as neutraceuticals—falcarinol in carrots and other root crops. In *Dietary Anticarcinogens and Antimutagens, Chemical and Biological Aspects*; Johnsson, I. T., Fenwick, G. W., Eds.; Royal Society of Chemistry: Cambridge, U.K., 2000; pp 386−391.

(29) Goris, M. A. The sugars in the root of the cultivated carrot (cultivar Nantaise demi-longue) seasonal and climatological variations, distributions in the tissues, alteration during storage. *Qual. Plant.* **1969**, *18*, 283−306.

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