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Lehrstuhl für Gemüsebau – Qualität pflanzlicher Nahrungsmittel,
Wissenschaftszentrum Weihenstephan, Technische Universität München

Essential oils as antioxidants of different coloured carrot cultivars (*Daucus carota* L. ssp. *sativus* Hoffm.)*

Ruth Habegger, W.H. Schnitzler

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Summary

Eight different coloured carrot cultivars, in orange, white, yellow, purple with orange or yellow and cream core, and red colour, were examined for their content and composition of essential oils and for their antioxidant capacity. For this purpose, two biochemical test systems were chosen (ABTS-test, MDA-test for lipid peroxidation). White and yellow carrot cultivars contained higher amounts of essential oils than orange cultivars. 'Nutri Red' had very high content of essential oils. There were significant differences between the cultivars in composition of their essential oils as shown by nine monoterpenes and two sesquiterpenes.

In both test systems essential oils of carrots showed antioxidant activity. The essential oils of purple and yellow coloured carrots had higher TEAC values than the essential oils of orange, white and red carrots.

Introduction

The presence of antioxidants in natural plants has received much attention as a disease preventive substance. Several herbs and spices have been investigated for the antioxidative activities of their essential oils, for example salvia, eucalyptus, basil, and thyme leaves. Some of the major aroma compounds found in these volatile extracts presented varying amounts of antioxidant effect, comparable to the known antioxidant α-tocopherol (Fellah et al., 2006; Lee and Shibamoto, 2001; Lee et al., 2005). Essential oil from *Pinus mugo* and some of its components showed antioxidative activity in several biochemical test systems (Grassmann et al., 2003).

There are vegetables like carrot, celery, celeriac, and parsnip, in which the essential oils serve as aroma relevant substances. Up to now, nutritional value of these plants was based on their content of minerals, vitamins, and dietary fibre. But furthermore their nutritional and healthy value may be extended by antioxidant capacity of secondary plant products.

The objective of this study was to analyse new carrot cultivars in comparison with orange carrots for the amount and antioxidant capacity of essential oils.

Materials and methods

In 2005 and 2006 eight different coloured carrot cultivars were cultivated in a research field of the Chair of Vegetable Science – Quality of Vegetal Foodstuff at Dürnast, Freising, according to good agricultural practice: 'Napoli' F_1 (orange; Bejo), 'Bolero' F_1 (orange, Nickerson Zwaan), 'White Satin' F_1 (white; Bejo), 'Mello Yello' F_1 (yellow; Bejo), 'Purple Haze' F_1 (purple with orange core; Bejo), 'Purple Rain' F_1 and 'Deep Purple' F_1 (purple with yellow or white-cream core; Bejo), 'Nutri Red' (red, Seminis).

Carrots were harvested at optimal ripening stage. After washing, roots were sorted according to the European Union external quality standard. A representative sample of at least 50 pieces was taken and deep frozen.

At analysis date the deep frozen material was crushed and mixed. In that way losses of the volatiles were reduced.

The essential oils were extracted by simultaneous steam distillation and pentane extraction (SDE) in Likens-Nickerson-apparatus. In the further text these volatiles were called essential oils, whereby they were no essential oils in closer sense. The amount of the essential oil was determined gravimetrically.

Separation and identification of components was carried out as described earlier (Habegger and Schnitzler, 2005). 110 of about 400 detected components were identified (Habegger and Schnitzler, 2000). Levels of separated components were calculated as area percent. Analyses were done with 4 replications.

For detection of antioxidant capacity a 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) decoloration assay was used (Cano et al., 2000; Yu and Ong, 1999). ABTS was oxidized by peroxidase (POD) and hydrogen peroxide (H_2O_2) to the green ABTS radical cation. By addition of antioxidants, here essential oils dissolved in ethanol, the ABTS-radical will be reduced to the original not coloured component. The reaction can be followed photometrically (wavelength 734 nm). The difference of absorption between 2 and 6 minutes is a measure of antioxidant capacity and is calculated into trolox equivalent antioxidant capacity (TEAC) value. Increasing values of TEAC correspond to a higher antioxidant capacity.

Antioxidant capacity of the essential carrot oil was measured in a further model system for lipid peroxidation, the malondialdehyde (MDA) test. Lipid peroxidation of cell membranes has been simulated by α-linolenic acid; oxidation has been induced by copper (Cu(II)) and H_2O_2. As an end product of lipid peroxidation malondialdehyde is quantified by adduct formation with 1-methyl-2-phenylindol giving a red to purple substance which is quantified photometrically at 586 nm. Values for inhibition of lipid peroxidation were calculated based on total oil amount of 100 g fresh matter (fm). Inhibition was compared to the control without addition of essential oil (Grassmann et al., 2001, 2002).

Analysis of variance was calculated by SAS, multiple mean comparison by Tukey-Test at 95 % probability. Equation of regression was calculated by Excel 2000.

Results and discussion

Total amount of essential oil

The carrot cultivars differ in the total amounts of essential oil content (Tab. 1). Cultivars with purple colour have about the same amount as the orange cultivars. 'White Satin' and 'Mello Yello' have significantly higher contents of essential oils than orange and purple cultivars. Alasalvar et al. (2001) reported highest total volatiles in white and lowest in yellow carrots. But names of carrot cultivars were not published.

In 2005 the oil content of 'Nutri Red' was significantly the highest; in 2006 it was similar to 'Napoli'.

'Napoli' tends to produce higher amounts of essential oil than 'Bolero' which shows the differences between orange coloured cultivars in general.

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Tab. 1: Essential oil content of different carrot cultivars (mg/100 g fresh matter)

cultivar	2005	2006
Napoli F_1	2.27 ± 0.19	3.56 ± 0.24
Bolero F_1	1.87 ± 0.20	2.27 ± 0.14
White Satin F_1	3.50 ± 0.31	4.84 ± 0.32
Mello Yello F_1	3.27 ± 0.21	4.27 ± 0.40
Purple Haze F_1	2.11 ± 0.17	1.73 ± 0.21
Purple Rain F_1	2.32 ± 0.22	2.81 ± 0.24
Deep Purple F_1	2.58 ± 0.08	2.24 ± 0.20
Nutri Red	4.44 ± 0.62	3.63 ± 0.20

Antioxidant capacity of essential oils

Essential oil of carrots has proved an antioxidant activity in ABTS-test. But the cultivars differ in their antioxidant capacity measured as Trolox equivalents (Fig. 1). Purple coloured cultivars have the highest values of TEAC per mg essential oil. 'White Satin' has the lowest Trolox equivalents. The orange cultivars and 'Nutri Red' are similar in the antioxidant capacity of their essential oil.
Because of the high differences in total oil content, values for antioxidant capacity are calculated in TEAC in oil of 100 g fresh matter (Fig. 2). Because of the high antioxidant capacity in 1 mg oil, 'Purple Rain' and 'Deep Purple' have the highest TEAC despite their lower total oil content. 'Mello Yello', 'Purple Haze' and 'Nutri Red' are very similar and have some higher TEAC than 'Napoli', 'Bolero' and 'White Satin'.

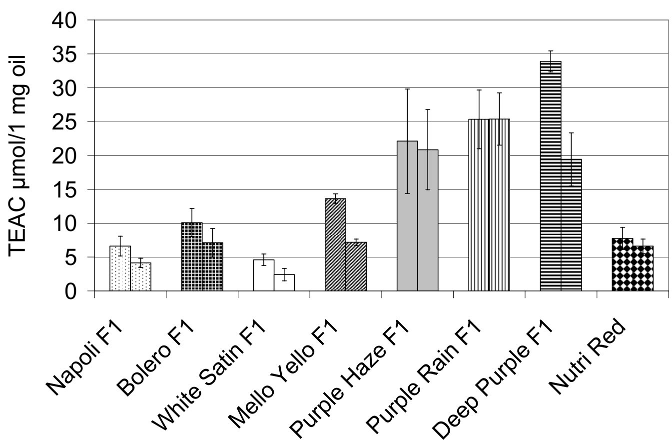

Fig. 1: Antioxidative potential of essential oil of different carrot cultivars in two years (2005 column left; 2006 column right).

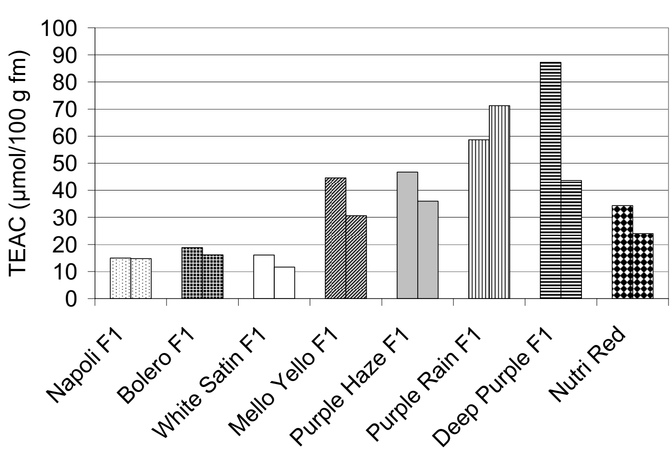

Fig. 2: Antioxidative potential of essential oil of different carrot cultivars calculated in TEAC (µmol/100 g fresh matter) in two years (2005 column left; 2006 column right).

Lipid peroxidation is inhibited by essential carrot oils (MDA-test); but there are no differences between the tested cultivars. The inhibition by essential oils of 'Bolero', 'Mello Yello' and 'Nutri Red' cultivated in 2003, is about 54 %, 57 % and 55 % compared to the control (VÖLKER, 2004).

Composition of the essential oils

The essential oil of carrots is mainly composed of mono- and sesquiterpenes. Monoterpenes are for example: α-pinene, β-pinene, sabinene, β-myrcene, α-terpinene, limonene, γ-terpinene, p-cymene, α-terpinolene.
Sesquiterpenes are for example: β-caryophyllene, *trans*-γ-bisabolene (tentatively). *Trans*-γ-bisabolene has been confirmed as an important carrot volatile in literature (TOTH-MARKUS and TAKACS-HAJOS, 2001).

These 9 monoterpenes and 2 sesquiterpenes amount to 70-90 % of total peak area (Tab 2). Additionally there are further components with a lower area percentage in the gas chromatogram.
The orange carrot cultivars differ in their quantitative composition of the essential oils. In general, the main monoterpene is α-terpinolene which corresponds to results of ALASALVAR et al. (2001). The main sesquiterpene is β-caryophyllene. In 2005 and 2006 the essential oil of 'Napoli' has higher area percentage of sabinene and β-myrcene than 'Bolero'.
The essential oil of 'White Satin' is characterized by high area percentage of β-caryophyllene, α-terpinolene and sabinene. The range of the last two components depends on cultivation conditions (HABEGGER and SCHNITZLER, 2005).
The area percentage of β-caryophyllene in essential oil of 'Mello Yello' is very low. The main substance is α-terpinolene, followed by sabinene in 2006. But this seems to be an exception because the results in 2003 and 2004 were similar to 2005 (HABEGGER and SCHNITZLER, 2005). Area of *trans*-γ-bisabolene is about 10 percentage.
ALASALVAR et al. (2001) published, that terpinolene (24 %), sabinene (23 %), β-caryophyllene (14 %) and γ-bisabolene (14 %) are the main terpenoids in yellow carrots.
In the gas chromatogram of the essential oil of 'Purple Haze' β-caryophyllene, *trans*-γ-bisabolene and α-terpinolene are the main terpenoids. The next one on the range is α-pinene, which is not usually for orange carrot cultivars.
Many similarities in composition of the essential oils are found for 'Purple Rain' and 'Deep Purple'. The area of *trans*-γ-bisabolene amounts to 20 percentage of total gas chromatogram area. Alpha-terpinolene and β-caryophyllene are the next components on the range.
The essential oil of 'Nutri Red' is dominated by α-terpinolene with about 40 to 50 area percentage. *Trans*-γ-bisabolene is a 'smaller' component like in the orange carrot cultivar 'Napoli'.

Between antioxidant capacity and the components of essential carrot oils there seems to be only one positive and high correlation between the TEAC values and the area percentage of *trans*-γ-bisabolene. Equation for regression are as following: in 2005 $y = 0.620x + 2.738$ ($R^2 = 0.696$) and in 2006 $y = 0.877x - 0.361$ ($R^2 = 0.932$). There is no improvement in the correlation when adding area percentage of one of the other ten mono- and sesquiterpenes.

The antioxidant capacity of the different components of essential oils has to be measured to explain the antioxidant effect of the total oils.

Tab. 2: Levels of compounds in essential oil of different carrot cultivars (GC peak area %).

2005

compound	Napoli F_1	Bolero F_1	White Satin F_1	Mello Yello F_1	Purple Haze F_1	Purple Rain F_1	Deep Purple F_1	Nutri Red
α-pinene	2.80	1.20	3.15	0.91	7.88	2.70	0.86	9.88
β-pinene	1.94	0.78	1.84	2.50	2.17	1.61	1.48	6.20
sabinene	10.11	4.50	18.38	9.56	0.62	0.59	0.09	0.27
β-myrcene	9.80	4.73	6.72	4.40	3.22	3.47	7.54	2.29
α-terpinene	0.16	0.10	0.22	0.19	0.06	0.06	0.05	0.08
limonene	2.38	2.18	4.19	2.20	1.43	1.63	2.21	3.28
γ-terpinene	2.08	2.24	2.18	5.10	3.48	5.91	4.80	6.19
p-cymene	0.45	0.45	0.46	1.47	0.48	1.23	1.22	1.07
α-terpinolene	18.87	19.86	8.55	32.31	13.00	19.12	17.48	43.66
β-caryophyllene	13.72	35.54	29.24	3.78	24.03	13.09	9.02	0.84
γ-bisabolene	8.37	3.74	0.23	14.92	22.23	23.14	21.01	8.45
sum of further identified monoterpenes	1.65	1.23	2.05	1.38	2.00	2.18	2.67	4.93
sum of further identified sesquiterpenes	3.16	5.39	5.40	3.28	3.99	3.29	3.68	1.34
myristicin	0	0.013	0	0	0.10	0.022	0	1.08
sum of total identified compounds	75.50	81.95	82.60	81.99	84.70	78.04	72.11	89.56

2006

compound	Napoli F1	Bolero F_1	White Satin F_1	Mello Yello F_1	Purple Haze F_1	Purple Rain F_1	Deep Purple F_1	Nutri Red
α-pinene	3.50	1.59	4.42	1.27	14.10	3.19	0.77	7.12
β-pinene	2.54	0.60	2.23	1.83	1.58	0.85	0.60	4.75
sabinene	23.31	9.51	13.88	25.45	0.36	0.13	0.09	0.15
β-myrcene	16.86	4.73	4.40	10.31	4.94	4.68	7.01	3.09
α-terpinene	0.15	0.14	0.12	0.24	0.04	0.05	0.04	0.05
limonene	1.99	1.81	3.10	1.68	1.38	1.37	2.04	2.34
γ-terpinene	1.91	3.70	1.67	5.43	4.39	7.26	4.35	4.81
p-cymene	0.25	0.49	0.25	0.83	0.37	0.82	0.91	0.65
α-terpinolene	23.44	28.42	20.28	33.42	20.66	26.07	25.55	57.53
β-caryophyllene	10.77	32.03	31.20	1.96	19.74	15.64	11.31	1.06
γ-bisabolene	5.68	2.19	0.11	7.48	16.35	22.14	18.03	6.75
sum of further identified monoterpenes	1.09	0.99	0.88	0.84	1.13	1.53	1.86	2.48
sum of further identified sesquiterpenes	1.56	2.85	3.73	1.53	1.93	2.56	3.18	0.88
myristicin	0	0.66	0	0	0.55	0.13	0	0.61
sum of total identified compounds	93.05	89.71	86.27	92.27	87.52	86.41	75.75	92.78

Because of its higher antioxidant capacity of essential carrot oil the nutritional and health value of the purple and yellow cultivars has increased compared to the orange cultivars.

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Address of the authors:

Dr. Ruth Habegger, Professor Dr. W.H. Schnitzler, Lehrstuhl für Gemüsebau – Qualität pflanzlicher Nahrungsmittel, Wissenschaftszentrum Weihenstephan, Technische Universität München, D-85350 Freising