The essential oil content in caraway species (*Carum carvi* L.)

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ABSTRACT: Caraway fruits contain 1–6% of essential oils consisting of about 30 compounds, from which carvone and limonene account form the main portion, about 95%. To evaluate the quality of various caraway cultivars, the amounts of essential oils and the carvone/limonene ratio were measured. The most common method of essential oil evaluation is steam distillation in accordance with Standard ČSN 58 0110, but an alternative method – supercritical fluid extraction (SFE) – was also investigated in this paper. Ground caraway fruits were extracted under different SFE conditions (pressure, temperature, use of modifiers). Released compounds (carvone and limonene) were quantified by GC.

Keywords: supercritical fluid extraction (SFE); caraway seeds; carvone; limonene; gas chromatography (GC); cultivars

Caraway is grown for its content of essential oils that are present in the whole plant, but their concentration is highest in achenes (KAMENÍK 1996). Nowadays, caraway is planted in this country on the average area of 2,400 ha (MINAŘÍK 2001). After the period of enormous fluctuations in 1995–1997, the size of caraway plantations has been stabilised during the last three marketing years. It is shown in comprehensive Table 1.

The varieties Kepron, Prochan, and Rekord, all of evergreen type, have been registered for growing. In the world, other varieties are registered, both deciduous and with shortened vegetation time, i.e. annual or perennial ones (Konczewicki variety). Caraway fruits (achenes) contain from 1 to 6% of essential oils that give the caraway its characteristic aroma. There are approximately 30 compounds contained in this plant species, while carvone and limonene account for about 95% of them. Seeds contain trace amounts of other compounds (acetaldehyde, furfural, carveole, pinene, thujone, camphene, phellandrene, etc.) that enhance dietetic and medical effects. Furthermore, seeds contain water (9–13%), fats (13–21%), nitrogen compounds (25–36%), 5–10% of extractive nitrogen-free compounds, 13–19% of crude fibre, 5–7% of ash, 1.5% of waxes and small amount of tannins and resin (ŠPALDON et al. 1986). The essential oil amount and composition is genetically conditioned and depends on climatic conditions during fruit formation and ripening.

The caraway essential oil composition was studied by MHEEN (1994), who first and foremost compared the content of carvone and limonene in caraway and dill. In caraway, the amount of carvone was 0.1%, or higher.

During the period 1996–2000, experiments with the evaluation of variety differences in caraway were performed in Česká Bělá, Keřkov and Šumperk. The results of 1997 harvest proved that variety Kepron achieved the best values in the following characteristics: plant height, number of 1st and 2nd order rays, number and weight of umbels on 1st order rays, yield of achenes per plant and per unit area. As for the Prochan variety, higher values were found only in the number of umbellules in the main umbel, number of main umbel achenes and essential oil content in the main umbel. Ethylene production was highest in Kepron achenes that had the lowest content of abscisic acid (ABA) (ONDŘEJ et al. 1997).

In experiments conducted in 1997 in the Agritec company in Šumperk and at a Field Experimental Station in Červený Újezd, the best morphological characteristics were found for Rekord variety. The results of experiments evaluated in 1998 proved that there were morphological differences between the varieties. In 1999, Rekord variety was the best as for plant height and weight of achenes per plant. In 2000, Kepron variety had the best values of morphological characteristics again, but in that year Prochan variety also showed some best characteristics: number of umbellules in the main umbel, weight of achenes per plant and number of 2nd order rays. From the qualitative point of view, Rekord variety was the best in all years (see Table 2).

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Table 1. The area and yields in individual marketing seasons

Marketing season	1996–1997	1997–1998	1998–1999	1999–2000	2000–2001
Harvest area (ha)	9,500	5,000	1,900	2,100	2,400
Average yield (t/ha)	1.00	1.00	0.80	0.85	0.75

The highest essential oil content was achieved in Rekord variety. However, it is obvious that the differences are dependent on plantation and weather conditions (ONDŘEJ et al. 1998).

The evaluation of caraway fruits according to current valid regulations brings large differences between individual analyses as the sample preparation prescription can be explicated in a different way. In the European Community, the amounts of essential oil are determined in whole fruits. That is why we started to deal with an alternative method, enabling faster analyses with comparable results.

At present, steam distillation according to Standards ČSN 58 0110 and ČSN 6571 (SEDLÁKOVÁ 1998) and hexane distillation (BOUWMEESTER et al. 1995) or a combination of both methods are used for the isolation and determination of essential oil content. Monoterpenes can also be isolated from a gaseous phase via Solid Phase Microextraction (SPME) followed by thermal desorption with on-line injection to a GC capillary column (MANI, WIOOLEY 1995). SFE is used as a new separation method.

For determination of carvone and limonene content, mostly gas chromatography with flame ionisation (FID) or mass spectrometry (MS) detection are used. But also the benefit of high performance liquid chromatography (HPLC) with UV (KOVAR, BOCK 1983; SEIF EL DIN et al. 1983) or polarimetric detection (KOVAR 1983), derivative spectrophotometry (SEIF EL DIN et al. 1983) and proton magnetic resonation (MOSSA 1980) can be applied.

Currently, SFE represents a dynamically evolving separation technique for essential oil determination. It is a new method of volatile compound isolation from complex natural matrices, comparable with older field-proven methods, like steam distillation, solvent extraction or hydrodistillation. Physicochemical properties of supercritical fluids represent transition between the properties of gases and liquids, which is the main reason for current increasing interest in SFE. In comparison with a liquid, supercritical fluid has one order higher diffusivity and one order lower viscosity with high solvent power maintained (CHURÁČEK 1992). From the aspect of mass transfer, the properties of gases are combined in supercritical fluids with solvation properties of liquids.

In SFE, carbon dioxide is used predominantly (KALLIO et al. 1994; ENGELHARDT, GROSS 1988; BOUNOSHITA et al. 1993) for its low critical temperature (31°C) and pressure (7.38 MPa), non-toxicity, incombustibility

Table 2. Morphological characteristics of caraway varieties during the project solution

Year	Variety	Plant height	Number of 1st order rays	Number of 2nd order rays	Number of umbellules per umbel	Achene weight per plant	TSW (g)
1997	Rekord	102.6 b	7.7 ab	16.9 ab	9.4 b	3.7 b	2.9
	Prochan	95.6 c	7.2 b	11.3 b	11.0 a	2.7 b	2.7
	Kepron	118.9 a	8.6 a	29.0 a	9.4 b	5.2 a	2.7
	MS	2,858.6 ***	10.0 *	1,626.5 ***	17.0 *	33.9 **	0.2
1998	Rekord	73.6 b	7.3	8.9	8.17	3.2	1.99
	Prochan	64.5 ab	6.56	8.86	8.07	2.6	1.6
	Kepron	62.3 a	6.2	6.93	8.67	4.6	2.07
	MS	358.9 *	6.5	26.02	2.018	33.9	1.2
1999	Rekord	59.71	7.2 ab	5.47 b	10.2	3.1	2.2
	Prochan	58.64	6.33 b	5.4 b	9.7	2.8	2.96
	Kepron	58.8	9.13 a	16.47 a	11.1	2.95	2.4
	MS	3.33	30.82 *	608.79 **	16.5		
2000	Rekord	79.46 a	9.08	3.38 a	10.0 a	2.0	1.8
	Prochan	86.59 ab	10.56	12.15 b	10.85 ab	2.2	1.7
	Kepron	84.44 b	11.85	11.11 a	8.85 b	2.1	2.23
	MS	223.34 *	35.04	363.4 *	27.11 *		

*MS – mean square

and low reactivity. Its polarity and extraction power are close to hexane and extraction efficiency decreases with growing analyte polarity. In the determination of volatile, reactive and thermosensitive terpenes, analysed in this paper, low critical temperature and nonpolar character of supercritical fluid are preferred.

A quality criterion determined in caraway is the content of essential oils, the amount of carvone and limonene in these oils and their mutual ratio. Since 1998, Regulation No. 330/97, an implementing rule of Food Act No. 110/97, has been obligatory for caraway quality. Czech codices from 1997 require the essential oil content of 3% and at least 50% content of carvone in the essential oil.

MATERIALS AND METHODS

Materials

Kepron, Prochan and Rekord, varieties of caraway (*Carum carvi* L.) planted in Agritec Šumperk (1998, 2000), were used to examine the effects of sample grinding and pretreatment, plant treatment and the time of harvest. New breeding selections of caraway plants from Huštěnovice plantations (1999) were used to determine essential oil content by the SFE method. The materials were newly-bred varieties with shortened vegetation period. To find out the best SFE modifier, Kepron variety was used, obtained from the plantation of plant breeding company ROLS Lešany (2000).

Prior to extraction and analysis, caraway seed samples were stored and preserved at a room temperature and protected from light.

Chemicals

l-carvone and *d*-limonene (Fluka, Switzerland) were used to test extraction efficiency. N-hexane (for HPLC, Merck, Germany) was used for the trapping of extracted substances. Liquid carbon dioxide for the food industry, nitrogen (4.0) and/or nitrogen (4.6), He (5.0) and medicinal oxygen (all AGA Ltd., Brno) were used for SFE and GC. As SFE modifiers, methanol (for HPLC), ethanol (for UV spectrometry), acetonitrile (for HPLC, all Merck, Germany), acetone (p.a., ONEX, Czech Republic), dichloromethane (p.a.), chloroform (p.a.), toluene (p.a., all LACHEMA Brno, CR) and ultra pure water (Millipore) were applied.

The investigated varieties were treated with growth regulator Roundup (Monsanto Europe S.A., Belgium). To treat the varieties against fungal diseases during the experiments the formulations Alert and Prelude 10 were tested.

Methods of analyte extraction and identification

Steam distillation

The steam distillation method was used in accordance with Standard ČSN 58 0110. The amount of sample, large enough according to the expected amount of essential oil, was weighed into a 1 l distillation vessel, 400 ml of water and some boiling stones were added. Distillation was carried out for 4 hours. Thereafter, cooling was turned off and distillation was performed without cotton for a while, to let the essential oil flow down quantitatively, whereupon heating was turned off and the amount of distilled essential oil was taken after 5 min. The essential oil was diluted in hexane and analysed by means of GC/MS.

Supercritical fluid extraction

Carvone and limonene were extracted from caraway seeds by means of supercritical CO_2 in the supercritical fluid extractor SE-1 (SEKO-K, s. r. o., Brno), taken up into n-hexane in a trapping vial and analysed via GC.

For the determination of essential oils in ground caraway, the pressure of 40 MPa, extraction cell temperature 80°C and restrictor temperature 120°C were used. The extracts were kept in refrigerator until the time of GC analysis.

Gas chromatography (GC)

To determine the mutual ratio of carvone and limonene in SFE extracts, gas chromatograph HP4890D was used. Separation was carried out on an HP-INNOWax column (polyethylene glycol, 30 m × 0.25 mm i.d. × 0.25 µm film thickness, all Hewlett Packard) at the helium flow rate 1 ml/min, injector temperature 220°C, detector temperature 240°C with temperature

Table 3. The effect of ripeness phase on the essential oil amount

	Kepron		Prochan	
	A	B	A	B
Essential oil (%)	1.26	3.36	1.50	3.94
RSD (%)	2.01	3.01	2.00	1.98
Limonene (%)	26.77	18.24	35.13	27.45
Carvone (%)	73.23	81.76	64.87	72.55

A – caraway samples collected before full ripeness
B – caraway samples collected before full ripeness

Table 4. The effect of Roundup treatment

	Caraway treatment		
	none	Roundup	MS*
Essential oil (%)	3.41	3.66	0.063**
RSD (%)	0.92	0.42	–
Limonene (%)	43.57	42.34	1.44
Carvone (%)	56.43	57.66	1.54

*MS – mean square calculated by means of statistical programme UNISTAT

programme 60°C, 40°C/min to 220°C, 2 min at 220°C. 1 µl of essential oil n-hexane solution was injected to the column. Resultant chromatograms were processed by means of CSW station (ver. 1.7, Data Apex, Prague).

RESULTS AND DISCUSSION

To determine the essential oil content in caraway, steam distillation is used as a classical method according to the Standard ČSN 58 0110, Pharmaceutical Code of Practice and the Czech Pharmacopoeia.

In 1999 KOCOURKOVÁ et al. (1999) ascertained that the essential oil determinations performed according to different methods bring large differences in results. That is why the aim of this study was to find an alternative method while SFE (supercritical fluid extraction) seems to be suitable.

In the first and second part of this study, the influence of sample pretreatment was investigated – whole vs. ground caraway seeds and the impact of different grinding methods was examined. Various types of mills were compared because the grinder type is not specified in the Standard ČSN 58 0110. In the third part, the effect of harvest time on the amount of essential oils was investigated. Caraway seeds were harvested before maturation and in full ripeness. Further, the influence of treatment on the amount of essential oil in individual varieties was investigated. The formulation Roundup was used as a ripening modifier, Alert and Prelude 10 as fungicides.

The alternative SFE method was used for essential oil determination in small samples, obtained during the plant breeding process. The analyses of 45 plants differing from the remaining plants were carried out, and the results were applied to further plant breeding.

The effect of sample grinding

The effect of sample grinding on essential oil recovery was investigated. In the case of steam distillation of whole caraway seeds, 2 weight % of essential oils were found, with RSD = 1.05%, and in the case of ground caraway, 2.6 weight % of essential oils were found with RSD = 1.25%.

As for the SF extraction of whole caraway seeds, 0.09 weight % of essential oils were found, with RSD = 1.56%, and in the case of ground caraway, 2.55 weight % of essential oils were found with RSD = 2.00%. It is obvious from these values that the SFE method is not suitable for essential oil determination in non-ground caraway seeds. Its recovery is one order lower in comparison with that of ground caraway SFE. These low recoveries are probably caused by poor analyte diffusion from the inner matrix volume. That is why we focused further only on essential oil determination in ground caraway seeds.

The ratio of carvone and limonene content is also different in the case of whole and ground caraway seeds extraction. In whole caraway extracts, the amount of carvone is 81.53% while in ground caraway extracts the content of carvone declined to 66.37%.

The effect of sample treatment (grinding)

Comparability of results of some sample grinding methods in different types of mills was investigated. Three types of mills were compared: ETA 0067 with millstones, splintery mill VIPO and cryogenic mill Vibrom 2, using liquid nitrogen (Jebavý, s. r. o., Czech Republic). The amount of extracted essential oil was found to depend on the type of mill. The best recovery was achieved in the splintery mill VIPO, 2.55 weight % with RSD = 2.00%, then in the ETA mill, 1.79 weight % with RSD = 1.36%, and finally in the cryogenic mill 1.72 weight % with RSD = 1.50%. It is obvious that both the type of mill and the grinding refinement can affect the amount of essential oil extracted.

The effect of harvest time

Essential oil content was determined in caraway seeds collected before (sample A) and after ripeness (sample B).

As shown in Table 3, samples (A) collected before maturation had lower essential oil content than samples (B) harvested in full ripeness. The difference between the samples was also visually apparent. The samples collected before harvest had elongate, narrow seeds while samples gathered after ripeness had rounder shapes. The amount of carvone increases during maturation by about 12%.

The effect of caraway treatment on essential oil amount

For this observation samples of caraway varieties Kepron, Prochan and Rekord (2000), planted in Šumperk,

Table 5. The effect of Alert and Prelude 10 treatment

Type of the treatment	Variety (essential oil %)		
	Kepron	Prochan	Rekord
None	3.41	3.22	3.32
Alert	6.75	5.56	5.39
Prelude 10	5.41	4.42	4.03

Table 6. Huštěnovice (1999) – Prochan

Sample number	*x*	RSD	L	K	Sample number	*x*	RSD	L	K
	(%)					(%)			
1	1.09	6.14	48.11	51.89	24	0.90	8.57	36.19	63.81
2	1.24	3.98	44.37	55.63	25	0.71	6.04	51.95	48.05
3	1.15	4.75	46.85	53.15	26	1.05	10.50	46.83	53.17
4	0.71	0.18	43.83	56.17	27	1.19	9.83	48.05	51.95
5	0.36	*	30.08	69.92	28	0.59	2.66	29.05	70.95
6	1.00	1.10	43.56	56.44	29	1.43	8.23	50.78	49.22
7	0.95	6.38	46.09	53.91	30	1.45	7.49	50.64	49.36
8	0.95	5.43	57.58	42.42	31	1.08	3.59	40.14	59.86
9	0.98	3.33	38.95	61.05	32	0.80	6.67	25.81	74.19
10	1.19	4.75	55.29	44.71	33	1.44	8.38	53.75	46.25
11	0.92	4.61	49.75	50.25	34	**1.83**	**3.84**	**44.87**	**55.13**
12	0.88	7.90	48.50	51.50	35	1.10	4.85	47.19	52.81
13	1.00	1.72	46.47	53.53	36	0.91	8.73	40.77	59.23
14	1.28	2.77	45.63	54.37	37	1.06	8.11	44.37	55.63
15	0.98	5.08	44.61	55.39	38	**1.80**	**9.06**	**38.29**	**61.71**
16	1.32	4.84	52.81	47.19	39	1.03	1.26	41.64	58.36
17	1.37	1.24	61.73	38.27	40	1.09	5.52	44.54	55.46
18	0.74	8.66	60.32	39.68	41	1.06	8.78	36.24	63.76
19	1.04	*	45.30	54.70	42	**1.56**	**8.87**	**34.49**	**65.51**
20	0.64	3.20	73.66	26.34	43	**1.59**	**8.00**	**16.79**	**83.21**
21	0.64	8.84	58.29	41.71	44	1.47	2.09	48.54	51.46
22	0.99	3.20	62.99	37.01	45	1.48	*	55.92	44.08
23	0.60	5.91	40.77	59.23					

*The experiment was not repeated due to a small amount of the sample

were used. Kepron variety was treated with Roundup used as a maturation regulator. The results are presented in comprehensive Table 2.

As it can be seen, the amount of essential oils increased after Roundup treatment, which is also proved by a highly significant difference of the variance analysis performed by the programme UNISTAT.

Further, two fungicides were used to treat the varieties Kepron, Prochan and Rekord (fungicide 1 – Alert and fungicide 2 – Prelude 10).

As shown in Table 3, after the treatment with fungicides the amount of essential oils increased significantly. The highest improvement was achieved after the vegetation Alert fungicide treatment, as proved by a highly significant MS value for the treatment in the analysis of variance (MS – 10.026***). No significant differences were found between the varieties (MS – 1.52), and the interaction and treatment of the varieties did not show any significant differences (MS – 0.297).

The use of SFE for determination of essential oils in small samples

The use of SFE is preferred for essential oil determination in small samples, gathered during vegetation. The essential oil content is a significant selection factor for plant breeders. The analyses of 45 plants that differed from the remaining plants (positively – taller habit, higher number of umbels etc.) were carried out. The analysis of essential oil content was performed in a half of the sample of seeds from the main umbel. The results are given in Table 6. The second half of seed samples was re-sown again.

The highest amounts of essential oil were found in samples No. 34, 38, 42 and 43. The lowest essential oil contents were found in samples No. 5, 20 and 21. The plants from which the seed samples were obtained underwent the morphological analysis, but no correlation was found between plant height, number of 1st and 2nd order rays and content of essential oils.

CONCLUSIONS

Due to different cultural practices and plant selection within the framework of further breeding caraway seeds contain various amounts of essential oil in the range of 1–6%.

Steam distillation was used to separate the essential oils from caraway seeds, and supercritical fluid extraction (SFE) as an alternative method. The following con-

ditions were used in SFE experiments: pressure 40 MPa, temperature 80°C, extraction time 120 min.

Whole and ground caraway seeds were extracted. The results proved that the amount of extracted essential oil increases with the quality of grinding. SFE is not suitable for the essential oil determination in whole caraway seeds because of poor analyte diffusion from the inner matrix volume. The mutual ratio of carvone and limonene is also different in the case of whole and ground caraway seed extraction. In the ground seed extract, the value of carvone portion decreased distinctly, about 15% in comparison with whole seeds. Thus, SFE is suitable only for the essential oil determination from ground seeds.

Further, the effect of grinding was investigated. Various grinding methods were compared because the Standard ČSN 58 0110 does not specify either the way of grinding or the mill type. The splintery mill VIPO seemed to be the most suitable of all three types used.

The applicability of SFE was verified in samples obtained from experiments where different cultural practices were monitored.

The content of essential oil in caraway seeds harvested at the beginning of maturation (sample A) and in full ripeness (sample B) was investigated. Samples (B) harvested in full ripeness have more essential oils. The difference between samples A and B is also visually evident. The samples harvested at the beginning of maturation had elongate, narrow seeds while the samples collected in full ripeness had more rounded shapes. Also the amount of carvone was higher in the sample harvested in full ripeness.

The content and quality of essential oils in plants treated in a different way were also examined. The effect of Roundup mixture on caraway maturation was investigated, to synchronize the maturation of caraway seeds in the main umbel and the first order umbels. The effect of fungicides (Alert and Prelude 10) on the content and quality of essential oils was also investigated. The use of both Roundup and fungicides was found to have a positive influence on the amount of essential oils in caraway.

Likewise, the possibilities of SFE use for the determination of essential oil content in small samples, collected during vegetation, were tested. Essential oil determination was performed in 45 plants obtained by means of positive selection in the plant cover of new varieties.

The results were applied as new criteria for the cultivation. The classical method for the essential oil determination does not allow anything like this.

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Obsah silic v odrůdách kmínu kořenného (*Carum carvi* L.)

ABSTRAKT: Nažky kmínu obsahují 1–6 % silic, v nichž je přítomno asi 30 látek, z nichž karvon a limonen tvoří asi 95 %. Zjišťovalo se množství silice a vzájemný poměr karvonu a limonenu u různých odrůd kmínu pro zjištění jejich kvality. Přestože nejčastější metodou pro stanovení silic je převážně destilace vodní parou (ČSN 58 0110), byla v práci také zkoumána alternativní metoda – superkritická fluidní extrakce (SFE). Sušené mleté nažky kmínu byly extrahovány při různých podmínkách SFE (tlak, teplota, použití modifikátorů). Uvolněné látky (karvon a limonen) byly kvantifikovány pomocí GC.

Klíčová slova: superkritická fluidní extrakce (SFE); semena kmínu; karvon; limonen; plynová chromatografie (GC); odrůdy

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