Determination of Essential Oils Content and Composition in Caraway (*Carum carvi* L.)

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Abstract

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Total content and composition of essential oils in caraway seed (*Carum carvi* L.) from a selection of caraway varieties grown in different production areas were determined after supercritical fluid extraction (SFE) and steam distillation. Different procedures of sample pretreatment (homogenization) and methods of isolation were compared from the point of view of efficiency, reproducibility and accuracy. The effect of storage period was also examined.

Keywords: carvone; limonene; *Carum carvi* L.; steam distillation; supercritical fluid extraction (SFE); gas chromatography

Caraway (*Carum carvi* L.) is grown for its high content of caraway oil, which is concentrated mainly in seeds (KAMENÍK 1996; KOCOURKOVÁ *et al.* 1999). Carvone and limonene are the essential components of caraway oil, in addition to trace amounts of other constituents (acetaldehyde, furfural, carveol, pinene, thujone, camphene, phelandrene etc.). Caraway seeds contain also lipids (13–21%), nitrogen compounds (25–35%), fibre (13–19%) and water (9–13%). In the last decade the area sown of this traditional crop in the Czech Republic is averaging 4500 ha (KOCOURKOVÁ *et al.* 1999). Total annual consumption of caraway exceeds 450 g per person (KOCOURKOVÁ 1996).

Caraway seed is used in meat, food and distillery industries due to its pleasant flavor and intense taste. It is also used in cosmetics. Its antibacterial and fungicidal properties are important in pharmaceutical applications and also in human and veterinary medicine (SEDLÁKOVÁ *et al.* 1998). Straw is an important feedstuff that increases milk production, improves taste and digestibility and reduces flatulence of cattle. Caraway oil is also used as a natural inhibitor of sprouting, mainly in stored potatoes.

Since 1998 the quality of caraway seed has been inspected following the State Directive No. 330/97 and the Food and Tobacco Products Act No. 110/97. The minimum content of caraway oil exceeding 30 g/kg and at least 50% of carvone in caraway oil is required by the Czech Pharmacopaeia, 1997 edition. The present legis-lation does not define exactly the analytical procedure for the determination of oil content. In practice the most often used procedures according to the standards ČSN 58 0110 (Metody zkoušení koření – Methods of space quality control), ISO-ČSN 6571 (58 0196 – Stanovení těkavých olejů [silic] – Determination of essential oils content) and the method described in the Czech Pharmacopaeia give different results and this could be the cause for discrepancies in quality control. Elaboration of an optimal simple method for the determination of total essential oils and of the carvone to limonene ratio, applicable for all routine laboratories, is of great importance.

Steam distillation according to the standard ČSN 58 0110, ISO-ČSN 6571 (58 0196), liquid extraction with hexane (BOUWMEESTER *et al.* 1995) or a combination of both procedures (MANI & WIOOLEY 1995) are the most widely used procedures for essential oils isolation from solid samples. Solid Phase Micro-Extraction (SPME) on polymeric sorbents and subsequent thermal desorption with a direct injection onto an analytical capillary GC column is a very suitable method for the isolation of monoterpenes from gaseous phase (KALLIO *et al.* 1994).

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Supercritical fluid extraction (SFE) of essential oils is a dynamically developing extraction technique that is a very good alternative to the classical extraction procedures using organic solvents. Supercritical carbon dioxide (polar modifiers like methanol, acetone, etc., are applied to increase polarity of the fluid) is the most widely used supercritical fluid in analytical SFE (KALLIO *et al.* 1994; ENGELHARDT & GROSS 1988; BOUNOSHITA *et al.* 1993), having low values of critical temperature (31°C) and pressure (7.38 MPa).

Gas chromatography with FID or MS detectors is mostly used to determine carvone, limonene and other constituents. Liquid chromatography with UV or polarimetric detection (KOVAR & BOCK 1983), derivative spectrophotometry in UV (SEIF EL DIN *et al.* 1983) and proton magnetic resonance (MOSSA *et al.* 1980) can be alternatively applied. Chromatographic methods allow the separation of enantiomers of carvone (BOWMEESTER *et al.* 1995; KALLIO *et al.* 1994; BOUNOSHITA *et al.* 1993; RAVID *et al.* 1992), limonene (BOWMEESTER *et al.* 1995) and other constituents (KALLIO *et al.* 1994), as well as the determination of origin by the so-called "finger-print" method (THIES 1984). Collection of GC chromatograms of different essential oils and MS spectra of many monoterpenes are available in a review and a book (MASADA 1976; Analytical Method Committee 1988). The identification of monoterpenes is usually based on the comparison of retention times and mass or infrared spectra of unknown components and standards.

The main aim of the paper is to elaborate an easy technique of sample preparation, i.e. grinding procedure and extraction, as well as of the determination of total content of oils and mainly of carvone to limonene ratios in the collected samples.

MATERIAL AND METHODS

Chemicals and Instrumentation*: l*-Carvone a *d*-Limonene (Fluka) were used to test extraction efficiency. Hexane (for HPLC, Merck) was used for the trapping of extracted substances. Liquid carbon dioxide for food industry, N_2 (4.0) and/or N_2 (4.6), He (5.0), H_2 (5.0) and medicinal oxygen (all AGA) were used for SFE and GC.

Different varieties of annual and biennial caraway (*Carum carvi* L.), mainly biennial varieties of Czech origin (Kepron, Prochan, Rekord) and the annual Polish variety Konczewicki, were studied. The samples were collected in different production areas of the Czech Republic (Šumperk – beet-growing Ř5, 1998–1999; Huštěnovice – maize-growing K5, 1999; Určice – beet-growing Ř4, 1999; Myslejovice – beet-growing Ř4, 1999; Bohuslavice – beet-growing Ř5, 1999; Česká Bělá – potato-growing type B2, 1997–1998). The production areas were characterized mainly by the differences in seasonal weather and moisture course, altitude, soil types and other factors that influence the content and composition of essential oils in caraway seed. Samples were stored in paper bags in hermetically closed brown bottles at ambient temperature.

An ETA 0067 grinder with grinding stones, a VIPO grinder and a Vibrom S2 (Jebavý, Třebechovice p. O., CR) single ball cryogenic grinder (liquid nitrogen) were tested for sample homogenization. A SE-1 (SeKo-K, Brno, CR) extractor for supercritical fluid extraction (SFE) and an apparatus for steam distillation according to ČSN 58 0110 and ČSN 6571 were applied for extraction and subsequent determination of the total content of caraway oil. Approximately 500 mg (± 0.1 mg) of exactly weighed sample was transferred into an extraction column for SFE extraction. Extraction was carried out for 60 min at 40 MPa, extractor temperature 80°C and restrictor temperature 120°C. The extract was trapped into a hexane layer in a trapping vessel.

The steam distillation method according to the standards ČSN 58 0110 and ČSN 6571 was used. Depending on the expected content of essential oil, an exactly weighed sample (10–25 g ± 0.1 mg) was transferred into a 1 l distillation vessel and 400 ml of water and boiling stones were added. The sample was boiled for 4 hours. After 4 hours the cooling was switched off and the distillation was prolonged for a while until all essential oils were quantitatively transferred into a calibrated tube. Then the heating was switched off and the volume of extracted essential oils was measured after 5 minutes. The extracted or distilled samples were stored in a refrigerator at 1–4°C (max. for 2 days), if necessary, and analyzed by GC.

A gas chromatograph HP 4890D (Hewlett Packard) with a FID detector was used to determine limonene-to-carvone ratio in the samples. Separation was performed using a HP-5 (Crosslinked 5% PH ME Siloxane, 15 m × 0.53 mm × 1.5 μm film) column at helium flow rate 2 ml/min, injector temperature 220°C and detector temperature 240°C using temperature program 60°C, 40°C/min up to 220°C, 2 min at 220°C. Portions of 2 μl of an essential oil solution in hexane were injected onto the analytical column. Resulting chromatograms were treated using CSW (Data Apex, Prague, CR) data station.

RESULTS AND DISCUSSION

Sample Preparation*:* According to the ČSN standard caraway seed has to be ground before analysis. Only those particles of ground seed that pass through a 1 mm mesh are used for subsequent extraction and determination. Results of a national inter-laboratory test of the steam distillation method according to ČSN 58 0110 and also poor reproducibility of our own results using SFE technique allow to conclude that the variation in sample preparation procedures (grinding, sieving) is the main source of differences in results obtained by respective laborato-

ries. The preparation procedures are not exactly specified in the standard mentioned. The extraction efficiency fully depends on the quality of grinding, particle size, temperature of the material ground and the grinding period. The use of an intact non-ground sample (the procedure is used in several countries like the Netherlands or Germany) seems to be the simplest way of total oil determination. These results initiated our interest in a detailed study of pretreatment procedures to develop a technique that could ensure most reliable results and make it possible to suggest an amendment of the ČSN 58 0110 standard.

Three different grinding procedures were tested using an ETA 0067 grinder, a VIPO grinder and a Vibrom S2 single ball cryogenic grinder. The extraction efficiencies for the grinding procedures were compared to the results obtained for the non-ground caraway seed samples (Table 1). Despite of the standard ČSN 58 0110 recommending the application of the ETA 0067 grinder with grinding stones, the highest efficiency was obtained in both isolation procedures (steam distillation and SFE extraction) when the VIPO grinder was applied.

Table 1. Influence of homogenization procedures on extraction efficiency

Homogenization	Distillation		SFE[1]	
	x	RSD	*x*	RSD**
Non-ground	2.18	0.96	0.09	1.56
ETA grinder	2.36	0.89	1.79	1.36
VIPO grinder	3.27	1.00	2.55	2.00
VIBROM 2S[2]	2.20	0.56	1.72	1.5

x – essential oils content in % (m/m); RSD – relative standard deviation for $n = 3$ in %; [1]supercritical fluid extraction; [2]cryogenic grinder

Steam distillation gives approximately the same results (the same order of magnitude). A non-ground mixed sample of caraway contained 2.18 g of caraway oil per 100 g (RSD = 0.96%), whereas the same ground sample (VIPO) contained 3.27 g/100 g with RSD = 1.00%. Efficiency of SFE in the non-ground sample was one order of magnitude lower (0.09 g/100 g with RSD = 2%) than in the ground sample (VIPO sample contained 2.55 g/100 g with RSD = 1.50%) and also in comparison with the method ČSN. Cryogenic grinding produces highly reproducible results (0.56 and 1.5%, respectively), but extraction efficiency is lower. The VIPO grinder was used for all other experiments.

***Extraction Methods**:* Two isolation procedures (steam distillation according to the standards ČSN 58 0110, ČSN 6571 (Stanovení obsahu těkavých olejů [silic] – Determination of essential oils content), ISO-ČSN 6571 and SFE extraction) were tested for the determination of total essential oils in caraway seeds. The steam distillation procedure (ČSN 58 0110) is suitable for both ground and non-ground samples. The steam distillation procedure ISO-ČSN 6571 (58 0196) differs from the previous method mainly by the construction of the apparatus and by trapping the essential oils in xylene. This procedure is not very suitable for everyday use since excessive evaporation of xylene (higher RSDs and lower accuracy) occurred during distillation. Lower efficiency of SFE extraction leads to lower values of essential oils content. Most pronouced differences were found in non-ground samples.

Comparison of total monoterpenes in different caraway varieties (Kepron, Rekord, Prochan, Konczewicki) showed that SFE extraction efficiency is obviously lower than the efficiency of conventional steam distillation procedure. As stated above in the section describing sample pretreatment, analysis of non-ground samples gives unacceptably lower results (one to two orders of magnitude). Despite of the lower efficiency of SFE extraction, the limonene-to-carvone ratios in different varieties are proportionate to those obtained by steam distillation.

Determination of Essential Oils Content in Samples from Different Production Areas: Samples from production areas Huštěnovice, Bohuslavice, Určice, Myslejovice and Šumperk were collected in 1998 and 1999. Both extraction procedures were used for the determination of content and composition of essential oils (Table 2). It was found that the sample from Šumperk contained the highest amount of caraway oil.

Table 2. Determination of total content of essential oils in caraway from different production areas

Production area	Homogenization procedure	Distillation		SFE[1]	
		x	RSD	*x*	RSD
Huštěnovice K5	non-ground	1.82	0.90	–	–
	ground	2.35	1.08	1.71	1.25
Bohuslavice	non-ground	2.89	0.12	–	–
	ground	3.42	0.29	2.54	1.59
Určice R4	non-ground	2.61	0.76	–	–
	ground	3.28	2.66	2.32	0.98
Myslejovice	non-ground	3.27	0.44	–	–
	ground	4.43	2.22	2.81	2.25
Šumperk 1/99 R5	non-ground	4.27	0.98	–	–
	ground	5.79	2.71	4.35	2.08

x – essential oils content in % (m/m); RSD – relative standard deviation in %; [1]supercritical fluid extraction

Variety Influence: A selection of caraway varieties (Kepron, Prochan, Rekord, Konczewicki) were compared concerning carvone and limonene content and their ratio in the final extract. The content of essential oils decreases from Rekord over Prochan and Kepron down to Konczewicki variety (Table 3). Despite the differences in essential oils content among different caraway varieties

Table 3. Comparison of essential oils content and their composition in different caraway varieties

Caraway variety	Distillation		SFE[1]		L to C ratio [%][2]
	x [%] m/m	RSD [%]	x [%] m/m	RSD [%]	
Prochan	8.67	4.08	6.65	3.76	36.1:63.8 32.6:67.4[a]
Rekord	8.94	4.95	7.02	3.70	34.4:65.6 31.1:68.9[a]
Kepron	7.98	1.40	5.71	4.82	35.9:64.1 32.0:68.0[a]
Konczewicki[b]	5.00				24.3:75.7
Prochan[c,d]	1.82	0.9	–	–	18.5:81.5
Prochan[c,e]	2.35	1.08	–	–	33.6:66.4
Winter variety [f,d]	1.85	0.72	–	–	30.6:69.4
Winter variety [f,e]	2.37	3.13	–	–	43.7:56.3

[1]supercritical fluid extraction, [2]distillation – limonene-to-carvone ratio, [a]SFE, [b]ŠS Česká Bělá, [c] Huštěnovice 1999, [d]non-ground, [e]ground, [f]Huštěnovice 1999, variety awaiting registion

the limonene-to-carvone ratio remains practically constant in all cases.

The results obtained by steam distillation in Prochan variety were compared with those in a winter variety (not yet registered) grown in the same area (Huštěnovice, K5, 1999). Although the isolation yields are approximately the same, the limonene-to-carvone ratio is higher in the winter variety. The ratio is lower in non-ground caraway seed than in ground samples.

Stage of Ripeness: The total content of essential oils was determined 14 days before full ripeness (A) and in full ripeness (B) in Kepron and Prochan varieties (Šumperk 1998) by steam distillation and SFE extraction using optimized experimental conditions. The total content of oils was lower in the samples (A), i.e. in seed collected before full ripeness, than in samples (B) collected in full ripeness. Also, conspicuous changes in color were observed visually. The total content of monoterpenes differed in different varieties. Limonene-to-carvone ratios were practically constant in the samples collected before (samples A) and in full ripeness (samples B). On the contrary, serious differences were found in SFE extracts (Table 4).

Effect of Storage Period: To control the effect of storage period on the content of essential oils, the samples were stored in hermetically closed brown glass bottles at room temperature. The bottles were protected from light. The samples 1/99–8/99 from Šumperk, Určice (Rekord variety) and Myslejovice (Kepron variety) were extract-

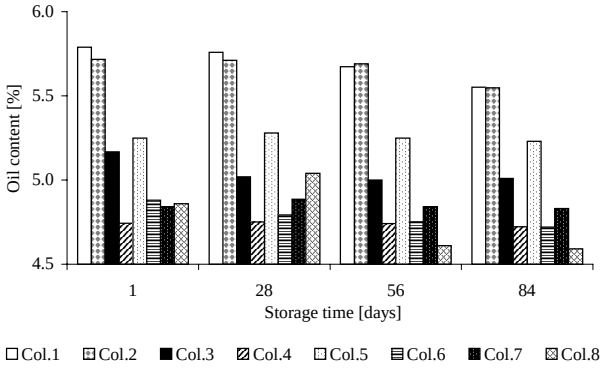


Fig. 1. Dependence of total essential oils content on the period of storage, samples from Šumperk production area 1/99–8/99 (columns 1–8)

Table 4. Comparison of total content and composition of essential oils from caraway in different vegetation stages

Caraway variety	Distillation	SFE [1]		L to C ratio (%)[2]	
	x [%])* m/m	x [%] m/m	RSD [%]	distillation	SFE [1]
Kepron – A	2.00	1.26	2.01	25.0:75.0	26.7:73.3
Kepron – B	5.27	3.36	3.01	25.0:75.0	18.2:81.8
Prochan – A	2.36	1.50	2.00	24.1:75.9	35.1:64.9
Prochan – B	6.18	3.94	1.98	24.9:75.1	27.5:72.5
Rekord – A**	2.40	–	–	24.5:75.5	–
Rekord – B**	6.90	–	–	24.3:75.7	–

[1]surercritical fluid extraction, [2]limonene-to-carvone ratios, *only single measurement due to insufficient sample size, ** ŠS Česká Bělá; A – before full ripeness; B – in full ripeness

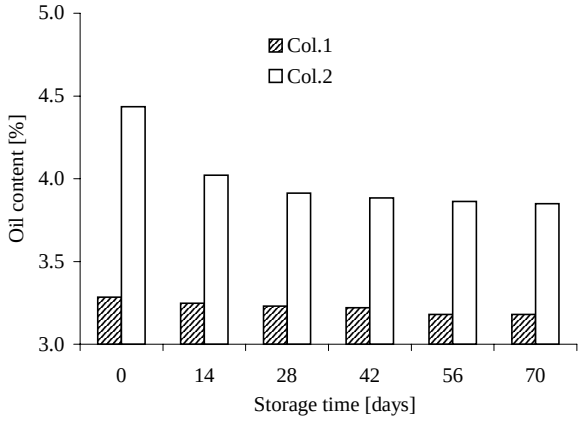
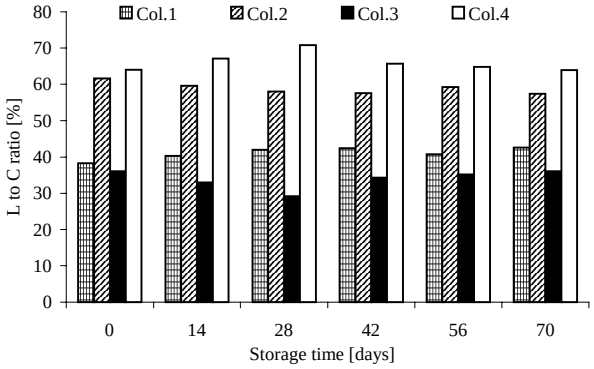


Fig 2. Dependence of total essential oils content on the period of storage, samples from Myslejovice production area – column 1 and Uhřice production area – column 2



Column 1– limonene, Určice production area, column 2 – car-vone, Určice production area, column 3 – limonene, Myslejovice production area, column 4 – carvone, Myslejovice production area

Fig 3. Dependence of carvone and limonene content (%) in ground (VIPO) samples on the period of storage

ed by steam distillation and SFE. The total content of essential oils was measured after two and four weeks. Results are summarized in Figs. 1–3. It can be seen that the content of essential oils decreases with increasing storage perion in all samples and both isolation procedures. Dependence of limonene-to-carvone ratio on the storage period was checked every two weeks (Figs. 2 and 3). The limonene-to-carvone ratio increased with time for both isolation procedures. Both substances evaporated from the seed during the storage period and were released into the ambient atmosphere.

CONCLUSIONS

It was found that different varieties of caraway contain 1–9% (m/m) of essential oil that produces typical caraway taste and fragrance. Approximately 30 substances are present, carvone and limonene forming approx. 95%. All other substances are present at trace concentrations. Their determination is more difficult, thus the quality of caraway is still rated by the total content of oils and by the ratio of the two monoterpenes. Athough it is not clear as yet which component(s) play the most important role in producing taste and fragrance, it can be expected that higher limonene-to-carvone ratio means better quality of caraway.

The VIPO grinder was found suit best for sample pretreatment in practical applications since the highest extraction efficiencies were obtained for both isolation procedures – steam distillation and SFE extraction – when using this grinder. The cleaning procedure is also simpler and the cross-contamination is minimized. Cryogenic grinding gives more reproducible results but is more time consuming and needs a special apparatus. It is also less convenient for practical purposes.

Steam distillation procedures (ČSN 58 0110, ČSN 6571 and ISO-ČSN) have been widely recognized and established procedures in practical applications for a long time. They yield good results for homogenized as well as non-ground samples. This fact simplifies sample pretreatment for analysis and can be recommended for the revision of pertinent national standards. Its application, in combination with the intact (non-ground) caraway seed, seems to be a very suitable alternative of the isolation of essential oils and subsequent determination of limonene-to-carvone ratio. Because of slightly lower extraction efficiency of this technique some changes in quality control legislation would be needed to fulfill the EU requirements (simplicity, accuracy, precission and absolute and relative contents of carvone and limonene).

SFE procedure is a new, very useful method for sample preparation and for the isolation of highly volatile substances from complicated matrices like plant materials. It can easily compete with the classical procedures like steam distillation and solvent extraction. Low critical temperature and non-polar character of supercritical fluid (carbon dioxide) is preferable especially for the isolation of highly volatile, highly reactive or thermo-sensitive compounds. The method is fast and precise.

The total content of essential oils increases with increasing ripeness, especially shortly before full ripeness. Two varieties (Kepron and Prochan) were examined. It was found that samples (A) collected 2 weeks before full ripeness containend less essential oils than those in full ripeness (samples B), in correlation with conspicuous color changes. The content of essential oils decreases with the time of storage, as does the limonene-to-carvone ratio.

Diverse varieties of caraway produced in the Czech Republic differ in both total content of essential oils and limonene-to-carvone ratio. The Rekord variety contained the

highest quantity of oil. Various varieties from different production areas differed in the content of essential oils according to the area (Huštěnovice, Bohuslavice, Určice, Myslejovice and Šumperk) and the variety. Difference between content of essential oils among spring or winter varieties was insignificant whereas spring varieties contained higher amount of carvone than winter varieties. Also in this case the color difference was conspicuous. This could make the production of spring varieties more desirable.

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Souhrn

SEDLÁKOVÁ J., KOCOURKOVÁ B., KUBÁŇ V. (2001): **Stanovení obsahu a složení silic kmínu kořenného (***Carum carvi* L.**).** Czech J. Food Sci., **19**: 31–36.

Celkový obsah a složení esenciálních olejů v kmínu kořenném (*Carum carvi* L.) byly stanoveny po superkritické fluidní extrakci a destilaci s vodní parou u různých odrůd kmínu pěstovaných v rozdílných produkčních oblastech. Z pohledu efektivity, opakovatelnosti a přesnosti byly srovnány různé postupy přípravy vzorků k analýze (homogenizace) a metody izolace. Byl rovněž studován vliv způsobu uchovávání vzorku na výsledky stanovení.

Klíčová slova: karvon; limonen; kmín kořenný; *Carum carvi* L.; destilace s vodní parou; superkritická fluidní extrakce (SFE); plynová chromatografie

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