Gas chromatography, sensory analysis and electronic nose in the evaluation of black cumin *(Nigella sativa L.)* aroma quality

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S u m m a r y

Essential oil composition of *Nigella sativa* L., the enantiomeric ratio of its chiral compounds as well as sensory characteristics were the subject of the study. The evaluation of several samples of *N. sativa* seeds purchased in retail was performed by gas chromatography, chiral gas chromatography, olfactometry, sensory profile analysis and the electronic nose. Eleven components were identified. Olfactometry revealed p-cymene, thymoquinone and carvacrol as the most important odorants. P-cymene occurred in all of the samples at highest amounts. Six pairs of enantiomers were isolated. All the components showed variability in their enantiomeric ratios, with exception of carvone, which was found with a stable optical purity of the (-)-isomer, not lower than 85%. The dominating attributes in the sensory profile were cardboard, herbal and spicy. Results of sensory profile analysis and the electronic nose, both interpreted by the Principal Component Analysis, gave similar, although not identical differentiation of the samples.

Key words: *black cumin, aroma, key compounds, sensory analysis, electronic nose*

INTRODUCTION

Nigella sativa L. as well as *Nigella damascena* L. are herbaceous annual plants of the family *Ranunculaceae* growing in grasslands, in temperature zones in Europe [1, 2]. Despite many bioactive components, seeds of *N. sativa* are the source of essential oil; its concentration may reach up to 1.5%. According to literature the main compounds of this oil include: α- and β-pinene, p-cymene, limonene, linalool, terpinen-4-ol, carvone, trans-anethole, thymoquinone and carvacrol [1-9].

N. sativa seeds have been used for many years as a spice and food preservative as well as a protective and curative remedy for various disorders, as their chemical components produce a variety of pharmacological actions [10]. Much of the biological activity of seeds has been shown to be due to thymoquinone [11].

The aroma of black cumin seed as well as some of its biological properties are influenced mostly by the composition of essential oil. Thymoquinone has generally been recognized as one of the most important components of oil [11-14], however, p-cymene is usually found in highest amount [6, 14, 15].

Aroma constituents of essential oils are often chiral compounds and they occur in the form of optical isomers. In some cases the enantiomeric ratio of these components is crucial for the aroma characteristics as a criterion of aroma authenticity [16, 17]. Among black cumin aroma constituents there are few chiral compounds, but there is a limited information on that subject in the literature.

Various methods can be used to characterize the spice aroma. The knowledge on oil composition is very important for quality characteristics of plant material, as the composition may show a variability due to many factors, such as climate, variety, the location of growing area [18, 19], which may be also observed in case of black cumin [1, 12].

The aim of this study was to apply various methods to evaluate the aroma of black cumin seeds supplied from four commercial firms operating on the market in Poland (one in Germany).

MATERIALS AND METHODS

Samples of seeds of *Nigella sativa* were purchased in retail on the local market (samples A, B, C, D), and from the market in Germany (E).

Standards of volatiles: (+)-camphor, (-)-carvone, (+)-carvone, p-cymen, carvacrol, (+)-2-carene, (-)-2-carene, (+)-limonene, (-)-limonene, (+)-linalool, (-)-linalool, (+)-α-pinene, (-)-α-pinene, (+)-β-pinene, (-)-β-pinene, terpinen-4-ol, (-)-terpinen-4-ol, thymoquinone, trans-anethol used for the identification of volatile compounds were purchased from Sigma Chemical Co. and Fluka. Other reagents, such as pentadecane, methanol and m-xylene, were of analytical grade for GC.

The essential oils were isolated from 1 g of black cumin seeds by the hydrodistillation method using a Deryng laboratory glass apparatus of Polish Pharmacopoeia [20]. Distillation took 3 hours. Prior to distillation 0.795 mg of the internal standard (pentadecane) was added together with 0.3 mL of xylene, used as the extraction solvent.

A Hewlett-Packard HP 6890 gas chromatograph with a split/splitless injector and FID detector was used for the analyses. Compounds were separated using a DB-5 capillary column (30 m x 0.32 mm x 0.25 μm) and two Restek chiral capillary columns (30 m x 0.32 mm x 0.25 μm) – RtβDex sa and RtβDex sm. The identity of the separated compounds was confirmed on a Hewlett Packard HP 5890 II gas chromatograph coupled to an HP 5971MSD quadrupole mass spectrometer. The injection of volatiles was performed in the split mode. Analysis parameters on the DB-5 column were as follows: initial temperature 60°C (held for 1 min.), then 10°C/min. to 200°C/min. (held for 10 min.), while on the chiral column it was as follows: initial temperature 40°C (held 1 min.), then 2°C/min. to 200 °C (held 4 for min.). The flow of hydrogen, used as a carrier gas, was 1.6 mL/min. The amount of volatiles were calculated on the basis of the known amount of the internal standard added to the sample prior to distillation. The enantiomeric ratio was calculated as the ratio of peak areas of the authentic sample.

Olfactometric analysis was performed using a HP 5890 gas chromatograph (Hewlett- Packard, Germany) equipped with an inlet splitter and a smelling port using a DB-5 column (30 m x 0.53 mm x 0.25 μm, Supelco Inc., Bellefonte PA). Separation conditions included: helium as a carrier gas, flow rate 1 ml/min., programmed column temperature: 1 min. at 40°C, gradient of 8°C/min. to 200°C, next an increment of 20°C/min. to 280°C and 5 min. at 280°C. Separated fractions were smelled in successive double dilutions of analyzed essential oils, until the last detectable aroma disappeared. In this way aroma was referred to respective retention indices and dilution factors (FD) for individual fractions [21-23].

A Fox 4000 electronic nose with 18 metal oxide sensors in three chambers was used for the analysis (Alpha M.O.S., Toulouse, France). Samples of ground seeds of black cumin (0.1 g) were placed in 10 mL vials, capped, and placed in a Combipal type autosampler (HS-100.). Samples were incubated for 5 min at 35ºC and then volatiles (500 μL) were transferred automatically to the electronic nose with a gastight syringe. A pure synthetic air flow of 150 mL/min was used to sweep samples through the electronic nose chambers. Each sample was analyzed in triplicate – three vials were prepared from each sample lot. Sensor optimization and data processing were performed using the Alpha Soft 0.8 software package (Alpha M.O.S.). Operations on signals included signal pre-processing to build libraries with the default values for statistical data processing, the selection of sensors providing the highest degree of sample differentiation and principal component analysis (PCA) of obtained data. For the sensor array data, the library was built with the values in ΔR/R0 and maximum of the sensor intensity. ΔR/R0 displayed the value of the sensors in relative resistance change. In that mode the displayed value was (R0 – R)/R0, where R0 was resistance at t=0 (baseline resistance) and R was resistance at a selected time. When the maximum value option was selected, the library was built with the value taken from the top of the response peak for each sensor (even if the maximum did not occur at the same time for all the sensors).

Sensory analysis was performed according to Zawirska-Wojtasiak et al. [19]. A panel of 10 people experienced in profile sensory analysis analyzed 5 samples of black cumin seeds. A vocabulary of descriptors was developed for the evaluation of their aroma. The following odor descriptors were offered for examined samples: (v1) cardboard, (v2) herbal, (v3) pepper-like, (v4) mint-like, (v5) flowery, (v6) sweet, (v7) spicy and (v8) petroleum-like. Seeds samples (1 g) mixed with 100 mL 4% starch solution were presented to panel members in closed 150 mL vials. The vials with samples were preheated at 40°C to liberate volatile compounds. Samples were evaluated during three sessions. Panel members marked the intensity of each odor descriptor on a 0–10 graphic line scale. Thirty replicates (3 sesions x 10 panelists) for each descriptor were processed using principal component analysis (PCA).

RESULTS AND DISCUSION

Eleven compounds were identified in the aroma isolates from black cumin seeds. Among them, the following were found in higher amounts: p-cymene, thymoquinone, carvacrol and carvone. Most of these compounds were reported as the primary compounds in *N. sativa* by other authors [6, 11-15]. γ-Terpinene was not identified, however, it was stated by other authors in essential oils of black cumin, by some of them in low quantity [12], but in the significant in others [6]. The similar situation was with cis-sabinen-hydrate or trans-sabinen-hydrate, not identified in this work, but found in the literature at a level from trace to 4% [4, 6, 9]. The content of the particular components as well as that of total volatiles varied among the samples. The sum of volatiles were calculated at the range from 15.44 mg to 21.57 mg per 1 g of seeds, while p-cymene and thymoquinone from 9.74 to 11.79 and from 0.97 to 4.35, respectively. Content of measured compounds in *N. sativa* seeds samples is presented in Table 1. In the available literature usually percentage composition of black cumin aroma compounds is presented [1, 6, 9, 12, 13, 15]. In this study p-cymene occurred even over 60% of the total compounds (samples A, B and C), while for thymoquinone the highest percentage was about 20% in sample E (from the German market). About 92% of total compounds were identified. The percentage composition of essential oils of analyzed samples is given in Figure 1.

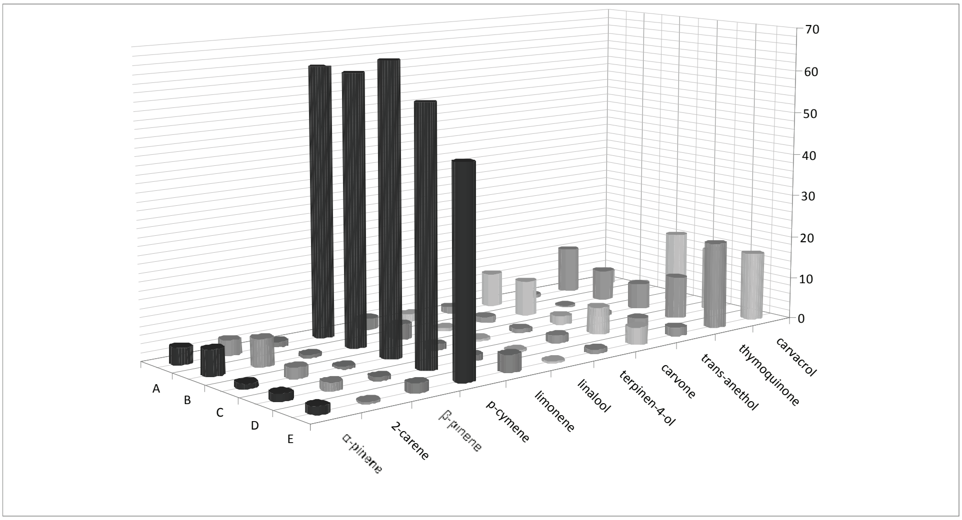

Figure 1. Percentage composition of volatile compounds in the samples of *N. sativa*

Table 1.

Volatiles content in the samples of *N. sativa* seeds

volatile compounds	Kovat's index	[mg/g]				
		A	B	C	D	E
α-pinene	935	0.61*	1.15	0.20	0.36	0.43
2-carene	940	0.56	1.20	0.40	0.38	0.13
β-pinene	968	0.17	0.13	0.09	0.17	0.69
p-cymene	1022	9.74	11.79	10.76	11.71	10.30
limonene	1026	0.41	0.68	0.22	0.25	0.34
linalool	1166	0.27	0.13	0.10	0.08	0.09
terpine-4-ol	1180	0.23	0.27	0.16	0.35	0.39
carvone	1202	1.22	1.55	0.34	1.27	0.92
transanethol	1259	0.12	0.07	0.15	0.36	0.45
thymoquinone	1297	1.63	1.35	0.98	1.95	4.35
carvacrol	1307	0.48	0.42	2.70	2.94	3.48
total		15.44	18.74	16.13	19.82	21.57

*Values represents mean of 5 repetitions. Coefficient of variation 2–5%

Six pairs of enantiomers were separated in the samples of black cumin essential oil. The enantiomeric ratios of the measured compounds showed variability, without a characteristic excess of one optical form (tab. 2). Carvone was the only component with a stable and rather high optical purity of the (-) form, not lower than 85%, which expresses mint-like aroma, while (+)-isomer is the typical caraway odor [17, 23]. In terms of optical purity of its minus form with the specific mint-like character, carvone can be used in authenticity control of this aroma, however, carvone is not a crucial component of black cumin aroma and it does not occur in large amounts. Several authors presenting aroma composition of *N. sativa* did not mention carvone at all [13, 15] or noticed only trace amounts of it [8]. The main odorants of *Nigella sativa* - thymoquinone and p-cymene - are not chiral compounds.

Table 2.

Enantiomeric composition of chiral components in *N. sativa*

compounds	RI	enantiomeric ratio (%)				
		A	B	C	D	E
(–)α-pinene	985	48.0	42.5	43.9	36.7	74.7
(+)α-pinene	990	52.0	57.5	56.1	63.3	25.3
(–)β-pinene	1034	58.4	62.3	67.2	37.7	65.3
(+)β-pinene	1043	41.6	37.7	32.8	62.3	34.7
(–)limonene	1082	7.1	15.9	5.9	21.8	20.3
(+)limonene	1084	92.9	84.1	94.1	78.2	79.7
(–)linalool	1237	31.8	48.0	37.7	37.2	24.7
(+)linalool	1244	68.2	52.0	62.3	62.8	75.3
(–)terpine-4-ol	1351	72.2	12.3	84.0	73.1	94.1
(+)terpine-4-ol	1360	27.8	87.7	16.0	26.9	5.9
(–)carvone*	1418	87.8	86.7	85.7	86.9	86.4
(+)carvone	1425	12.2	13.3	14.3	13.1	13.6

*carvone isomers were separated on Rt βDex sa chiral column while all the rest on Rt βDex sm

Olfactometric measurements taken for the analyzed samples revealed thymoquinone, p-cymene and carvacrol as the most important odorants in black cumin aroma. However, the FD values of these compounds varied depending on the sample, mostly for thymoquinone (tab. 3).

Table 3.

FD-values from olfactometry measurement of *N. sativa* samples

name of compound	odor	A	B	C	D	E
		FD-values				
α-pinene	pine-like	128	128	16	64	64
2-carene	spicy	64	64	32	32	8
β-pinene	turpentine	8	8	8	16	32
p-cymene	cardboard	512	512	512	512	256
limonene	citrus-like	32	16	16	8	64
linalool	flowery	8	128	64	64	128
terpine-4-ol	woody-earthy	64	64	32	32	32
carvone	mint-like	128	128	64	128	128
transanethol	anise-like	64	128	8	16	32
thymoquinone	medicinal	512	512	256	512	1024
carvacrol	petroleum-like	128	128	256	256	256

The sensory profile odor estimation performed for all the tested seed samples revealed differentiation between samples which were more or less aromatic seeds, with different odor characteristics. The dominating descriptors in the sensory profile analysis were v1 – cardboard-like, v2 – herbal, v7 – spicy and v4 – mint-like. Figure 2 presents the PCA projection of the sensory data. Samples p3 (C) and p4 (D) were low aromatic in contrast to p5 (E), p1 (A) and p2 (B). However, the last three samples also significantly differed in character. Flavour of p5 (E) was intensive cardboard, while p1 (A) and p2 (B) were herbal, spicy and mint-like.

The PCA interpretation of the electronic nose data is shown in Figure 3. The differentiation of the samples was similar to that obtained by sensory profile, interpreted by the same statistical method. The most similar were samples A and B, significantly different from all the others. These two samples were also similar, according to sensory data. A similar distribution of the other three samples can also be stated. A good correlation was observed for the data from the electronic nose sensors and the sensorily estimated intensities of mint-like (0.9773) and cardboard (0.9618) aromas.

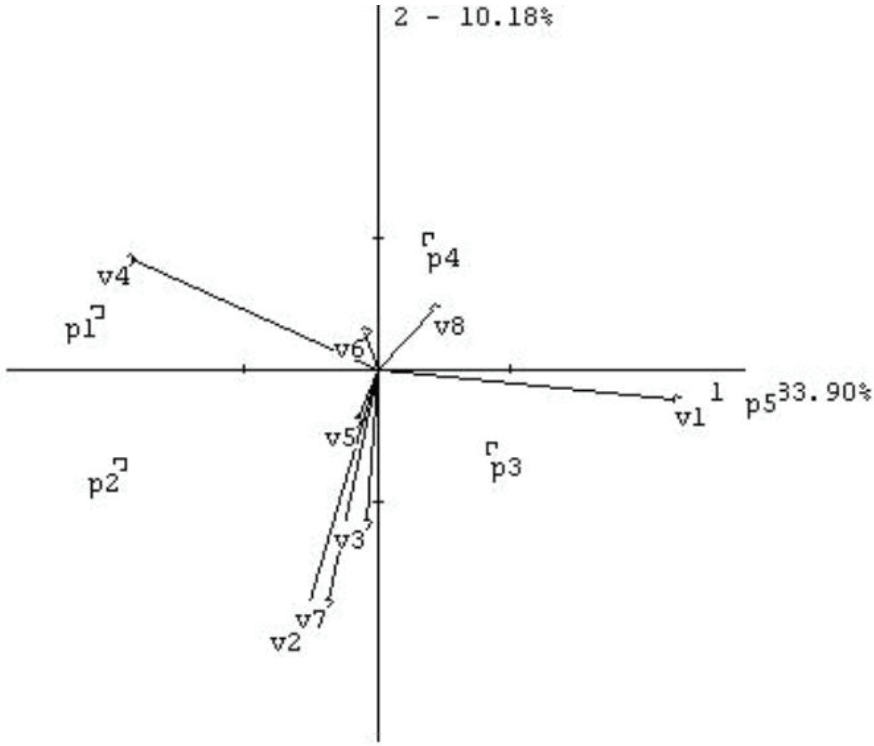

Figure 2. PCA plots of sensory data. Sample codes: p1 – A, p2 – B, p3 – C, p4 – D, p5 - E: Descriptors: (v1) cardboard, (v2) herbal, (v3) pepper-like, (v4) minty, (v5) flowery, (v6) sweet, (v7) spicy and (v8) petroleum-like

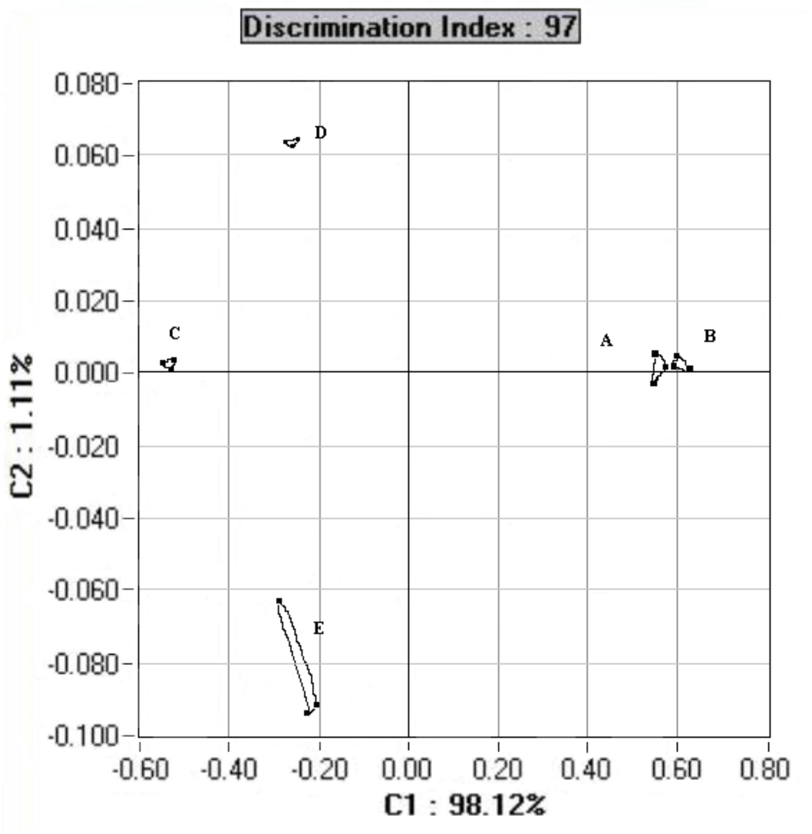

Figure 3. The PCA interpretation of electronic nose data of *N. sativa* samples using combination of sensors

CONCLUSIONS

Concerning all the data it can be stated that in the examined samples p-cymene was recorded in the highest amount, but the content of this component as well as that of thymoquinone and carvacrol, i.e. two other black cumin main odorants varied between the samples. Differentiation in the total volatiles and the proportions between them reflects sensory properties expressed in different sensory profile as well as in distinctive electronic nose data. The aroma of black cumin contains several chiral components, although, in general, they did not occur in characteristic enantiomeric ratios with exception of carvone which is not a very important component of this aroma.

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CHROMATOGRAFIA GAZOWA, ANALIZA SENSORYCZNA I NOS ELEKTRONICZNY W OCENIE AROMATU CZARNUSZKI (*NIGELLA SATIVA* L.)

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S t r e s z c z e n i e

Przedmiotem badań był skład związków zapachowych olejku eterycznego z nasion *Nigella sativa* L., stosunki enancjomerów związków chiralnych oraz charakterystyka sensoryczna. Ocenę kilku prób nasion czarnuszki pozyskanych na rynku wykonano z zastosowaniem chromatografii gazowej, spektrometrii mas, chiralnej chromatografii gazowej, olfaktometrii, profilowej analizy sensorycznej oraz nosa elektronicznego. Zidentyfikowano jedenaście składników aromatu. Analiza olfaktometryczna wykazała, że głównymi odorantami były p-cymen, tymochinon i karwakrol. Najwyższą zawartość we wszystkich próbach stwierdzono dla p-cymenu. Rozdzielono sześć par enancjomerów. Wszystkie te związki wykazywały zróżnicowanie stosunku enancjomerów w poszczególnych próbach, za wyjątkiem karwonu, który występował zawsze w formie lewoskrętnej (-)-karwon, o czystości optycznej nie mniejszej niż 85%. Dominującymi atrybutami w ocenie sensorycznej były zapachy kartonowy, ziołowy i korzenny. Sensoryczna analiza profilowa i nos elektroniczny wykazały podobne zróżnicowanie między próbami. Wyniki otrzymane obiema metodami były interpretowane z zastosowaniem Analizy Składowych Głównych (PCA).

Słowa kluczowe: *czarnuszka, aromat, związki kluczowe, analiza sensoryczna, nos elektroniczny*