Research Article

Headspace solid-phase microextraction and gas chromatography–olfactometry analysis of raw spirits of different organoleptic quality

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ABSTRACT: The aim of this research was to determine which compounds contribute to the flavour of agricultural distillates and to indicate those compounds which are responsible for a deterioration of sensory quality. Aroma profiling was carried out by headspace solid-phase microextraction (HS-SPME) and gas chromatography-olfactometry (GC–O). Aroma profiles were obtained using the fingerspan method. It was ascertained that the aroma profiles of agricultural distillates contain about 40 odours, some of which may serve in discriminatory analysis as markers of sensory quality. Some of the aroma compounds could be identified with the use of a mass spectrometer. It was found that the greatest amount of information on quality was obtained through analysis of the most intense odours detected in all or almost all samples, and that two odours formed the most radical indicators of sensory quality, being the result of the presence of two compounds, dimethyl trisulphide and geosmin. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: volatile compounds; aroma; raw spirits; solid-phase microextraction; gas chromatography–olfactometry

Introduction

Sensory analysis is a factor which is often decisive regarding the suitability of agricultural distillates for the production of rectified spirits. It is known from the literature and from direct contacts with representatives of the spirit industry that, in spite of the fact that the majority of currently produced distillates and spirits conform to standards, they differ significantly with respect to aroma and taste. An organoleptic evaluation is sometimes the only way to detect sensorially active contaminants in seemingly pure spirits. Because of the great influence of the human factor it has, after all, numerous shortcomings, such as poor repeatability and reproducibility of results. A limitation is also caused by the impossibility of identifying analytes and conducting their quantitative analyses.

In effect, there are still no unequivocal methods of distinguishing agricultural distillates produced from poor-quality raw materials or through improper technology, from qualitatively good distillates.[1,2] Processing of poor-quality raw spirits leads to obtaining low-quality rectified spirit and alcoholic beverages and, in effect, to reduced demand and financial loss. Thus, the key stage in the production of spirits and alcoholic beverages in a distillery is the selection stage (acceptance or rejection) of agricultural distillates for further rectification and the production of beverages such as pure or flavoured vodkas. Because of the indicated limitations of sensory analysis methods, there is the necessity to carry out research work which would lead to more precise identification and quantitative evaluation of sensorially active impurities occurring in agricultural distillates, spirits and alcoholic liquors.

The problem of determination of volatile aroma compounds in alcoholic products is not new.[3] Numerous publications are known, connected with studies on aroma compounds in alcoholic beverages such as wine, beer, brandy, whisky or other flavoured vodkas, that indicate that already a few hundred chemical compounds are known which can appear in the volatile fraction of such products. However, most often studies published heretofore were related to the identification of volatile compounds found in some characteristic or regional wines and flavoured vodkas with a specific bouquet, and they concentrated mainly on the choice of an appropriate extraction method and conditions of the analytical procedure. Furthermore, it is difficult to find publications on the relationship between the constitution of the volatile fraction of a product and its quality. To sum up, heretofore not many analytical methods have been developed which fulfilled the expectations of Polish producers in the spirit industry and which would provide useful information for this industry.

Determination of aroma components with the use of instrumental techniques is carried out in two stages. The first stage of the analysis is of particular importance, i.e. the isolation of analytes from the matrix. The form of the olfactograms closely depends upon the isolation procedure, as the use of various

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sample preparation methods, even various solvents in the case of solvent extraction, results in differing composition and content of isolated compounds. The isolated extract has to be possibly representative, therefore the choice of an appropriate sample preparation method is essential. Heretofore, the most often used technique has been liquid–liquid extraction. Isolates obtained by exhaustive extraction methods, e.g. methods using solvent extraction or distillation, do not always reproduce the aroma composition that reaches the olfactory and flavour receptors when eating or drinking. It should be kept in mind that the aroma of beverages and victuals includes only part of the volatile aroma compounds. Depending on their solubility and properties and on the composition of the matrix itself (e.g. saccharide content), the constitution of the volatile fraction of products can vary; thus, most favourable is the use of such extraction methods which rather exemplify the liberation of volatile components from the matrix than allow determination of their full component content, and which can be thus easier correlated with the results of sensory analysis. Methods of this type include methods using headspace analysis, in this a solid-phase microextraction (HS-SPME).[4–6] Such technique has been already used for analysis of aromas of alcoholic beverages, linked with the detection frequency method[4] and the dilution to threshold method—aroma extract dilution analysis (AEDA).[5,6]

In the research presented here, on the aroma profiling of raw spirits of various organoleptic qualities, a combination of the HS-SPME technique with the direct intensity method (the so-called fingerspan method) has been used[7]. While in the detection frequency methods and the dilution to threshold methods each of the evaluating persons notes only the presence or absence of an olfactory stimulus, direct intensity methods measure the intensity of the stimulus and its duration. The fingerspan method allows a larger amount of data to be obtained, because the olfactogram obtained is near to typical chromatograms achieved with the use of conventional detectors and shows the dependence of aroma intensity as a function of retention time.

Experimental

Samples and Chemicals

Raw grain spirits with an ethanol concentration of approximately 90% v/v were obtained from the Sobieski Distillery (Destylarnia Sobieski S.A., Starogard Gdański, Poland). Samples were delivered by local agricultural distilleries (Pomeranian region). The raw spirits selected for this study (39 samples) differed in organoleptic properties; 13 of them reached the highest rating from sensory analysis performed in accordance with Polish Standard PN-A-79 528-2:2002 (samples 27–39); 13 spirits received diverging ratings during the sensory analysis—some of the evaluating panelists found them to satisfy the standard, some rated otherwise (samples 14–26); the remaining 13 samples did not fulfil the organoleptic requirements (samples 1–13). Preliminary sensory analyses of samples were carried out in the laboratory of Sobieski Distillery.

High-purity deionized water (MilliQ A10 Gradient/Elix System, Millipore; Bedford, MA, USA) was used for the preparation of samples. *n*-Alkanes with a chain length of C_5–C_{20} (Sigma-Aldrich Poland, Steinheim, Germany) were used for the calculation of retention indices. During the preparation stage of the evaluating team, the following substances were used for olfactometric analysis: 95% rectified spirit (Polmos, Lublin, Poland) and 10 selected reference substances: 2-phenylacetaldehyde (≥90%), 2-phenylethanol (≥98%), 2,3-butanedione (≥99.4%), ethyl propanoate (99%), ethyl butanoate (99%), ethyl hexanoate (≥99%), 3,7-dimethylocta-1,6-dien-3-ol (linalool; 97%), hex-3-en-1-ol (98%), 3-methylbutanal

(≥98%) and furan-2-carbaldehyde (furfural; 99%) (Sigma-Aldrich, Steinheim, Germany); and for identification of aroma substances: ethyl acetate (≥99.5%), 3-methylbutanal (97%), ethyl propanoate (99%), ethyl butanoate (99%) dimethyl disulphide (≥99%), 3-methyl-1-butanol (≥99%), ethyl hexanoate (≥99%), dimethyl trisulphide (≥98%), ethyl octanoate (≥99%), ethyl decanoate (≥99%), 2-phenylethyl acetate (99%), ethyl dodecanoate (99%), 2β,6α-dimethylbicyclo[4.4.0]decan-1β-ol (geosmin; 100 µg/ml in methanol; Sigma-Aldrich).

Sensory Analysis

For the purpose of determining the general aroma character of the tested samples of agricultural distillates, a classical sensory analysis was carried out. Sensory analyses of samples were conducted with the use of the profiling method, carried out by a three-person team. On the basis of the known literature, nine sensory descriptors were chosen, often used in the analysis of alcoholic beverages: onion/vegetable, earthy/mouldy, bread/toast, fruity/sweet, chemical/solvent, nauseating, acrid/penetrating, green/plant and tart.[8–18] A four-point scale of intensity of a given odour was used to evaluate quantitatively the intensity of each of these descriptors: 3 points, very intense odour; 2 points, odour of medium intensity; 1 point, not intense odour; 0 points, no odour. The samples of raw spirits were diluted to 20% ethanol concentration. The measurement was carried out at a temperature of approximately 20 °C in an odour-free room. All the samples were analysed twice in various sequences.

SPME Conditions

An SPME holder for manual use and divinylbenzene/carboxen/poly-dimethylsiloxane (DVB/CAR/PDMS)-coated fibres (50/30 µm thickness, 2 cm length) were purchased from Supelco (Bellefonte, PA, USA). The fibre was conditioned daily before the experiments by inserting it into the GC injector for 30 min. Sample preparation parameters were the subject of an earlier investigation[19] and the SPME conditions given below were determined as optimal parameters used in volatiles isolation. In the optimized procedure, 6.25 ml distilled water, 1.75 ml raw spirit and 80 µl hex-3-en-1-ol standard solution (833 mg/l) were placed in a 15 ml vial and the vial was then tightly capped with an open top closure with PTFE/silicone septa. The sample was heated to 45 °C for 10 min before the extraction and agitated using a magnetic stirrer (700 rpm). The HS-SPME of the sample was carried out at 45 °C for 40 min with constant stirring. When the extraction step was finished, the fibre was removed from the vial and inserted into the injection port of the GC for thermal desorption of the analytes. The desorption temperature was 250 °C.

Gas Chromatography Conditions

For determination of extracted aroma compounds, a TRACE GC 2000 (Thermo Finnigan, Waltham, MA, USA) gas chromatograph equipped with a split/splitless injector GC–O measurement system (Sniffer 9000 System, Brechbühler, Houston, TX, USA) and a TRACE DSQ quadrupole mass spectrometer was used. The injection was performed for 5 min (in splitless mode for 1 min and in split mode for the rest of time at split ratio 1:10) using a 1 mm i.d. split liner. Separation was achieved on a Stabilwax-DA (Restek, Bellefonte, PA, USA) polar capillary column with a modified polyethylene glycol-bonded phase (30 m × 0.32 mm i.d., 0.5 µm film thickness). The column temperature programme was as follows: 45 °C, held for 1 min and then ramped up at 6 °C/min to 120 °C, then increased at 5 °C/min to 180 °C and once again ramped up at 8 °C/min to 240 °C and held for 7 min. The total run time was 40 min. The carrier gas was helium at a flow rate of 1.5 ml/min. The GC–O transfer line temperature was 240 °C. The make-up gas was humidified nitrogen at a flow rate of 12.5 ml/min. The MS detector operated in the electron impact mode (70 eV) at 220 °C. The transfer line temperature was 240 °C. Detection was carried out in the scan mode in the range of *m/z* 40–400.

Table 1. Sensory properties and concentrations of 10 standard aroma compounds used in the training solution

Compound name	Density (g/ml)	Volume[a] (µl)	Concentration[b] (mg/l)	Odour description
3-Methylbutanal	0.797	10	781	Aldehydic, bitter chocolate, cacao
Ethyl propanoate	0.891	10	882	Sweet, rum, fruity
2,3-Butanedione	0.985	10	979	Butter, creamy, pleasant
Ethyl butanoate	0.878	10	869	Pineapple, sweet, fruity, pleasant
Ethyl hexanoate	0.873	10	864	Fruit drop, sweet, fruity, pleasant
Hex-3-en-1-ol	0.85	10	833	Fresh cut grass, green
Furan-2-carbaldehyde (furfural)	1.16	10	1150	Sweet, almond
3,7-Dimethylocta-1,6-dien-3-ol (linalool; two peaks)	0.87	5	422	Citrus, tea, green
2-Phenylacetaldehyde	1.075	10	968	Flowery, hyacinth, pleasant
2-Phenylethanol	1.02	10	1000	Flowery, pleasant

[a]Added to 10 ml standard solution.
[b]In standard solution.

GC–Olfactometry Training

To achieve reproducible and mutually compatible results of analyses, it is indispensable that the evaluating team has some experience. As the evaluating team consisted of three persons, only one of whom had experience with carrying out analyses with the use of an olfactometric detector, before commencing with testing real samples a training was conducted, consisting of two tests. Both tests were carried out with the application of a specially prepared solution of 10 selected standard aroma compounds. The content and properties of the aroma compounds composing the standard solution are presented in Table 1. The standard solution was prepared in 95% rectified spirit and kept at a temperature of 5 °C. Reference samples containing aroma compounds were prepared in 15 ml vials; like real samples and they contained 6.3 ml deionized water, 1.7 ml rectified spirit (95%) and 40 µl, 80 µl or 160 µl of the standard sample solution, achieving final concentrations of aroma compounds in samples further marked as c/2, c and 2c, respectively. Both the applied conditions of extraction and the chromatographic conditions were the same as during the analyses of agricultural distillate samples.

Results

Profiling with the Use of Classical Sensory Analysis

The results obtained by conducting a classical sensory analysis showed the existence of essential differences in aroma profiles of agricultural distillates belonging to the individual quality classes. Almost all distillates of very poor or poor organoleptic quality were characterized by high values of the onion/vegetable descriptor (Figure 1). It was also found that almost always when such an odour appeared, it was accompanied by a nauseating odour descriptor. In some samples, instead of an onion/vegetable odour, an earthy/mouldy odour appeared. This odour appeared in several agricultural distillates of very poor or poor organoleptic quality and it was most often accompanied by an acrid/penetrating descriptor which did not appear in distillates of very high quality. In such distillates, descriptors such as onion/vegetable and earthy/mouldy did not appear, thus probably just these odours were the reason for disqualification of samples and low classification in the initial sensory analysis.

Descriptors such as chemical/solvent and fruity/sweet appeared in almost every sample, so that most often they had no essential influence on the organoleptic worsening of quality of the distillates,

unless these odours were exceptionally intense (e.g. sample 13). Other descriptors, such as green/plant and bread/toast appeared only sporadically in distillates of various kinds.

Elements of Validation of the HS-SPME/GC–O Method

The aim of the first test carried out during the training of the evaluation team was to make them familiar with the proper way of sniffing at the samples and drawing peaks with the use of the so-called fingerspan, so that results obtained for successive samples of the same composition and concentration were comparable. During this test, standard samples of invariable composition were analysed, thus both the areas and heights of peaks of individual aroma compounds should in this case remain at a similar level. Figure 2a shows olfactograms obtained by one of the evaluating persons during the analysis of samples of identical concentration of aroma compounds. Table 2 puts together the results of the whole panel in the form of average areas of the surface of peaks and the obtained coefficients of variation for three repetitions, with regard to both the height and the surface areas of the peaks.

The test showed that even an unqualified team of evaluators without prior basic training is capable of performing repetitive olfactometric measurements—already after approximately two chromatographic analyses familiarity with the apparatus and a kind of intuitive autocalibration of the potentiometer dial occurred in the measurement of intensity of olfactory stimuli. It was also observed that each evaluating person used an own scale, therefore data processing is important before averaging the results. When comparing the differences in heights and surface areas of peaks it was seen that the obtained coefficients of variation (CV) lay within a few points at about 30%. It is worth noting that in the case of all three evaluating persons, lower values of CV were obtained when comparing peak heights, thus the measurement of aroma intensity was more repeatable than that of its duration. This is in agreement with previous publications[20].

The aim of the second test was to obtain skill in discerning between the intensity of aromas, so that the obtained olfactograms would depict these differences. This time, samples of the same composition but with varying concentrations of the tested aroma compounds were analysed. Thus, with changing concentrations
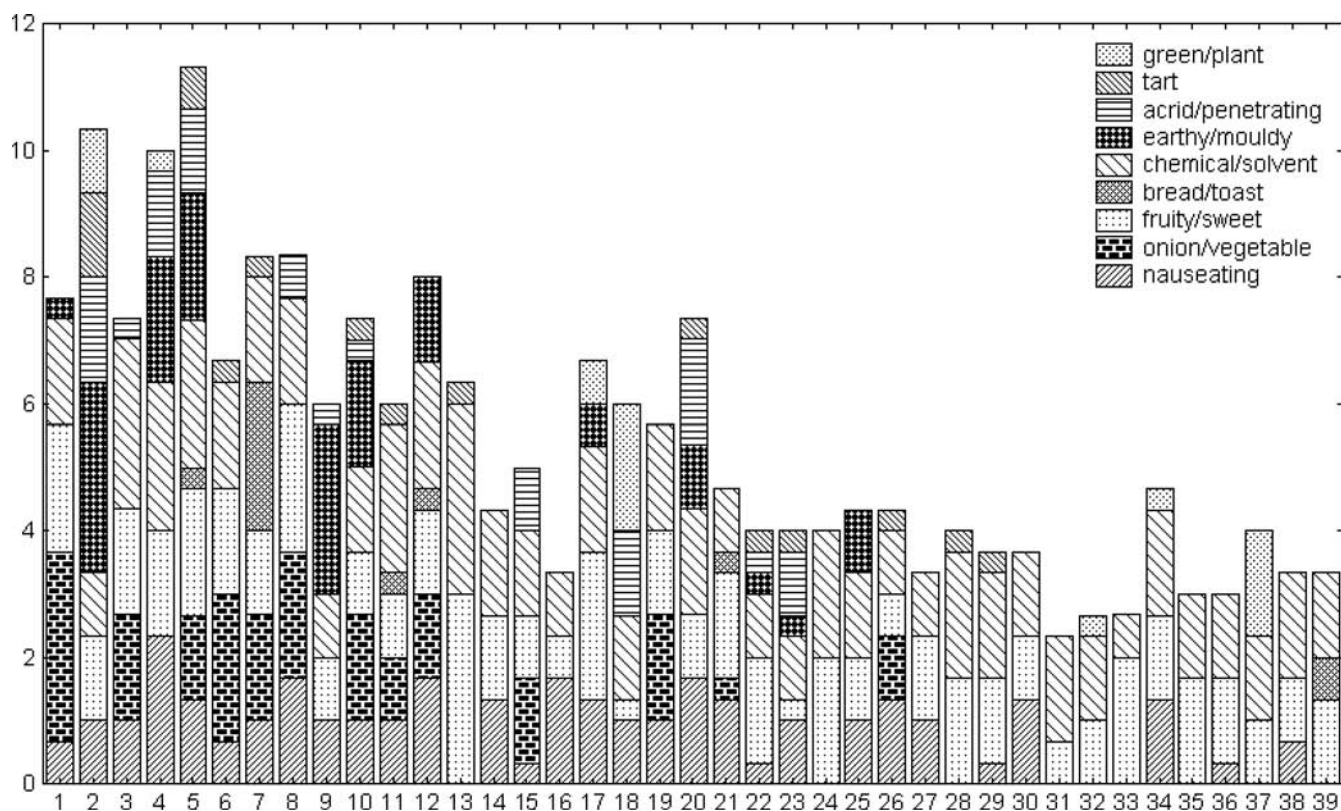
Figure 1. Sensory profiling of raw spirit samples of different qualities: 1–13, very bad; 14–26, medium-bad; 27–39, good

Table 2. Results obtained during repeatability training of sensory panel (ND—not detected)

| Compound	Peak area						Peak height					
	Average (10^8)			CV (%)			Average (10^7)			CV (%)		
	1	2	3	1	2	3	1	2	3	1	2	3
3-Methylbutanal	2.64	2.36	2.85	8.2	15	4.8	5.97	5.07	2.03	2.2	3.9	14
Ethyl propanoate	2.10	4.23	2.51	11	16	17	5.93	6.01	2.98	4.8	6.6	26
2,3-Butanedione	1.61	4.35	0.99	15	1.1	41	5.54	6.44	1.21	4.2	12	12
Ethyl butanoate	3.00	5.60	1.35	23	8.0	33	6.48	6.80	2.18	4.0	11	14
Ethyl hexanoate	4.52	7.26	1.79	15	22	8.9	7.20	7.96	1.95	3.9	8.5	11
Hex-3-en-1-ol	1.62	1.38	0.87	16	10	14	4.74	3.23	1.48	14	8.8	9.6
Furan-2-carbaldehyde	ND	ND	ND	—	—	—	ND	ND	ND	—	—	—
3,7-Dimethylocta-1,6-Dien-3-ol I	1.40	2.73	0.90	13	16	29	5.25	5.31	1.25	8.3	14	15
3,7-Dimethylocta-1,6-Dien-3-ol II	3.06	4.87	1.70	15	23	8.6	6.83	6.47	2.25	3.3	7.2	2.3
2-Phenylacetaldehyde	5.70	13.0	2.51	28	6.3	16	6.96	7.87	1.59	6.8	6.3	16
2-Phenylethanol	3.91	7.66	4.49	17	8.7	13	6.77	6.50	1.97	8.0	7.4	27

of the tested aroma compounds the heights and surface areas of peaks ought to change. Each of the evaluating persons performed several repeated analyses within each test until satisfactory results were obtained. Figure 2b shows olfactograms obtained by one of the evaluating persons during the analysis of samples with concentration of aroma compounds changing in proportions c/2, c and 2c.

The olfactograms obtained from samples with different compositions of the basic solution differed explicitly between each other. It can be observed that both an increase of peak height and surface area is perceivable with increasing concentration of

investigated compounds in the sample. A greater difference in intensity of the perceivable odour occurred between samples with concentrations c and 2c than between c/2 and c.

Aroma Profiles of Agricultural Distillates

The analysis carried out with the use of olfactometric detection was conducted with the aim of determining which aroma compounds form the aroma of distillates and to indicate those which were responsible for the worsening of sensory quality. In the results of the investigations it was found that there were about
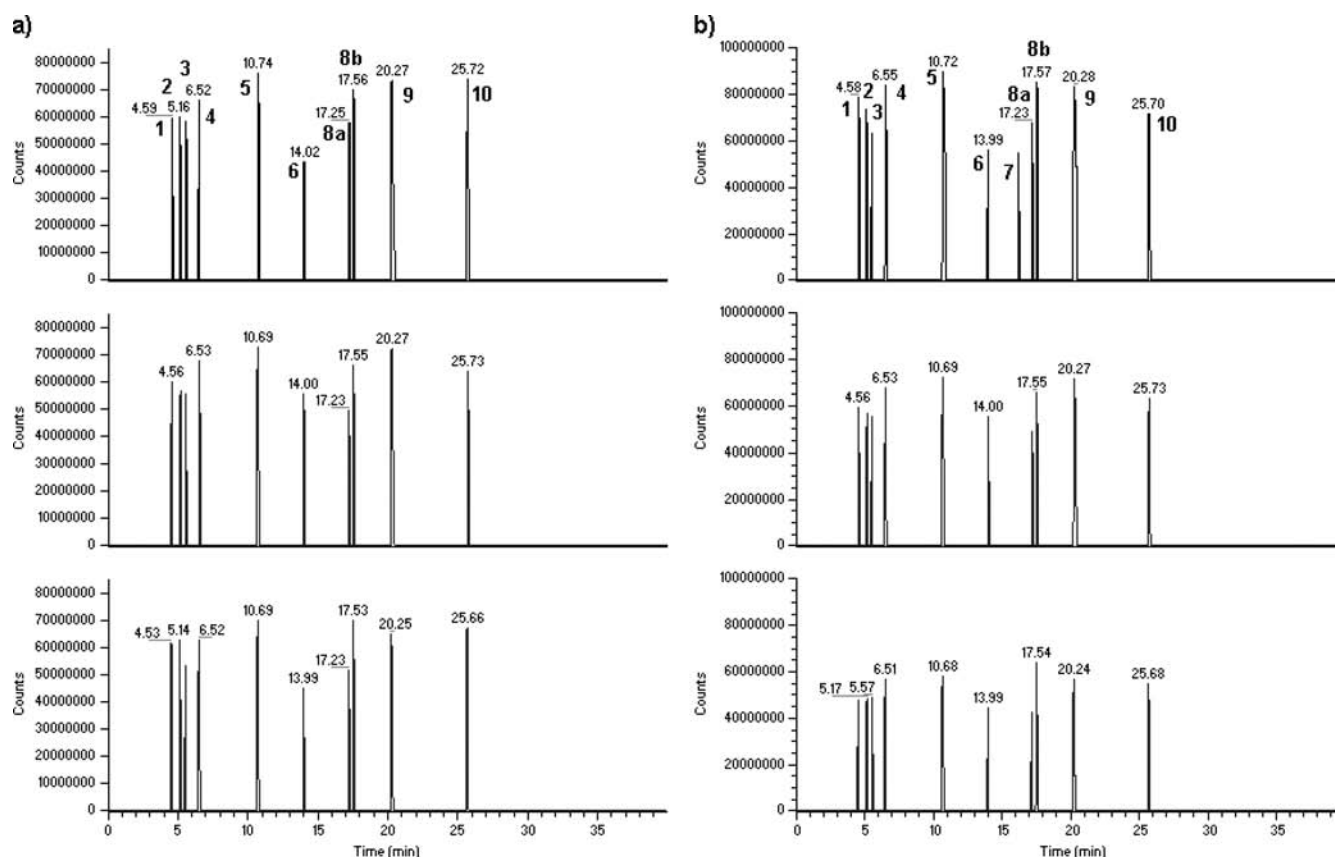
Figure 2. Examples of olfactograms obtained during analyses of samples with (a) the same and (b) different concentrations of aromatic compounds

40 odours included in the aroma profiles of raw spirits. Part of the aroma compounds could be identified on the basis of comparison of olfactograms and chromatograms obtained with the use of a mass spectrometer. Some of the compounds were detected using only olfactometric detection and were not observed on the chromatograms. Compounds were identified on the basis of comparing their mass spectra with spectra available in the National Institute of Standards and Technology (NIST) spectrum library. The identification was confirmed on the basis of uniformity of retention times and mass spectra with standard substances. Additionally, retention indexes were also calculated on the basis of a homologous series of *n*-alkanes with a chain length from C_5 to C_{20}. GC–MS and GC–O analysis were performed separately and identification was done by comparison of the retention times of particular peaks on the olfactograms and chromatograms. Because the retention times differed (peaks on the olfactograms had longer times than peaks on the chromatograms), before the analysis of real samples the solutions of 20 standard aroma compounds were injected in the same chromatographic conditions in order to establish the values of shifting time. To confirm the performed identification of aroma compounds in real samples, the odours found in real samples were compared with descriptions found in the literature and with odours detected empirically from standard compounds. The average retention times, retention indices of identified compounds and descriptions of odours detected in volatile phase extracts of agricultural distillates are presented in Table 3.

In the results of the analyses it was found that the olfactograms drawn by the individual evaluating persons differ from one another. This refers to both the individual fingerspan of each evaluating person and to the intensity of perceptible odours and the number of detected sensorically active compounds. While the differences in the range of intensities of perceptible odours can be levelled through relatively simple mathematical procedures, the two remaining observations may present a greater problem. Differences in intensity of odours felt by individual evaluating persons are practically unavoidable. Every man has distinct olfactometric abilities. A common fact is the occurrence of so-called specific anosmia, which manifests itself in loss or reduced ability to detect some odours or odour categories[21]. A specific increased susceptibility to some odours is also possible. In the studies under discussion, such situations are connected with e.g. the smell of mouldy cheese (average time of retention 18.85 min), which was perceptible in almost any sample by only one of evaluating persons, and also odour groups which could be described as the smell of cooked vegetables, which one of the persons perceived considerably more often than the other two. It should be kept in mind that even the sensitivity of individual persons may be subject to changes, due to a change of the state of health or comfort. Eventually, it was noted that, along with acquired experience, a phenomenon of adaptation and a kind of inurement occurs, particularly with regard to unpleasant odours. To eliminate errors which might result from changes in sensory susceptibility of panel members in the course of investigations, a constant amount of an aroma

Table 3. Odours found in raw spirits HS–SPME extracts determined by GC–O (odours which appeared most often in aroma profiles are in bold type)

RT (min)	Odour description	Compound name
4.07	Sweet, fruity	Ethyl acetate + 1,1-diethoxyethane
4.54	Musty, sweetish, aldehydic	2-Methylbutanal + 3-methylbutanal
5.20	Sweet, rum	Ethyl propanoate
5.83	Synthetic, sweet	2-Methylpropyl acetate
6.53	Sweet, fruity	Ethyl butanoate
6.93	Stewed cabbage, vegetable	Dimethyl disulphide
8.17	**Sweet, fruity, fruit drop**	2-Methylbutyl acetate + 3-methylbutyl acetate
9.62	Sweet, fruity	
10.05	**Musty, sweetish, cheese**	2-Methyl-1-butanol + 3-methyl-1-butanol
10.60	**Sweet, fruity, pineapple**	Ethyl hexanoate
11.52	Sweetish, nauseating	
12.00	Sweet-and-sour	
12.13	Sweet, citrus, fresh, pungent	
13.49	Synthetic, acrid	
14.00	Green, cut grass, fresh	Hex-3-en-1-ol (IS)
14.34	**Stewed cabbage, onion, vegetable**	Dimethyl trisulphide
14.87	Synthetic, plastic, sweetish	
15.16	**Sweet, citrus, fruit drop, pungent, acrid**	Ethyl octanoate
15.28	**Green, green peas, grass**	
15.73	**Synthetic, dust, bread crust**	
15.84	Stewed cabbage, vegetable	
16.13	**Synthetic, bread crust**	
16.66	**Bread crust, pungent**	
17.20	Green, plant, geranium	
17.45	Musty, acrid	
18.89	Blue cheese	
19.61	**Green, plant**	
19.76	**Sweet, pungent, acrid**	Ethyl decanoate
20.50	**Animal, mousy, unpleasant**	
20.66	Green, geranium	
20.73	**Medicinal, vitamin-like, boiled chicken**	
21.34	**Green, wet soil, fresh, geranium**	
22.13	Stewed cabbage, vegetable	
21.55	Musty	
23.84	**Flower, sweet champagne**	
24.06	**Pungent, sweetish, acrid, green**	Ethyl dodecanoate
24.12	**Earthy, dank cellar, mouldy**	Geosmin
25.10	**Anise, acrid**	
25.20	Cheese, creamy	
27.00	Synthetic, almond	
27.52	Green, plant, geranium	

compound was always added–hex-3-en-1-ol, serving as an internal standard (IS). It was determined that variation coefficients of surface areas of the peak of the internal standard, individual for each person and calculated for all 39 samples, were 12.4%, 19.4% and 26.3%.

Differences in the amount of odours perceptible by different evaluating persons from the same distillate samples can be explained by the respiratory cycle[22]. The possibility of perceiving an odour exists only during inspiration. The duration of expiration is sufficiently long that an odour may be omitted, particularly when it is not intense and long-lasting, or when a subsequent odour appears within an insignificant time interval from the preceding one. If inspiration starts already in the course of appearance of an odour, only part of the odour is registered and the real intensity of the odour is being lowered; this can cause much greater difficulties in the interpretation of results. Thus, in a situation when aroma profiles of samples are unknown, it is necessary to use repetitionsor, as in the case of the discussed investigation, to engage several persons to evaluate the same sample. If the method is to be used for routine determination, the knowledge of retention times of known odours can significantly increase the repeatability of results.

Organoleptic Quality Factors of Agricultural Distillates

The obtained aroma profiles were used for identification of quality factors of agricultural distillates, i.e. compounds whose presence in a profile may indicate low quality of the product and be the

cause of low classification during sensory analysis. Above all, the odours which were detected most often in the majority of agricultural distillate samples by a maximum number of evaluating persons were evaluated for suitability as quality markers (Table 3). From the olfactograms the areas of the investigated odour peaks were read and subsequently calculated to percentage values as ratios of surface areas to the average surface area of all samples, in order to eliminate differences caused by varying ranges of scale between the evaluating persons.

An analysis of the obtained olfactograms showed that, in spite of numerous similarities between aroma profiles of good and poor sensory quality, a few dependences can be found between the quality of distillates and the kind and intensity of odours occurring in profiles. The majority of aroma substances appeared in the tested extracts above the perceptibility threshold, both in poor and high-quality samples, but some of them were perceived only in a few samples of bad and medium quality. Such odours include, for example, the odour described as synthetic, acrid [average retention time (RT) = 13.49 min], the odour of boiled cabbage (average RTs = 15.84 and 22.13 min) and also a musty, acrid odour (average RT = 17.45 min). These odours were detected only sporadically, therefore mostly by part of the evaluating panel; nevertheless, their participation in worsening the sensory quality of some samples cannot be excluded.

Among the odours whose presence was being detected in both poor and high sensory quality samples, two groups could be singled out: odours of small and medium intensity, most often detected in a given sample only by part of the evaluating team; and very intense odours whose detectability was very high in all samples. In both the first and the second group, potential discriminants of sensory quality of agricultural distillates could be pointed out. In the case of the first group, results obtained with the use of an olfactometric detector (in relation to odours which could be identified) still did not always correlate with the results obtained through a mass spectrometer. This was probably caused by the low intensity of the odours, the subsequent small precision of the evaluating persons and the often-occurring omission of the whole or part of an aroma signal, which led to lowering of the results. For this reason, the possibility of using the quality factors from this group was limited to samples in which the concentration of the aroma compound was relatively high. Such a situation occurred, for example, in the case of a sweet fruity aroma (average RT = 4.07 min) originating probably from ethyl acetate and 1,1-diethoxyethane (both compounds that are not subject to resolution in the applied chromatographic conditions), and also from aldehydic odour of bitter cocoa originating from 2- and 3-methylbutanal. Although the chromatographic peaks in many poor-quality samples were higher than in the remaining ones, only part of these samples could be singled out with the use of an olfactometric detector. Of course, one should not forget the possibility of the effects of mutual masking, elimination or enhancement by aroma compounds appearing side by side, and even of odourless compounds eluting along with aroma compounds[21]. Besides, it should be remembered that the detection limits and linearity ranges of the two detectors were completely different (in the case of a human nose this meant rather thresholds of sensory perceptibility and the course of psychometric functions of individual compounds), therefore no high correlation between surface areas of chromatographic and olfactometric peaks should be expected. In the case of odours which often appeared in extracts of the volatile fraction of distillates but which were

usually not intense, the feature which indicated a low sensory quality thus may have been the detection of a relatively high intensity of an odour in a given sample or its detection by the whole evaluating team.

Most information about the organoleptic quality of distillates came from the analysis of the most intense odours detected in all or almost all samples. Then the feature which allowed discrimination between distillates of poor and high sensory quality was the intensity of and odour in a given sample. Table 3 presents in bold type such odours which were detected in all or almost all samples. It was determined that the most essential relationship between the intensity of individual odours and the organoleptic quality of samples occurred in the case of unpleasant odours, particularly two: the odour of boiled cabbage, onion, vegetable (RT = 14.34 min); and the odour described as earthy, mouldy, humid cellar (RT = 24.12 min). Using mass spectrometry it was found that these odours originated from dimethyl trisulphide and geosmin (2β,6α-dimethylbicyclo[4.4.0]decan-1β-ol). Both compounds are characterized by very low sensory thresholds. The odour of dimethyl trisulphide is described in the literature as that of rotten food, rotten cabbage, garlic, onion, musty, sulphuric, pungent. This compound has been identified in beverages such as wine, tequila and Yanghe Daqu (Japanese grain alcoholic drink) and its sensory perceptibility threshold in a 10% ethanol solution is 0.2 µg/l.[6,23–25] Geosmin is a compound of earthy and musty smell which is detectable practically in ultravestigial amounts—its sensory threshold in wine is 60–90 ng/l.[26] Neither of these compounds are typical fermentation products and they constitute volatile metabolites produced by various undesirable microorganisms, such as moulds of the *Botrytis cinerea* or *Penicilium expansum* kind, and many species belonging to the Actinomycetes growing on raw materials, or as the result of infection during the fermentation process itself. It results from the conducted investigation that their increased content in agricultural distillates, recorded by the GC–O method, is in most cases simply a disqualifying defect. At the same time, dimethyl trisulphide also appears in inconsiderable amounts in medium- and high-quality samples; on the other hand, geosmin peaks appear only in chromatograms of distillates of the worst and medium quality. It has also been observed that the results of olfactometric profiling are consistent with the results obtained with the use of the GC–MS method. Figure 3 presents diagrams depicting dependencies of average intensity of both odours on the organoleptic quality of the sample. The numbers on the abscissae denote the numbers on distillate samples in accordance with rising sensory quality. As seen, both the results obtained for both compounds with the use of an olfactometric detector and a mass spectrometer showed a falling tendency with an increase of the sensory quality of samples. In the case of the diagram related to dimethyl trisulphide (Figure 3a), it is clearly seen that in the first sample the peak of this compound on the chromatogram of the total ion current was about 10 times higher than on the average in the remaining samples, thus the concentration of this compound was in this sample much higher; on the other hand, such a large difference was not observed in the intensity of this odour, although the result obtained with the use of GC–O was also the highest for the first sample. Probably in this case the concentration was exceeded at which the human nose undergoes a kind of "saturation" and does not perceive a rise in odour intensity in spite of increasing concentration, which is typical for the course of psychometric functions. Next, the opposite can be observed in the case of
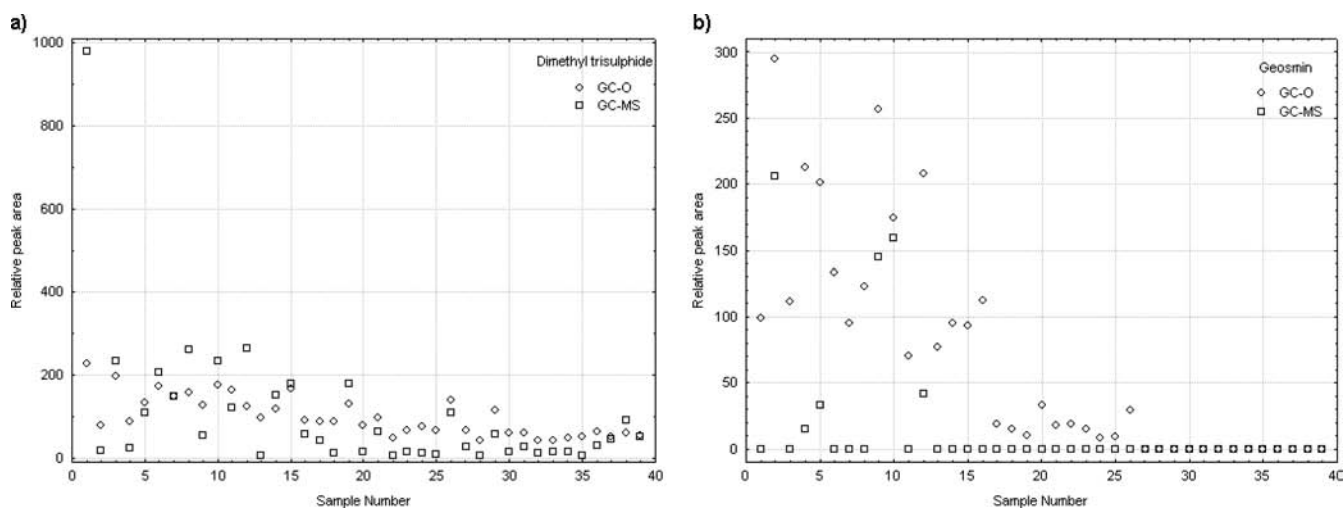
Figure 3. Relationships between (a) dimethyl trisulphide and (b) geosmin relative peak areas obtained by GC–O and GC–MS and the organoleptic quality of raw spirit samples

aroma profiles of geosmin (Figure 3b). While with the use of the mass spectrometer this compound was detected only in some samples of the worst sensory quality, through the use of an olfactometric detector it was detected with much greater sensitivity, i.e. in all samples of worst quality and in insignificant amounts in all or almost all medium-quality samples. This attests to a kind of superiority of the human nose over conventional detectors with respect to some analytes.

From the remaining odours of high intensity and frequency of detection, only in the case of four (with average RT of 8.17,

14.87, 20.50 and 20.73 min) were no dependences found between the sensory quality of the distillates and the intensity of a given odour for the sample. The remaining odours showed an increased intensity at least in some samples of very bad or medium quality, although the differences between distillates of good and worse quality usually were not great. Figure 4 shows the obtained dependencies for identified compounds. For comparison, results are also presented as obtained with the use of a mass spectrometer. The great analogy between results obtained with the use of an odour detector and a conventional one
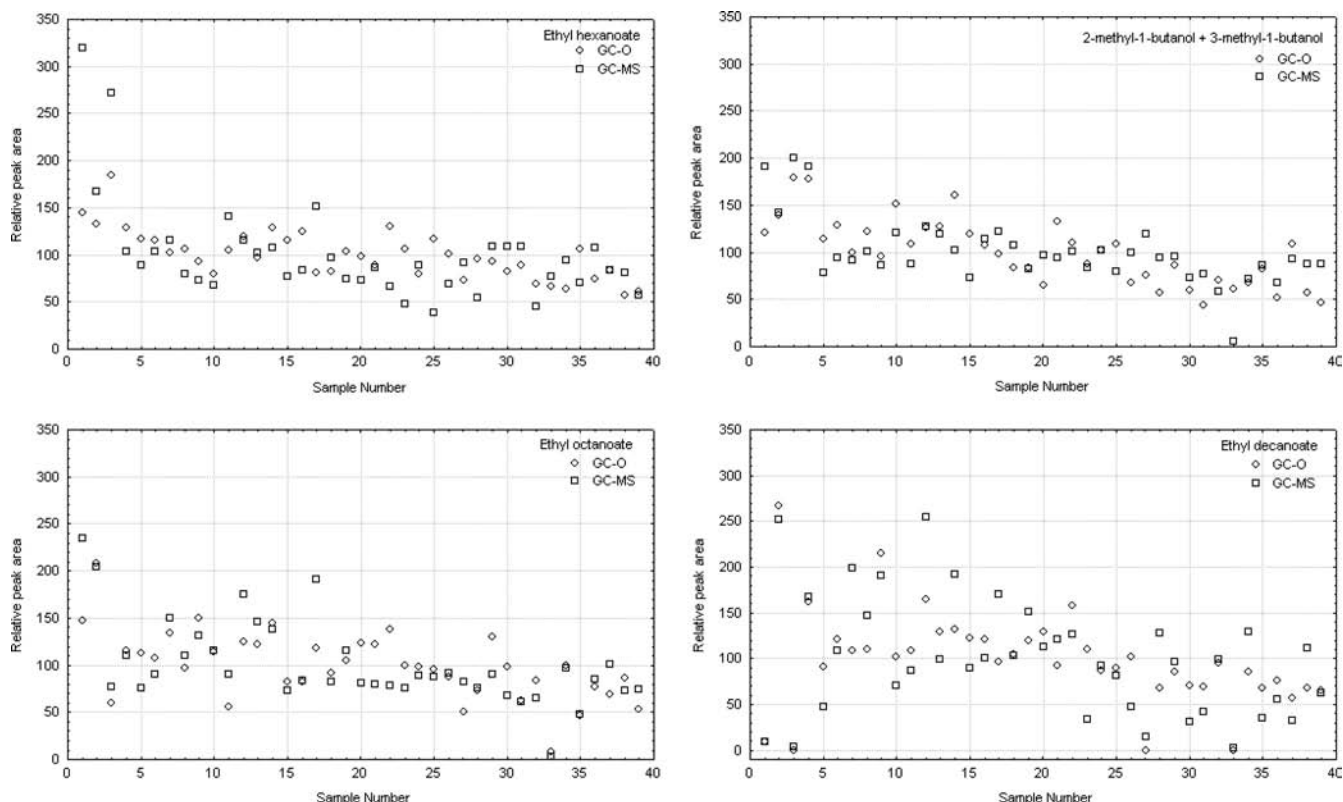
Figure 4. Relationships between relative GC–O and GC–MS peak areas of identified aromatic compounds and the organoleptic quality of raw spirit samples

Discussion

The aim of the discussed investigations was to determine which aroma compounds are included in the aroma of raw spirits and to indicate those which are responsible for the worsening of sensory quality. Before commencing the analyses of real samples, a training session of the evaluating team was conducted, which showed that, in spite of the fact that the fingerspan method—considered to be the most difficult to be properly carried out—variant of methods with the use of an olfactometric detector, even unqualified persons are capable after a few tests of learning how to use the so-called fingerspan in a reproducible way and to react to changes in odour intensity. This is undoubtedly an advantage of this method, permitting its use in industrial applications.

In the results of the investigations it was found that the composition of aroma profiles of agricultural distillates includes about 40 odours, some of which can serve as discriminants of sensory quality. The greatest amount of information on the organoleptic quality of distillates came from the analysis of the most intense odours detected in all or almost all samples. Then the feature which allowed distillates of poor and good sensory quality to be discerned was the intensity of odour in a given sample. The most essential indicators of sensory quality were found in the form of two odours which were the result of the presence of two compounds in the sample, dimethyl trisulphide and geosmin. The presence of at least one of these compounds in the tested extracts of poor and medium sensory quality samples in a concentration exceeding the perceptibility threshold, as well as the fact that the results of olfactometric profiling were consistent with results of sensory analysis and the results obtained with the use of the GC–MS method, testifies to the possibility of exploiting the determination of these compounds for quality control of distillates in production.

Acknowledgements

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