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## INFLUENCE OF GEOSMIN, DIMETHYL TRISULFIDE, 2- AND 3-METHYLBUTAN-1-OL ON RAW SPIRITS QUALITY[*]

**Summary.** Nowadays, organoleptic quality of alcoholic beverages is a very important factor for the consumers. Distilleries, to produce high quality alcoholic beverages have to estimate which compounds are responsible for unpleasant aroma thus decreasing the quality of obtained alcohols. The main purpose of this research was to create aroma profiles of the raw spirits differing in their organoleptic quality and indicate compounds which presence could be the cause of poor quality of alcoholic beverages. To conduct these studies GC-O and GC-MS techniques were chosen. Results indicated about 40 different odours. Three flavours: "earthy, mouldy wet basement", "boiled cabbage, boiled onion", and "sweet, cheesy, mouldy", were identified as: geosmin, dimetyl trisulfide, and 2- and 3-methyl-1-butanol. The correlation between the odour intensity and quality of raw spirit was observed. With increasing quality of raw spirits the concentration of these compounds was decreasing.

**Key words:** volatile compounds, raw spirits, GC-O, GC-MS, organoleptic quality

## Introduction

In accordance to the type of alcoholic beverage, type and quality of raw materials used, applied method and technological process conditions, some by-products are formed. In alcoholic beverages compounds such as esters, fusel alcohols, aldehydes, organic acids and sulphur compounds can occur. These pollutants are formed in biological, microbiological, thermal, enzymatic and chemical processes that can take place during processing. For example esters are formed mainly during fermentation process and they are strongly dependent on the process temperature as well as on the type of yeast used in processing and worth sugar profile (SAERENS et al. 2008, YOUNIS and

STEWART 1998). Also some of by-products are caused by raw materials spoilage or are formed during storage. All of these compounds, their content, presence and composition, determine overall organoleptic quality of the product. Even small changes in concentration of one compound can influence the flavour.

The problem of determining volatiles in alcoholic beverages is well known (FERREIRA and HOGG 2003, LEE and NOBBLE 2003, MALLOUCHOS et AL. 2003, ROCHA et AL. 2004, DIÉGUEZ et AL. 2005, FAN and QIAN 2006, LEDAUPHIN et AL. 2006, CÂMARA et AL. 2007, CRUPI et AL. 2007, VANDERHAEGEN et AL. 2007). There are many useful methods for analysing volatile fraction of alcoholic products. To determine volatile compounds few extraction techniques, such as solid phase extraction (SPE) (CASTRO et AL. 2008), liquid-liquid extraction (LLE) (CASTRO et AL. 2004), or solid phase microextraction (SPME) (GÜRBÜZ et AL. 2006) were applied. Gas chromatography and electronic noses (GARCÍA et AL. 2006) can be also used for this purpose.

According to the literature about few hundred chemical compounds are known in alcoholic beverages. However, the alcoholic products which were investigated are mostly beverages such as wine, liqueur, whisky, brandy and beer. The investigation is mostly focused on volatile compounds caused by process condition in different stages of the production. There are also commonly conducted studies on aroma profiles and specific bouquet of wines from different origins. Almost all works are focused on the examination of final volatile compounds in product, not in semi-product like raw spirit. Volatile compounds are responsible for final aroma of the product and their organoleptic quality. The qualitative composition of by-products in raw spirits influence the final product quality. To produce premium quality alcoholic beverages distilleries have to use good quality raw spirits. Acceptance of raw spirits as a substrate to further processing is the important stage of producing high quality alcohols.

Nowadays defining the usefulness of agricultural distillates as a substrate in producing rectified spirits is achieved mostly by classical sensory analysis. This approach is not sufficient enough and still the new methods are necessary for proper quality and quantitative determination of compounds responsible for contamination of agricultural distillates, rectified spirits and alcoholic beverages. The European regulations and Polish Standards methods are limited and the information are not enough detailed, that's why these studies were conducted. Sensory analysis gives only information about overall quality, doesn't point out compounds which are responsible for it. Knowing quantitative information about occurring by-products in agricultural distillates could let on more precise quality evaluation of products and could help with selection of appropriate process condition. The products such as raw spirits were not appropriately investigated to date.

The main aim of the presented work was to characterize volatile fraction of agricultural distillates and establish relation between their quality and volatile fraction composition.

## Materials and methods

### Samples and reagents

For the research 39 samples of raw spirits obtained in small distilleries in Pomeranian region were selected. The ethanol concentration in the raw spirits samples was

approximately 90% (v/v). All the samples were categorized by the assessors in distilleries into three groups. Categorizing raw spirits in groups helped to estimate which distillates can be used for further processing into rectified spirits. Among all samples three categories were distinguished:

- samples with bad organoleptic quality which didn't fulfilled Polish Standard PN--A-79528-2:2002 organoleptic quality requirements, the results were obtained after sensory analysis performed by the group of the trained assessors (samples from 1 to 13),
- samples with good organoleptic quality with differentiations in evaluation marks during sensory analysis; some of the assessors reckoned them in accordance, others without accordance to Polish Standard demands (samples from 14 to 26),
- samples with the highest organoleptic quality which fulfilled the Polish Standard demands (samples from 27 to 39).

In the analysis, to monitor adaptation effect on the assessors, to level changes in sensitivity and control analysis digressed from others, an internal standard solution of cis-3-hexen-1-ol was used as well as high purity water (MilliQ A10 Gradient/Elix System, Millipore; Bedford, MA, USA) and alkanes with a chain length from $C_5$ to $C_{20}$ (Sigma-Aldrich Poland, Steinheim, Germany).

**HS-SPME conditions**

The holder for manual use and tested fibers was purchased from Supelco (Bellefonte, PA, USA). For the research, the optimal parameters found in previous investigation were applied (PLUTOWSKA and WARDENCKI 2008). Thus in the research DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) coated fiber (50/30 μm thickness, 2 cm length) was used. Before each analysis the fibers were conditioned for half an hour in the injector. The optimal composition of the sample were as follows: 6.3 ml – high-purity water (MilliQ A10 Gradient/Elix System, Millipore; Bedford, MA, USA), 1.75 ml – raw spirit, 80 μl of internal standard solution of cis-3-hexen-1-ol (concentration 8.33 ppm) were placed in 15 ml vial and tightly capped with an open top closure with PTFE/silicone septa purchased from Supelco (Bellefonte, PA, USA). Samples prepared in such way were agitated using magnetic stirrer (700 rpm) and thermostated for 10 min. at 45°C. After the heating, the analytes were extracted at 45°C for 40 min with a constant stirring. When the extraction step was finished, the fiber was removed from the vial and inserted into injector for thermal desorption for 5 min.

**Gas chromatography conditions**

Gas chromatography analyses were performed using a TRACE GC 2000 (Thermo Finnigan, Waltham, MA, USA) gas chromatograph equipped with a split/splitless injector, an olfactometric detector, Sniffer 9000 (Brechbühler) and a TRACE DSQ quadrupole mass spectrometer. Desorption step least 5 min (in splitless mode for 1 min and split mode in 1:10 ratio for the rest of this time) with the use of a 1 mm I.D. split liner. Separation of volatiles were achieved on polar capillary column, a Stabilwax-DA (Restek, Bellefonte, PA, USA) with a modified polyethylene glycol bonded phase (30 m × 0.32 mm I.D., 0.5 μm film thickness). The total runtime was 40 min and temperature

program of the analysis was as follows: the initial oven temperature was 45°C and held for 1 min. After that time the temperature increased at 6°C/min to 120 ºC, then ramped up at 5°C/min to 180°C and once again ramped up 8°C/min to 240°C and then held for 7 min in this temperature. The injector temperature was 250°C. The carrier gas was helium with 1.5 ml/min flow rate. The detector operated in electron impact mode (70 eV) at 220°C. The transfer line temperature to both detectors was set at 240°C. Detection was carried out in scan mode in 40-400 range of m/z. The GC-O make-up gas was nitrogen humidified by water vapour with a flow rate of 12.5 ml/min.

## Results and discussion

The analysis of raw spirits samples were conducted with the use of two techniques, capillary gas chromatography-olfactometry (GC-O) – finger span method and capillary gas chromatography mass-spectrometry (GC-MS). Olfactometric detection was performed by a group of three assessors. Before the real samples analyses the training was conducted. During the training each of the assessor has learned proper responses for odour intensity changes. It was kind of self autocalibration during generation of peaks.

During GC-O analysis of raw spirits samples about 40 different flavours were found whereas the GC-MS analysis indicated the presence of over 200 different compounds although only about 20 of them were identified in olfactometry analysis. All the flavours revealed during this study are shown in Table 1.

Table 1. Flavours revealed during GC-O analysis
Tabela 1. Zapachy wyczuwane podczas analizy GC-O

| RT | Aroma description | Compound(s) |
|---|---|---|
| 1 | 2 | 3 |
| 4.09 | Sweet, fruity | Ethyl acetate + 1,1-diethoxyethane |
| 4.53 | Musty, sweetish | 2-Methylbutanal + 3-methylbutanal |
| 5.23 | Sweet, rum | Ethyl propionate |
| 5.85 | Sweet, synthetic | 2-Methylpropyl acetate |
| 6.59 | Sweet, fruity, pineapple | Ethyl butyrate |
| 7.00 | Vegetable, boiled cabbage, onion | Dimethyl disulfide |
| 8.23 | Sweet, fruit drop, fruity* | 2-Methyl-1-butyl acetate + 3-methyl-1-butyl acetate |
| 9.69 | Sweet, fruity | |
| 10.08 | Sweet, cheesy, musty* | 2-Methylbutan-1-ol + 3-methylbutan-1-ol |
| 10.66 | Sweet, fruity, pineapple* | Ethyl hexonate |
| 11.57 | Sweet, unpleasant, sickening | |
| 12.07 | Sweet and sour | |
| 12.15 | Fresh, citrus, sweet | |

Table 1 – cont. / Tabela 1 – cd.

| 1 | 2 | 3 |
|---|---|---|
| 13.51 | Pungent, acrid | |
| 14.00 | Green, fresh cut grass | Hex-3-en-1-ol (IS) |
| 14.41 | Vegetable, boiled cabbage, boiled onion* | Dimethyl trisulfide |
| 14.77 | Sweet, plastic, synthetic | |
| 15.18 | Sweet, pungent, citrus, fruit drop* | Ethyl octanoate |
| 15.30 | Green, peas, grass* | |
| 15.48 | Bread peel, synthetic* | |
| 15.74 | Pungent, bread peel | |
| 16.21 | Cabbage | |
| 16.45 | Synthetic, bread peel* | |
| 16.76 | Bread peel, pungent* | |
| 17.21 | Green, geranium | |
| 17.45 | Musty, pungent | |
| 17.54 | Green tea, citrus | |
| 18.90 | Mould-ripened cheese | |
| 19.67 | Green, floral* | |
| 19.79 | Sweet, pungent* | Ethyl decanoate |
| 20.56 | Unplesant, mousy, animal* | |
| 20.58 | Green, floral, geranium | |
| 20.79 | Medicine, witamin, boiled chicken* | |
| 21.36 | Fresh, wet soil, geranium,green* | |
| 22.14 | Boiled cabbage, vegetable | |
| 23.55 | Musty | |
| 23.87 | Flowery, sweet champagne* | Acetic acid phenylethyl ester |
| 24.10 | Green, sweet, pungent* | Ethyl dodecanoate |
| 24.22 | Wet basement, mouldy, musty, wet earth* | Geosmin |
| 25.15 | Pungent, aniseed* | |
| 25.20 | Creamy, processed cheese | |
| 26.95 | Almond, synthetic | |
| 27.61 | Floral, green, geranium | |

*Flavours occurred most often in almost all the samples.

Some of the flavours were identified by comparison of the retention times from ol-factograms and chromatograms from mass detection. To confirm the results the empiri-cal aroma description was compared with the literature aroma description. The olfacto-metric analysis has shown that in spite of similarities in volatile fraction composition some relationships in raw spirits quality were observed. There are some dependences between organoleptic quality of raw spirit, composition and intensity of some com-pounds occurring in volatile profile of the sample. Majority of the compounds occurs in samples above their thresholds and are easy noticeable whereas some of the flavours are presented only in few samples with low and medium quality. This group include fla-vours like: "pungent, synthetic" (RT = 13.51), "boiled cabbage" like odour (RT = 16.21, RT = 22.14) and "musty, pungent smell" (RT = 17.45). These smells were detected sporadically and mostly by the part of the assessors but their effect on decreasing qual-ity of agricultural distillates cannot be neglected. Among odours appearing in bad qual-ity and good quality samples we can distinguish nearly intensive fragrances which were detected only by the part of the panelist team. From this group some of the markers can be chosen but not all were in good correlation with the results obtained from mass de-tector. The reason for that could be too low aroma intensity and resulting consequences – less precision of the assessors and not noticing all of the flavours. Therefore only aromas with relatively high intensity can be taken into account from this group. For example "sweet, fruity" aroma originated from ethyl acetate + 1,1-diethoxyethane (RT = 4.09) and "sweet, musty, aldehydic" odor from 2-methylbutanal + 3-methylbu-tanal (RT = 4.53). Despite the fact that in bad quality samples peak areas are bigger than in better quality samples, their presence in olfactometric detector was not frequent. These odours were detected only in few samples. Masking of odours and mutual effect on odours should be also taken into account.

The other group of compounds were high intensity flavours which occurred in every or mostly in every samples. This group gives a lot of information about quality markers in raw spirits samples. The intensity of the peak areas lets discriminate bad and good quality raw spirits. The flavours most often detected marked by star are presented in Table 1. Some relationships between few compounds intensity and the quality of raw spirit sample were found. Three of identified compounds have big influence on agricul-tural distillates quality. The most important compounds having good correlation with the organoleptic quality that were identified with the use of mass spectrometer are geosmin, dimethyl trisulfide and 2- and 3-methylbutan-1-ol (these two compounds weren't sufficiently separated in applied chromatographic conditions). Geosmin (RT = 24.22) gives sample unpleasant, wet basement like and musty odour. Presence of this compound can be noticeable at ultra trace concentration. Its threshold in wine is in the range of 60-90 ng/l (DARRIET et AL. 2000). Another very significant compound is di-methyl trisulfide (RT = 14.41) which gives vegetable, boiled, spoiled cabbage like odour. Its sensory threshold in 10% ethanol solution equals 0.2 μg/l (GUTH 1997). These compounds are not the typical product of fermentation process. Their presence can be explained by the metabolism of different microorganisms such as *Botrytis cine-rea*, *Penicillium expansum* as well as *Actinomycetes* growing on the raw materials sur-faces. After comparison of peak areas from two detectors (olfactometric and mass spec-trometer) some dependences were found. GC-O results are comparable with GC-MS data. Conducted studies have shown that increased peak area of these compounds (GC-O) is mostly disqualifying feature.

Every assessor has its own scale of generating peak areas by GC-O. In order to minimize the differences in generating peak areas by each assessor, the results were presented in relative scale of peak areas (%) As it is shown, with increasing quality of raw spirit the peak areas from both detectors decreases. In Figure 1 we can see that geosmin was detected only in few low quality samples whereas it was highly observable by human nose detection. This flavour was observed in all low quality samples. Smaller amounts appeared also in almost every medium quality samples. However, wet basement like odour was not observed in good quality samples. This result indicate predominance of human sense of smell in comparison with conventional detectors.
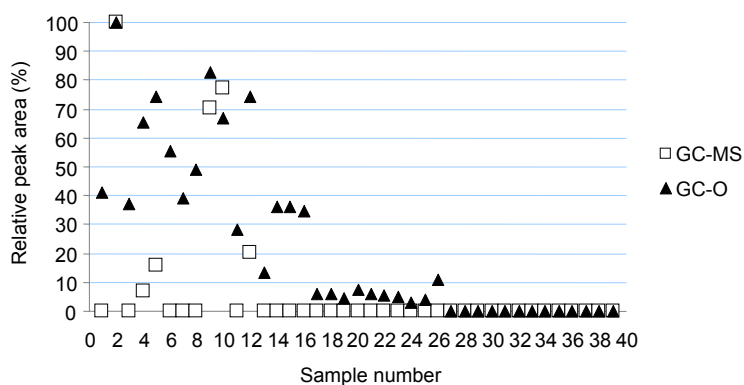


Fig. 1. Dependence between relative peak areas of geosmin obtained by GC-O and GC-MS and organoleptic quality of raw spirit sample
Rys. 1. Zależność między względnymi polami powierzchni geosminy otrzymanymi techniką GC-O oraz GC-MS a jakością organoleptyczną destylatu rolniczego

Different situation was observed with boiled cabbage description originated from dimethyl trisulfide. It is worth to mention that besides of dimethyl trisulfide presence in the lowest quality samples, their odour is also observable in small amounts in medium and good quality raw spirits, whereas the geosmin odour is not present in samples with good quality. Figure 2 has shown that in the first sample (the lowest quality) the GC--MS peak area is few times bigger than in the rest of samples. It meant that in this sample concentration of dimethyl trisulfide is the highest. However, the GC-O analysis shows that peak area in first sample is also the biggest but that high discrepancies between the amount of this compound in other low quality samples weren't observed. This result can be caused by psychometric function which show relationship between concentration of the compound and perceived flavour intensity. Different compounds have different characteristic of psychometric function. Generally, with increasing concentration of the compound the intensity of the perceived odour increases and human nose can easily notice intensity changes. Nevertheless, this effect is limited. Human perception decreases with higher concentrations (DELAHUNTY et AL. 2006). This effect is probably characteristic for dimethyl trisulfide. The differences in peak areas for medium and good quality samples were not high, even though the decreasing tendency in organoleptic quality was also found.
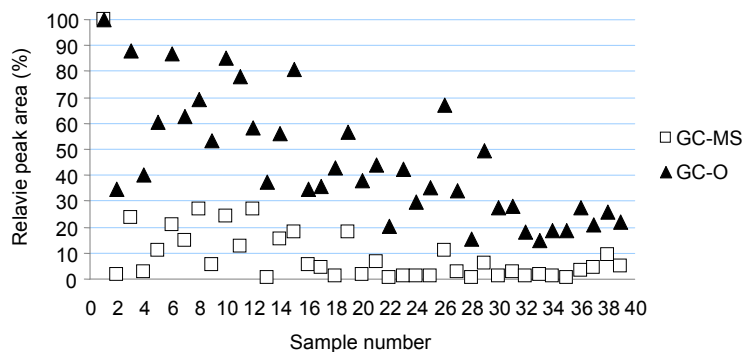
Fig. 2. Dependence between relative peak areas of dimethyl trisulfide obtained by GC-O and GC-MS and organoleptic quality of raw spirit sample
Rys. 2. Zależność między względnymi polami powierzchni trisiarczku dimetylu otrzymanymi techniką GC-O oraz GC-MS a jakością organoleptyczną destylatu rolniczego

It is worth to mention that similar relationship was noticed in case of 2- and 3-methylbutan-1-ol. Differences in peak areas were smaller than in case of geosmin and dimethyl trisulfide but relation quality-peak area for both detectors was preserved. This relation is shown in Figure 3.
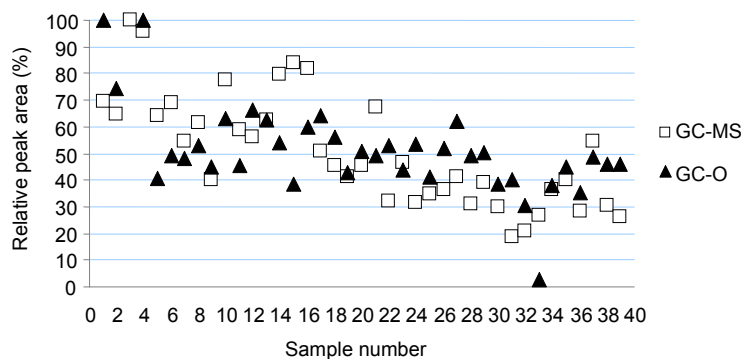


Fig. 3. Dependence between relative peak areas of 2- and 3-methylbutan--1-ol obtained by GC-O and GC-MS and organoleptic quality of raw spirit sample
Rys. 3. Zależność między względnymi polami powierzchni 2- i 3-metylobutan-1-olu otrzymanymi techniką GC-O oraz GC-MS a jakością organoleptyczną destylatu rolniczego

Samples qualified as the lowest quality were mostly dependent on dimethyl trisulfide and geosmin presence. Even small amount of these compounds was the reason to reject sample for further processing. Figure 4 shows dependences between quality of the sample and GC-O intensity of odour of two compounds, dimethyl trisulfide and geosmin.
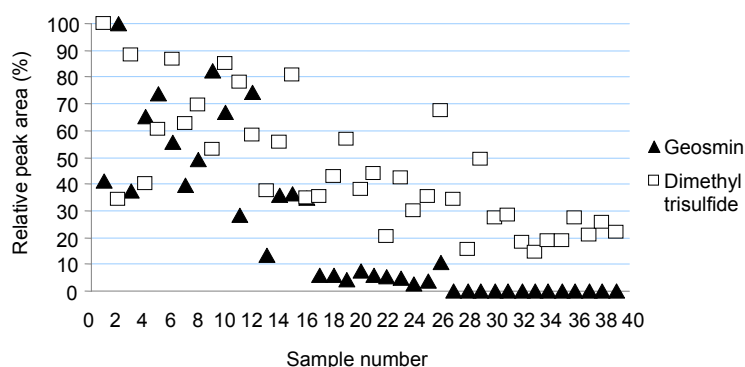
Fig. 4. Dependence between relative peak areas of geosmin and dimethyl trisulfide obtained by GC-O and organoleptic quality of raw spirit sample
Rys. 4. Zależność między względnymi polami powierzchni geosminy i tri-siarczku dimetylu otrzymanymi techniką GC-O a jakością organoleptyczną destylatu rolniczego

Apart from high intensity odours found most often during GC-O analysis there are only four which did not reveal relationship between quality of the sample and their peak areas. Their retention times were as follows: 8.23, 14.77, 20.58, 20.79. The rest of odours which were present almost in every sample indicate intensive odour at least in few bad quality samples. Nevertheless, the differences between medium and good quality samples were not high. These studies have shown high analogy between conventional detector results and "flavour" detector data. It indicates that GC-O technique can be succesfully applied for quality control of raw spirits in distillaries.

This research reavealed that the reacher volatile profile, the lower organoleptic quality of agricultural distillate is. Some of the revealed compounds can be used as quality markers. GC-O technique detected geosmin with higher sensitivity than conventional detector did. Performed studies allow to conclude that GC-O technique can be used as a quality control tool, as well as process condition coordination in agricultural distillaries and qualifying its to further processing into rectified spirits and vodkas.

## Conclusions

The main aim of the presented research was to isolate aroma profiles of raw spirit samples differing in their organoleptic quality and establish dependence between the composition of volatile compounds and organoleptic quality of the sample. Headspace solid phase microextraction (SPME) and capillary gas chromatography-olfactometry and mass spectrometry technique (GC-O and GC-MS) allowed not only the identification of some flavours occuring in volatile fraction but also indicate those compounds which cause poor quality of agricultural distillates.

Three compounds (i.e. geosmin, dimethyl trisulfide and 2- and 3-methylbutan-1-ol) which triggered off decreasing quality of the samples were identified in these studies.

These compounds are not taken into account in quality control whereas they strongly affect raw spirits organoleptic quality. Polish and European regulations recommend to quantify only total content of particular groups of undesired ingredients. Even trace quantity of these by-products presented in agricultural distillates influence the quality of rectified spirits, as well as alcoholic beverages obtained from them.

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WPŁYW GEOSMINY, TRISIARCZKU DIMETYLU ORAZ 2-
I 3-METYLOBUTAN-1-OLU NA JAKOŚĆ DESTYLATÓW ROLNICZYCH

**Streszczenie.** Jakość napojów alkoholowych jest bardzo istotna dla konsumentów. Destylarnie, chcąc wytwarzać wysokiej jakości trunki, muszą określić, które składniki są odpowiedzialne za nieprzyjemny zapach pogarszając jakość otrzymywanych alkoholi. Głównym celem przeprowadzonych badań było określenie profili zapachowych próbek destylatów rolniczych różniących się jakością organoleptyczną oraz wskazanie tych składników, których obecność może być przyczyną złej jakości napojów alkoholowych. W badaniach zastosowano dwie techniki chromatografii gazowej: z detekcją olfaktometryczną oraz z detekcją mas. Badania wykazały obecność około 40 różnych zapachów. Trzy zapachy: ziemisty – pleśni, wilgotnej piwnicy; warzywny – gotowanej kapusty, cebuli oraz stęchły – serowy zostały zidentyfikowane jako: geosmina, trisulfid dimetylu oraz 2- i 3-metylobutan-1-ol. W przypadku tych zapachów występowała zależność pomiędzy jakością próbki a ilością tych związków. Wraz ze wzrostem ich stężenia można było zaobserwować spadek jakości organoleptycznej destylatu rolniczego.

**Słowa kluczowe:** składniki lotne, destylaty rolnicze, GC-O, GC-MS, jakość organoleptyczna

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