APARATURA
BADAWCZA I DYDAKTYCZNA

Profiles of organic volatile compounds in different types of litters in laying houses in Poland

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ABSTRACT

The paper presents results of analyses of the volatile compound profile provided by GC/MS for three types of litters from laying houses (poultry houses for layer hens). Among 115 identified volatile compounds 46 were found to be metabolites of microscopic fungi, predominantly aldehydes and ketones accounting on average for 0.52 (RU – peak area/area of internal standard – tridecane) for wheat straw up to 2.75 (RU) for chopped chaff, as well as hydrocarbons ranging from 0.18 (RU) for chopped chaff up to 1.09 (RU) for rye straw. Discriminant analysis showed the greatest role of 2-methylbutanal, 1-heptanol, naphthalene and trichodiene in the population distribution of groups of analyzed litter samples based on their profiles of volatile compounds.

Profil organicznych związków lotnych różnych rodzajów ściółek pochodzących z kurników zlokalizowanych w Polsce

Słowa kluczowe: zanieczyszczenie środowiska, ściółka, związki lotne

STRESZCZENIE

Przedstawiono wyniki analizy profilu związków lotnych za pomocą GC/MS trzech rodzajów ściółek pochodzących z kurników w których utrzymywane są kury nioski. Zidentyfikowano 115 związków lotnych, 46 należało do grupy metabolitów grzybów mikroskopowych. Wśród nich dominowały aldehydy i ketony – średnio od 0,5222 (RU) dla słomy pszennej do 2,7536 (RU) dla sieczki słomiastej, oraz węglowodory – średnio od 0,1825 (RU) dla sieczki słomiastej do 1,0918 (RU) dla słomy żytniej. Przeprowadzona analiza dyskryminacyjna wykazała, że największe znaczenie podczas rozdziału populacji grup analizowanych prób ściółek na podstawie profilu związków lotnych mają następujące związki: 2-metylobutanal, 1-heptanol, naftalen oraz trichodien.

1. INTRODUCTION

Odor nuisance of livestock housing facilities is connected with the fact that during animal rearing processes various substances are released to air, mainly organic acids, ammonia and phenols, which by stimulating olfactory epithelial cells cause unpleasant olfactory sensations [1]. For many aroma substances found simultaneously in air, as it is the case with air expelled from livestock housing facilities, we may observe synergism, masking or neutralization of olfactory stimuli [2].
Among animal housing facilities poultry houses are characterized by high odour emissions to the ambient atmosphere. Several elements affecting the amount and quality of formed odorants have been identified in the poultry house environment. One of the primary elements determining the profile of formed volatile compounds is the litter. On most farms mainly chopped chaff litters are used. Material to be used for litter needs to be dry, clean, free from mechanical and microbiological contaminants, releasing negligible odour levels and exhibiting good hygroscopic properties [3]. Microscopic fungi developing in the litter are one group of the many microorganisms commonly found in the poultry house environment. The application of antibiotics and other attempts to limit bacterial growth have resulted in a disturbed microbial balance and in many cases – to a strong expansion of microscopic fungi, which are difficult to eradicate. Microscopic fungi, accounting for as much as 35% whole population of microorganisms contained in the litter microflora, in their metabolic processes produce e.g. organic volatile compounds (VOCs) [4]. Among them a significant role is played by trichodiene from the trichothecene group [5], a volatile precursor for the formation of mycotoxins. Mycotoxins are secondary metabolites exhibiting toxic effects towards humans, animals and plants. One of the best methods to analyze the profile of volatile compounds is to isolate them from the atmosphere by stationary phase extraction on carbon microfibre, followed by qualitative and quantitative analysis by GC/MS or the electronic nose. The application of this method in industrial practice is frequently connected with the detection of adulteration in agri-food produce [6].
The aim of this study was to determine profiles of organic volatile compounds (VOCs), focusing on metabolites of microscopic fungi, from different types of straw litters used in poultry houses for layer hens (laying houses). These analyses may lead to the identification of the profile of volatile compounds in litter contaminated with microscopic fungi and in the future may be applied to assess microbial purity and quality of litter used on poultry farms.

2. MATERIAL AND METHODS

2.1 Material

Experimental material comprised samples of 3 litter types: chopped straw (n=150), rye straw (n=110) and wheat straw (n=150) from poultry houses of large farms (stocking: 250-300 hens) located in Poland.

2.2 Analytical methods and calculation

Volatile metabolites (VOCs) were extracted from grain using the solid-phase microextraction method (SPME). Litter samples (8 g) were placed in 20 ml vials and extracted by means of headspace SPME for 30 min. at 50°C with 200 mm -53/30 μm divinylbenzene/Carboxene/polydimethylsiloxane StableFlexTM (DVB/Carboxen/PDMS) fibre. The analyses were run on a gas chromatograph (Agilent 7890A) hyphenated to a mass spectrometer (TruTOF HT, LECO), using a RTX-5 (0.20mm x 10 m) capillary column. The amount of VOCs was estimated by comparing the area of their total ion current (TIC) peaks with the internal standard (tridecane, 25 ng in pentane) and expressed as their ratio (RU). Compounds were identified by comparing their mass spectra with spectra from the NBS 75K and NIST 98 libraries and retention indices were compared to data available in the literature. Volatile metabolites were isolated from litter by solid phase microextraction, which in this case was a 100-μm layer of polydimethylsiloxane (PDMS) bound on carrier fibre. Extraction of volatile compounds from the headspace phase of litter samples was run at 50°C for 30 min. Volatile compounds were analyzed using a gas chromatograph (Hewlett Packard 6890) equipped with a mass detector (Hewlett Packard 5972 A) and a HP-5MS column (30.0 m, 0.25 μm).

Results were subjected to statistical analysis in the STATISTICA ver. 8.0 software. Analyses of the profile of volatile compounds were conducted in triplicate for each sample. Results presented in graphs represent mean values and the calculated standard deviations (±SD) are marked in the form of error bars. Significance of differences were evaluated using the analysis of variance. The Levene test was used to determine homogeneity of variance. Tukey's test was applied to estimate statistically significant difference between means. The step-wise discriminant analysis (SLDA) was used to separate groups of analysed populations. Additionally, the classification matrix and multivariate significance tests were also applied.

3. RESULTS AND DISCUSSION

Qualitative analyses of organic volatile compounds made it possible to identify 115 compounds, of which 46 are metabolites of microscopic fungi. Odour compounds may be divided into several major groups. They include volatile organic acids (with simple and branched chains), aroma compounds (indoles, phenols), nitrogen compounds (ammonia, volatile amines) and sulfur compounds (hydrogen sulfide and mercaptans) [7]. Among all volatile metabolites of microscopic fungi identified in this study aldehydes and ketones predominated, amounting on average to 0.52 (RU – peak area/area of internal standard – tridecane) for wheat straw up to 2.75 (RU) for chopped straw, as well as hydrocarbons ranging from 0.18 (RU) for chopped straw up to 1.09 (RU) for rye straw (Tab. 1). Profiles of analyzed litters differed significantly, both qualitatively and quantitatively, in detected compounds (Fig. 1). Among tested litters chopped straw was characterized by the greatest diversity of identified compounds and contained their highest concentrations. Stepwise discriminant analysis (SLDA) was applied in order to assess whether the applied analytical method will make it possible to separate analyzed populations of litters based on their profiles of volatile compounds. The separation of litters was obtained at the significance level $p=0.001$ to provide as follows: chopped straw 100%, rye straw 79% and wheat straw 65% (Fig. 2). Analysis of the discriminant function showed that among 46 compounds introduced to the SLDA statistical model 2-methylbutanal (Lambda Wilk's test: 0.3151, F-statistic: 34.3364), 1-heptanol (Lambda Wilk's test: 0.1352, F-statistic: 3.8860), naphthalene (Lambda Wilk's test: 0.1406, F-statistic: 4.8011) and trichodiene (Lambda Wilk's test: 0.1368, F-statistic: 4.1572) exhibit the greatest discriminatory power, thus playing a significant role in the differentiation of individual litters depending on the degree of their contamination with microscopic fungi. One of the important detected compounds is trichodiene, a volatile precursor for the formation of one of the most common mycotoxins found in the climate of central Europe, i.e. deoxynivalenol (DON) belonging to the group of trichothecenes (Fig. 3). The profile of formed volatile compounds by microscopic fungi depends on the type of substrate, in this case the type of litter.

The profile of volatile compounds is characteristic of the cereal species, which straw was used to produce litter, and it is a compilation of volatile compounds being secondary metabolites formed as a result of degradation of nutrients found in straw through the metabolism of microorganisms

Table 1 A comparison of averaged profiles of volatile compounds produced by microscopic fungi on three litter types. Contents of individual compounds are expressed in RU (peak area/area of internal standard – tridecane)

No.	Compound	Type of litter		
		Rye straw	Wheat straw	Chopped straw
1	methanol			1,12
2	ethanol		0,01	
3	propanol			0,52
4	1-decanol	0,14		
5	1-heptanol	0,17	0,08	1,49
6	1-hexanol			0,06
7	1-octen-3-ol		0,01	
8	2-ethyl-1-hexanol	0,18	0,08	0,75
9	2-phenoxyethanol			0,05
10	acetone	0,25	0,07	0,15
11	butanone		0,02	0,28
12	pentanone	0,01	0,04	0,04
13	methylpentan-2-one	0,05		
14	(E)-6,10,-dimethyl-5,9-undecadien-2-one	0,46	0,26	0,54
15	heptanal			0,29
16	nonanal		0,04	0,01
17	1,3,5-trimethylbenzene	0,02		
18	1-ethyl-2-methylbenzene	0,01	0,01	
19	2-methylnaphthalene	0,03		
20	benzene		0,03	
21	toluene			0,03
22	1,2,4-trimethyl-benzene	0,04		
23	cyklahexane	0,03		0,02
24	1-methyl-4-prop-1-en-2-yl-cyclohexene			0,01
25	2-methyl-1-propene	0,03	0,06	0,06
26	cyclopentane	0,16	0,16	0,35
27	tridecane		0,02	0,23
28	indane		0,01	
29	propylbenzene	0,16	0,15	0,07
30	p-xylene	0,89	0,17	0,04
31	styrene	0,03	0,02	
32	dodecane	0,57	0,30	0,07
33	eicosane	0,13		
34	pentadecane	0,10	0,06	0,03
35	tetradecane		0,02	
36	triacontane		0,01	
37	undecane	0,16	0,27	0,19
38	3-carene	0,33	0,35	0,04
39	cymene		0,01	
40	lilial		0,01	
41	limonene	0,02	0,02	
42	5-butyldihydro-2(3H)-furanone	0,08	0,05	0,02
43	diethyl phthalate		0,02	
44	dichloromethane	0,01	0,02	
45	trichloromethane	0,01	0,02	
46	chloroethane	0,03	0,02	

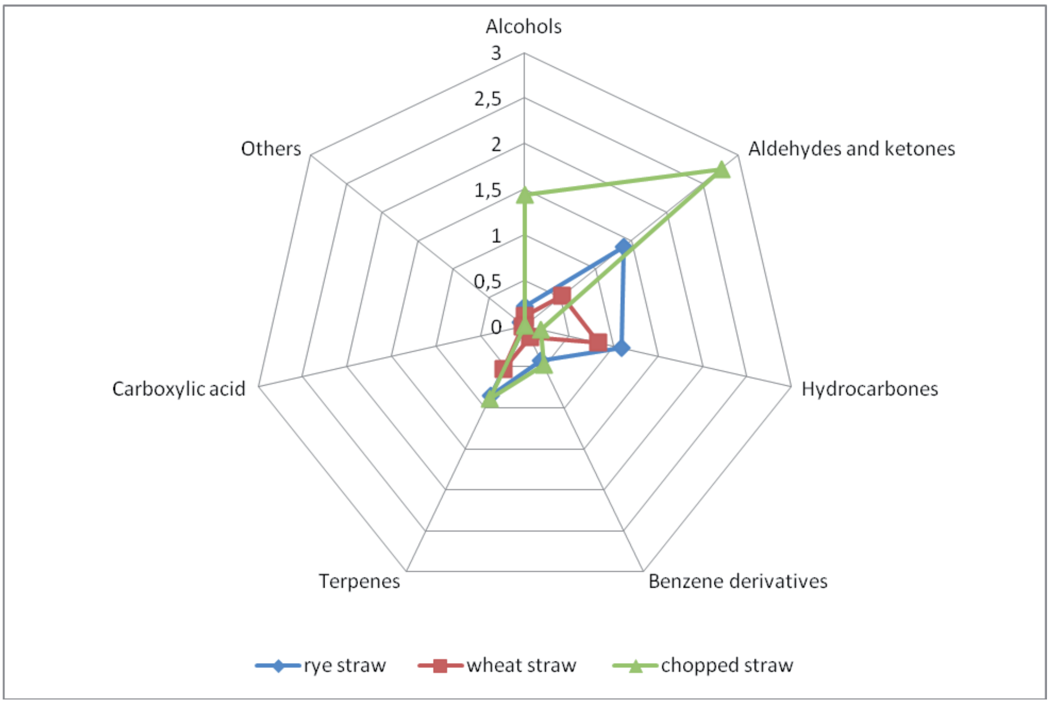

Figure 1 Mean contents of volatile compounds (RU) in terms of the division into groups of chemical compounds in samples of different litter types

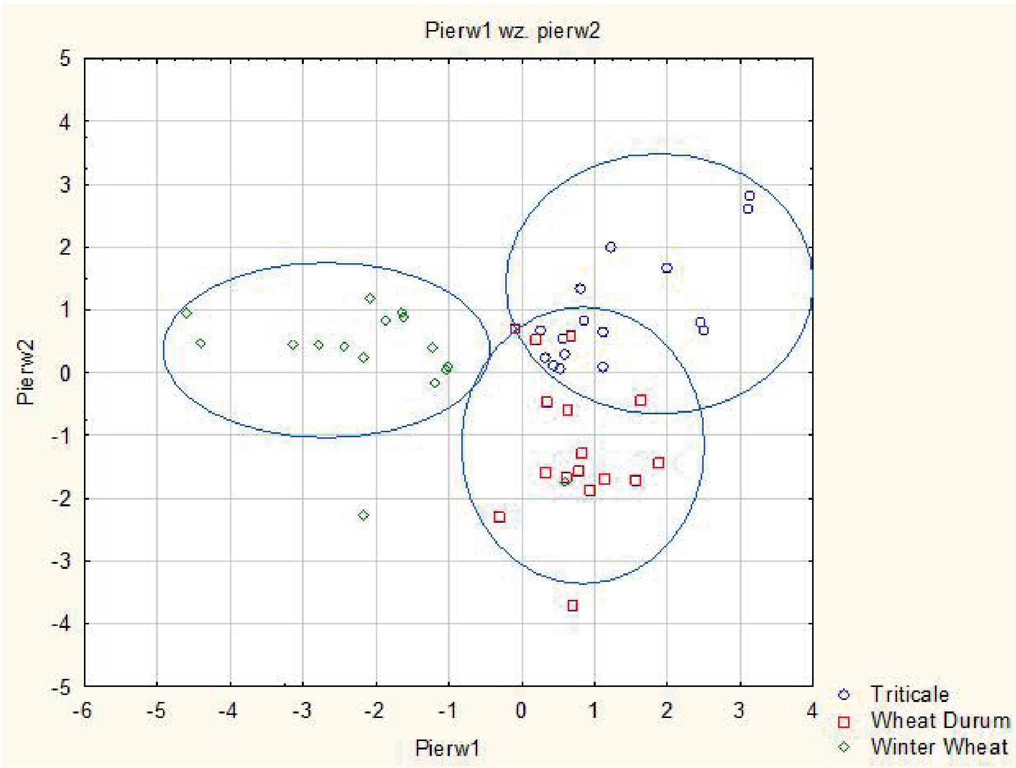

Figure 2 Analysis of the discriminant function of the profile of volatile compounds for different litter types

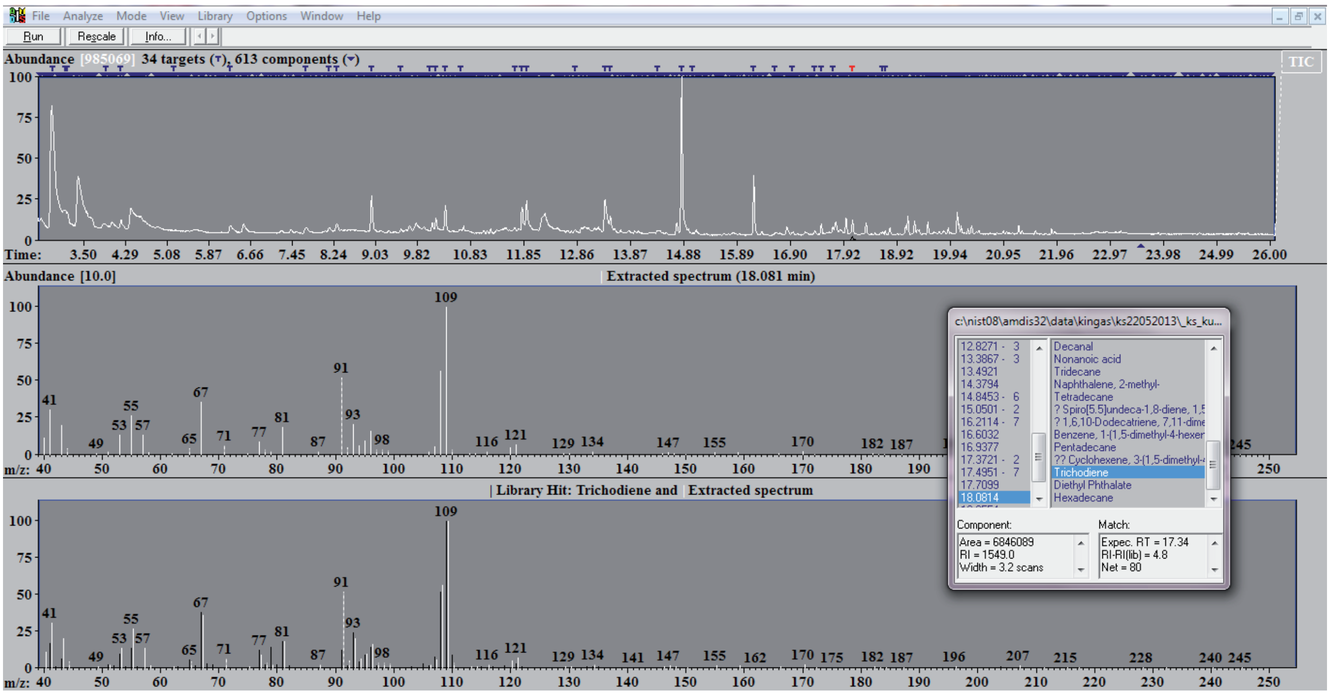

Figure 3 An example chromatogram of volatile compounds identified in chopped chaff litter focusing on the identified trichodiene

colonizing it and as a result of chemical processes taking place in plants [8]. 2-methylbutanal, found in cereals and produced through lipid oxidation by microorganisms during the formation of mycotoxins, is the compound with the greatest discriminatory power. Another compound, 1-heptanol, was identified as a product of fatty acid degradation by microscopic fungi. Naphthalene and p-xylene are also considered to be compounds formed through the metabolism of microorganisms colonizing the surface of different anatomical parts of cereals. In turn, cymene is a compound produced by *C. flexuosus* during storage of *M. leucadendron* via synthesis of aflatoxins. In turn, the detected 2-methylnaphthalene is a compound originating from the contamination of soil and air by compounds formed as a result of combustion of petroleum derivatives and very often accumulated in cereals [9].

4. CONCLUSION

Aroma, and thus nuisance posed by odorant compounds, depend on various types of factors, such as the structure of the chain or carbon ring, the presence of unsaturated bonds, as well as the presence and type of functional groups and their distribution in the molecule. Thus, compounds of different structures may be characterized by a very similar aroma, or vice versa. To date profiles of organic volatile compounds from the poultry house environment have not been investigated in such a comprehensive manner. Based on this study it was found that the type of used litter may have a significant effect on the contamination of the poultry house environment with microscopic fungi as well as organic volatiles produced during their metabolic processes. Both elements play a significant role in the maintenance of health security both for farm workers and animals. Odors may be eliminated through various types of treatments such as e.g. a change of physico-chemical properties of odors or the application of different types of sanitizing additives. The objective of engineering and zoohygienic methods is to optimize the microclimate in animal housing facilities and to improve litter quality [10-11].

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