

VOLATOLOMIC ANALYSIS APPLIED TO FARM ANIMALS.

II. VOLATILE COMPOUNDS EMITTED FROM THE FAECES OF CATTLE

ANALIZA VOLATOLOMICĂ APLICATĂ ANIMALELOR DE FERMĂ.

II. COMPUȘI VOLATILI EMIȘI DIN FECALELE BOVINELOR

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ABSTRACT | REZUMAT

Volatolomics opens new possibilities for the study of the biological systems. The volatility distribution of highly- and semi- volatile organic compounds released from the faeces of cattle was studied. Samples were collected from farm animals in three regions of Romania, namely Râmnicu Vâlcea, Bistrița Năsăud and Constanța. Special procedures for sample collection, storage, transportation and analysis were developed. Organic compounds were found in a broad boiling point range, from n-C₆ (36 °C) up to n-C₁₇ (302 °C), but the highest concentration of about 80 % was found in the range of n-C₉-n-C₁₂ (151–216 °C), with a peak at n-C₁₀ (151–174 °C). Only slight variations were observed among the samples collected from different geographical regions.

Keywords: volatolomics, cattle, faeces

Analiza volatolomică deschide noi posibilități de studiu al sistemelor biologice. În această lucrare a fost studiată distribuția volatilității compușilor organici volatili emiși din fecalele bovinelor. Probele au fost colectate de la animale de fermă din trei regiuni din România, anume Râmnicu Vâlcea, Bistrița Năsăud și Constanța. Au fost elaborate proceduri specifice de prelevare, conservare și transport a probelor. Compușii organici au fost identificați într-un interval larg al punctelor de fierbere, de la n-C₆ (36°C) la n-C₁₇ (302°C), dar concentrațiile cele mai mari, de aproximativ 80%, au fost identificate în intervalul n-C₉-n-C₁₂ (151–216°C), cu un maxim la n-C₁₀ (151–174°C). Între probele colectate din regiuni geografice diferite ale țării au fost observate doar ușoare variații ale compoziției.

Cuvinte cheie: analiza volatolomică, bovine, fecale

"Volatolomics", which is the study of volatile metabolites, has valuable applications, such as in medicine, for early stage diagnosis and monitoring of human diseases, or in agriculture, for pest control or for the characterization of the plant – fungi – bacteria microsystems (1, 9, 13). From ancient times, the odour, colour and other characteristics of human faeces were subject of interest for early physicians to determine the health status of the patients (15, 16). In recent times, gas chromatography or electronic-nose systems were used in attempts of early-stage detection of several diseases from the analysis of human faeces (10-12, 14). Metabolites in faeces are mainly non-volatile, however more than 300 VOCs could be also identified (6-8). The application of volatolomics could be extended for the study of faeces from farm animals

as a non-invasive, rapid and cost-effective tool for monitoring their health status. We previously presented the volatility distribution of the VOCs identified in the respiration of cows in farms from different locations in Romania (4). Here we present the results on the VOCs from the faeces of cows, while the third part of the study will deal with VOCs emitted by cows through skin.

MATERIALS AND METHODS

10 healthy cows from three cattle farms across Romania, namely 2 in Bistrița Năsăud, 4 in Râmnicu Vâlcea and 4 in Constanța counties were selected. Faeces collected from the animal were placed inside of a plastic jar. The jar was connected through a Tygon tube to a small, portable and settable vacuum pump that forced the gases to pass through a biological PTFE filter then through a Tenax tube that captures the VOCs (Fig. 1). The Tenax tubes contain a porous polymer sorbent that is able to adsorb compounds with medium to high boiling points, it has a low background and is hydrophobic, so that it does not adsorb humidity, which is usually high in the gaseous emissions of animals. The Tenax tubes were stored and transported at a low temperature of 4 °C.

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Fig. 1. Sampling of VOCs from faeces of farm cows

Analysis techniques such as gas chromatography coupled with mass spectrometry, ion mobility spectroscopy, or electronic noses are valuable tools for the analysis of VOCs (2-4). A 6890N gas chromatograph (GC) from Agilent, with a HP5-MS column (30 m x 250 mm x 0.25 mm), was used for the chromatographic separation of the volatile organic compounds.

The VOCs trapped in the Tenax tubes were thermally desorbed in a laboratory-designed system. This consists of a small electric furnace connected to a temperature controller, which allowed precise programming of the heating rate and of the final temperature.

The volatile compounds desorbed from the Tenax tubes were concentrated on a solid phase microextraction (SPME) fibre, from which they were thermally desorbed in the injection port of the gas chromatograph, for analysis.

RESULTS AND DISCUSSIONS

The gas chromatographic analysis showed the presence of about 130 organic compounds in the samples collected from the faeces of cows. It was observed that faeces contained a higher number of volatile compounds when compared with exhaled breath air, in which only about 100 compounds were found. Due to the higher number of compounds, the concentration of the main ones, with retention time around 7.6 and 7.7 min (compounds marked with "*" in Fig. 2), decreased to about 35 %, compared with about 40 – 45 % for breath samples. The compounds with retention times of 8.1, 9.5 and 11.9 min were found in much higher amounts compared with samples from exhaled air.

These are accompanied by a small number of compounds with a peak area above ~1%, while many o-

ther compounds were found in very small amounts, making difficult their correct identification.

Fig. 2 shows the chromatograms of three samples, one from Râmnicu Vâlcea, one from Bistrița Năsăud and one from Constanța. Although the chromatograms look similar, some differences could be observed between the samples. For example, the compounds with retention time around 2.6 and 14.4 min were found in higher amounts in the sample collected from Râmnicu Vâlcea, while the compounds with retention time around 11.2 and 11.6 min were found in higher amounts in the sample collected from Constanța (compounds marked with "+" in Fig. 2). On the other hand, the compounds around 12.0 and 12.5 min (compounds marked with "-" in Fig. 2) were found in much lower amounts in the sample from Constanța (c) compared with the samples from Râmnicu Vâlcea (a) and Bistrița Năsăud (b). Some differences in the relative amount of the various compounds along the chromatograms were also observed.

The position of normal paraffins from n-C₆ (hexane) up to n-C₁₆ (hexadecane) in the chromatogram is also marked in Fig. 2. This shows the relative position of the VOCs to the normal paraffins, which are used as references of the boiling points, marking the volatility ranges. The volatility range of compounds, based on the boiling points of normal paraffin, is presented in Fig. 3. The evolution of boiling points of normal paraffins with the number of carbon atoms in the molecule is not linear, and the larger the molecule is, the lower is the increase in its boiling point.

The sum of the peak area percentage of all compounds appearing in the chromatogram in the n-C_{x-1} – n-C_x range of retention time is graphically represented versus the carbon number, "x". The obtaining curve – Fig. 4 is called np-gram (np-standing for normal paraffins) and is an easy way to describe the volatility distribution of compounds in a mixture. More details on the meaning and construction mode of np-grams were given in a previous paper, the first one in the series. The np-grams in Fig. 4 show the average of the values determined for the samples in each region in Romania, that is 2 samples from Bistrița Năsăud, 4 from Râmnicu Vâlcea and 4 from Constanța, as well as the range of these values (minimum and maximum) for each region. It was found that the VOCs from the faeces of cows consisted of compounds distributed in the range of n-C₆ – n-C₁₇, which corresponds to the boiling point range of 36 – 302 °C. All three np-grams had a peak of about 50 % at n-C₁₀, which, according to Fig. 2 and to Fig. 3, corresponds mainly to the compounds marked with "*" at about 7.6 and 7.7 min in the chromatograms, that have the volatility in the range of 151 – 174 °C. Additionally, higher amounts of compounds, of about 10 – 12 %, were found and n-C₉, n-C₁₁ and n-C₁₂, having the volatility in the range of 126 – 151, 174

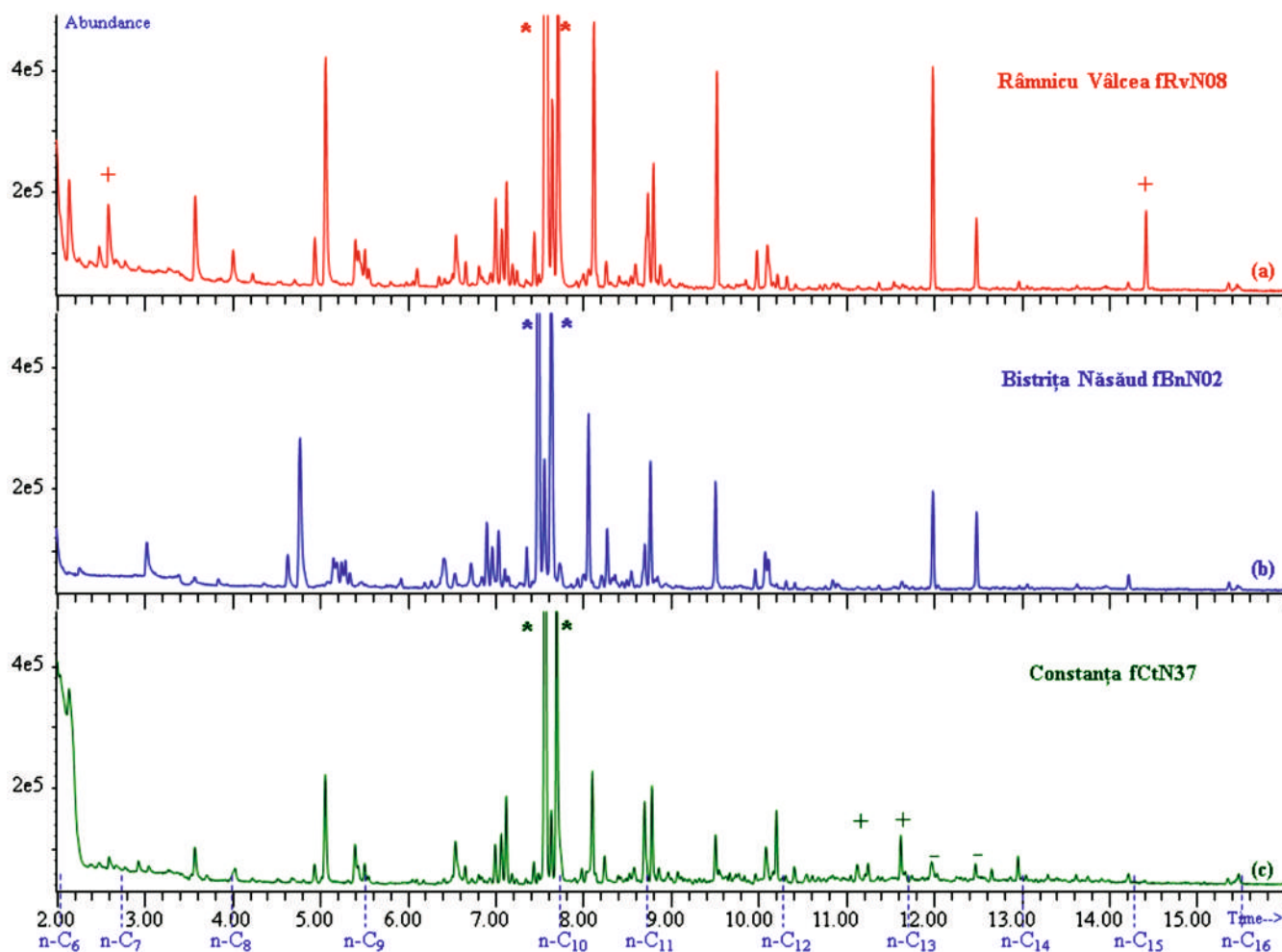


Fig. 2. The chromatograms of several samples of volatile compounds from the faeces of farm cows in three regions on Romania

– 196 and 196 – 216 °C, respectively. These amounts are higher than for breath samples, and led to the decreased amount of the peak at n-C₁₀. While some differences were observed in the chromatograms in Fig. 2, of the samples collected from various regions in Romania, these are less significant in Fig. 4.

The np-grams show good overlapping, with some exceptions, for example, the slightly lower amounts of compounds at n-C₁₄ and slightly higher amounts of compounds at n-C₆ for the samples from Constanța.

The samples from Râmnicu Vâlcea have greater variation among the collected samples, as shown by the dotted red segments representing the range of calculated values, especially for the peak at n-C₁₀; this was similar with the exhaled breath.

CONCLUSIONS

VOCs samples were collected from faeces of 10 cows in farms from three regions in Romania. Around 130 organic compounds were found released from faeces, more than for the exhaled breaths samples. These

compounds had the volatility in the range of boiling points from 36 to 302 °C. Two main compounds were found at n-C₁₀, with boiling point in the 151–174°C range, and in lower amounts compared with the respiration samples. Some differences in the relative amounts of compounds were found between the samples collected in different regions of Romania, but these had low impact on the global distribution of the volatility ranges.

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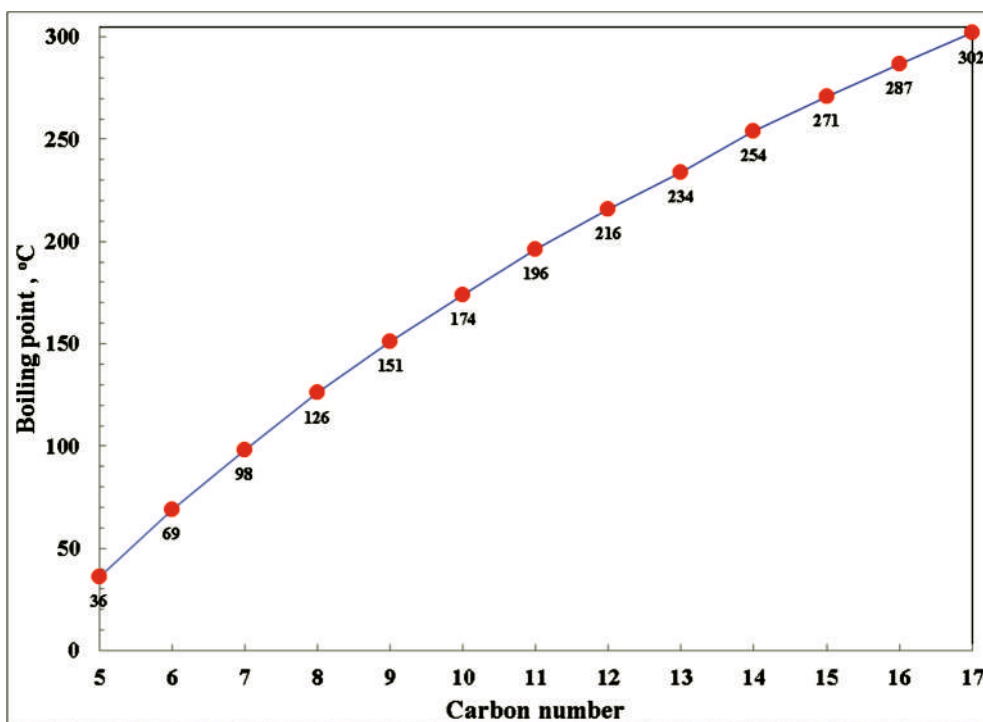


Fig. 3. The volatility ranges based on the boiling point of normal paraffins

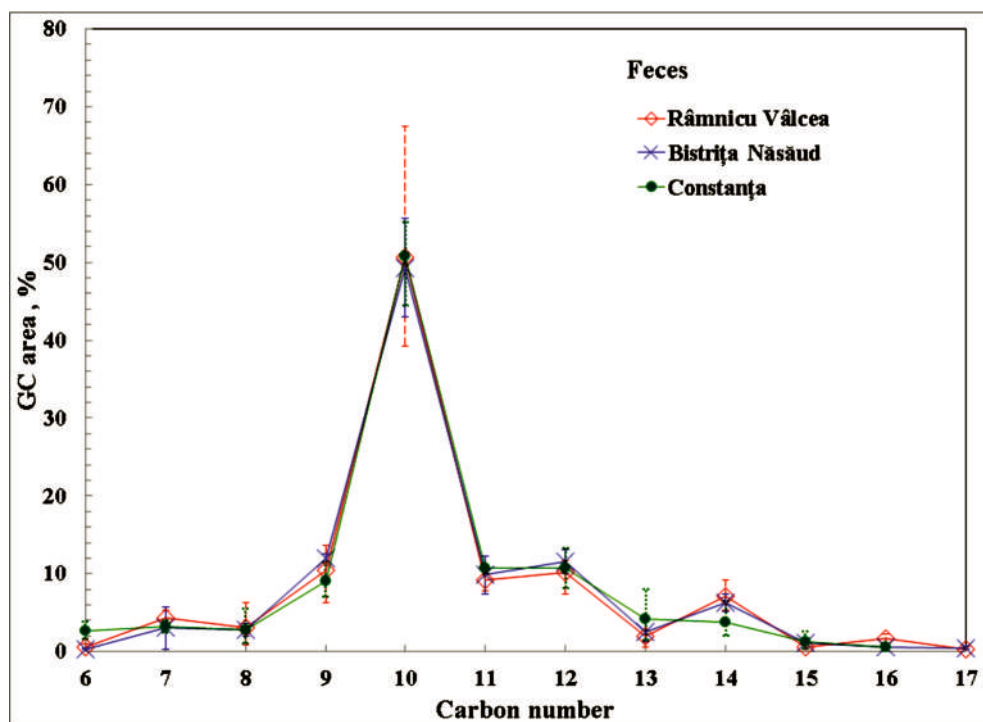


Fig. 4. The volatility distribution of organic compounds from faeces of farm cattle

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