ISSN: 1220-3173; E-ISSN: 2457-7618

VOLATOLOMIC ANALYSIS APPLIED TO FARM ANIMALS. III. VOLATILE COMPOUNDS EMITTED THROUGH SKIN OF CATTLE

ANALIZA VOLATOLOMICĂ APLICATĂ ANIMALELOR DE FERMĂ. III. COMPUȘI VOLATILI EMIȘI DIN PIELEA BOVINELOR

M. BREBU[1),*),
Lisset MOGOLLÓN[2)], R. IONESCU[3)],
D. TURCU[4)], P. GRIGORESCU[4)],
A. IORDACHE[5)], A.A. ENACHE[6)]

ABSTRACT

Volatolomics opens new possibilities for the study of biological systems. Volatility distribution of highly- and semi-volatile organic compounds emitted through the skin 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. About 62 –68 % of organic compounds emitted through skin had the volatility in the boiling point range of n-C_{10} normal paraffin (151–174 °C) and another 16–18 % was in the range of n-C_{11}– n-C_{12} (174–216 °C). Only slight variations were observed among samples collected from different geographical regions.

Keywords: volatolomics, cattle, skin

REZUMAT

Analiza volatolomică deschide noi posibilități pentru studiul sistemelor biologice. În această lucrare s-a studiat distribuția volatilității compușilor organici foarte și semi-volatili emiși prin pielea bovinelor. Au fost recoltate probe de la animale de fermă din trei regiuni ale României, respectiv Râmnicu Vâlcea, Bistrița Năsăud și Constanța. Au fost elaborate proceduri speciale pentru colectarea, stocarea, transportul și analiza probelor. Aproximativ 62-68% din compușii organici emiși prin piele au avut volatilitatea în intervalul punctului de fierbere al parafinelor normale n-C_{10}(151-174 °C) și alți 16 -18% au fost în intervalul n-C_{11}- n-C_{12} (174-216 °C). Între eșantioanele colectate din diferite regiuni geografice au fost observate doar variații ușoare.

Cuvinte cheie: analiza volatolomică, bovine, piele

Volatile organic compounds (VOCs) are important chemicals involved in the communication of plants and animals. They can have unintentional releases (chemical cues) or can serve evolved functions (chemical signals) in recognition, sexual attraction, or predator deterrence between specimens. Several recent studies have shown the particular utility of volatile organic compounds (VOCs) for detecting health disorders (1-3, 9-14).

Investigations of the human VOCs from the skin, in a metabolomics context (volatolomics), are useful both for the identification of individuals (as in forensic science) and for the understanding of the relations between human hosts and mosquitoes or microflora on the skin (4-6). The human cells, the resident skin microflora, and external factors affecting the abundance and distribution of skin microbiome have strong effects on the VOCs from the skin (7, 8).

The application of volatolomics could be extended to the study of the VOCs emitted from the skin of 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 released through respiration or emitted from feces, by cows in farms from different locations in Romania. Here we present the results on the VOCs emitted by cows through the skin.

MATERIALS AND METHODS

Ten healthy cows from three cattle farms across Romania, namely 2 from Bistrița Năsăud, 4 from Râmnicu Vâlcea and 4 from Constanța counties were selected. A specially designed funnel was placed on the skin of the animal after removing the hair (Fig. 1).

The funnel 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 and through a Tenax tube that captures the VOCs. The Tenax tubes contain a porous polymer sorbent that can adsorb compounds with medium to high boiling points, has a low background and is hydrophobic, so that it does not adsorb humidity, which is usually

1) "Petru Poni" Institute of Macromolecular Chemistry, Iași, jud. Iași, România
2) Universidad de Pamplona, Pamplona, Colombia
3) Uppsala University, Uppsala, Sweden
4) "Spiru Haret" University, Faculty of Veterinary Medicine, Bucharest, România
5) CSV Tetoiu, jud. Vâlcea, România
6) Apel Laser, Mogoșoaia, jud. Ilfov, România
*) *Corresponding author: bmihai@icmpp.ro*

high in the gaseous emissions of animals.

The Tenax tubes were stored and transported at a low temperature of 4 °C.

Fig. 1. The sampling of VOCs emitted through the skin of farm cows

Analysis techniques such as ion mobility spectrometry and gas chromatography were found to be suitable for the detection of human skin volatiles (9, 10). A 6890N gas chromatograph (GC) from Agilent, with an HP5-MS column (30 m x 250 mm x 0.25 mm), was used for chromatographic separation of 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 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 80 volatile organic compounds emitted through the skin of cows. This is a much lower number compared with about 100 VOCs in exhaled breath samples and about 130 VOCs released from feces. The abundance scale of chromatograms shows that the intensity of signals, which is proportional to the concentration of compounds in the sample, is much lower compared with the samples from breath and feces. About 50–65 % of the total area of the chromatogram is represented by the two main compounds, with retention time of about 7.6 and 7.7 min (compounds marked with "*" in Fig. 2). This is higher than for exhaled breath or feces. 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 samples. For example, the compounds with retention time of about 8.7 and 11.5 min. (compounds marked with "+" in Fig. 2) were found in higher amounts in the sample collected from Bistrița Năsăud (b), while the sample from Constanța (c) had lower amounts of heavier compounds. There are also differences in the relative amount of various compounds along the chromatograms.

The retention times of normal paraffins, from n-C_6 (hexane) up to n-C_{16} (hexadecane), marked in blue in Fig. 2, are used as standard values for the volatility range. The volatility range of compounds, based on the boiling points of normal paraffins, is presented in Fig. 3. The volatility range of normal paraffins decreases with increasing molecular weight of the molecule.

The compounds in the chromatograms leaving the column before a normal paraffin n-C_x but after the previous normal paraffin n-C_{x-1} have the boiling point, and consequently the volatility, in the range of the two normal paraffins, and are regarded as having the carbon number "x". The sum of peak area percentage of all compounds 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.

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 emitted by cows through skin are distributed in the range of n-C_6 – n-C_{17}, which corresponds to boiling point range of 36 – 302°C. All three np-grams had a peak of about 62–68 % at n-C_{10}, which, according to Fig. 2 and Fig. 3, corresponds mainly to the compounds marked with "*" around 7.6 and 7.7 min in the chromatograms, that have the volatility in the range of 151–174°C.Amounts of about 6–10 %, were found at n-C_{11} and n-C_{12}, and slightly lower at n-C_9, having the volatility in the range of 174–196, 196–216 and 126–151 °C, respectively.

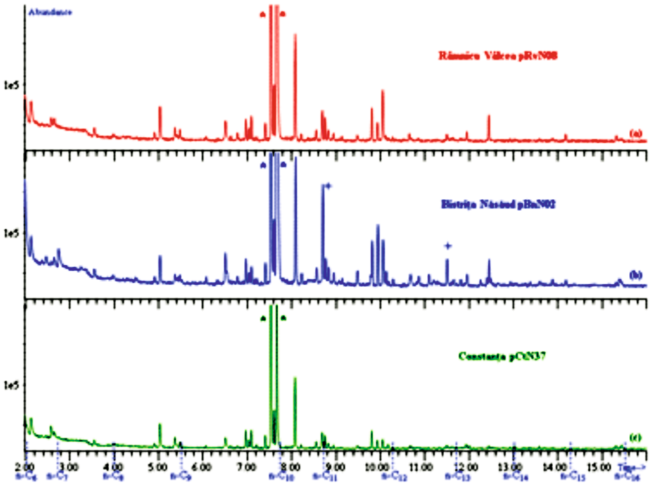

Fig. 2. The chromatograms of several samples of volatile compounds emitted through skin by farm cows in three regions on Romania

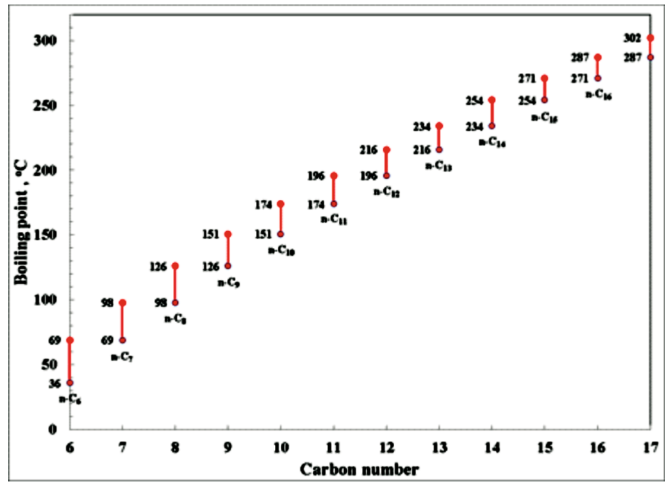

Fig. 3. The volatility ranges based on boiling point of normal paraffins

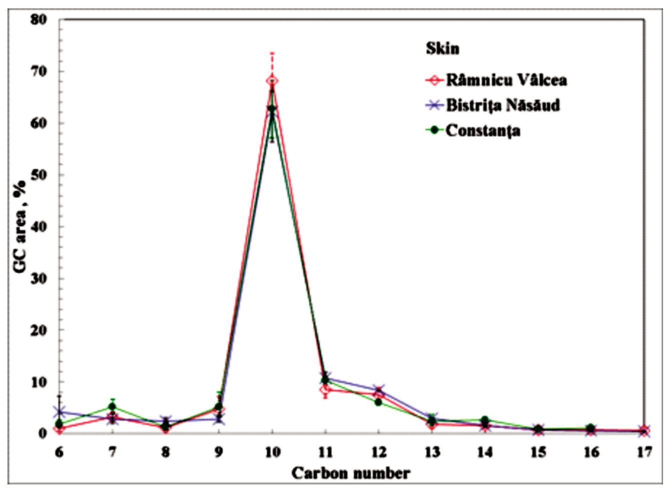

Fig. 4. The volatility distribution of organic compounds emitted through the skin of farm cattle

Some significant differences could be observed among samples collected from various regions in Romania. For the case of Râmnicu Vâlcea, the peak at n-C_{10} is taller and shows higher variation among collected samples, as indicated by the dotted red segments representing the range of calculated values. These samples also had slightly lower amounts of compounds at n-C_{11}. The samples from Bistrița Năsăud had a slightly higher amount of compounds at n-C_6 and a slightly lower amount of compounds at n-C_9, while the samples from Constanța had slightly more compounds at n-C_7 and slightly fewer compounds at n-C_{12}.

CONCLUSIONS

VOCs emitted by cows through the skin were collected from 10 animals in farms from three regions in Romania. Around 80 organic compounds were found, less than from feces or respiration, with volatility in the range of boiling points from 36 to 302 °C. Two main compounds were found at n-C_{10}, having the boiling point in the 151–174 °C range. Some differences were found between samples collected in different regions of Romania.

Acknowledgments.

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement Volatolomics test for the diagnosis of bovine tuberculosis No 777832. The financial support of European Social Fund for Regional Development, Competitiveness Operational Programme Axis 1 – Project "Petru Poni Institute of Macromolecular Chemistry - Interdisciplinary Pol for Smart Specialization through Research and Innovation and Technology Transfer in Bio(nano)polymeric Materials and (Eco)Technology ", InoMatPol (ID P_36_570, Contract 142/10.10.2016, cod MySMIS: 107464) is gratefully acknowledged.

REFERENCES

1. *Baraitareanu S., Vidu L.,* (2019), The preventive medicine of bovine viral diarrhoea - mucosal disease in dairy farms: a review. Revista Romana de Medicina Veterinara, 29(2): 61-64.
2. *Brebu M., Beleno K., Ionescu R., Turcu D., Dinu H., Caciandone H., Enache A.A.,* 2020, Volatolomic analysis applied to farm animals. II. Volatile compounds emitted from the faeces of cattle. Revista Romana de Medicina Veterinara, 30(1):34-38
3. *Brebu M., Beleno K., Ionescu, R., Turcu D., Daneș M., Bogolin I.,* (2019), Volatolomic analysis applied to farm animals. I. Volatile compounds in respira-

tion of cattle, Annals of Spiru Haret University, Veterinary Medicine Series, 20(1):5-10
4. *Curran A.M., Prada P.A., Furton K.G.*, (2010), The differentiation of the volatile organic signatures of individuals through SPME-GC/MS of characteristic human scent compounds. J Forensic Sci, 55:50-57
5. *de Lacy Costello B., Amann A., Al-Kateb H., Flynn C., Filipiak W., Khalid T., Osborne D., Ratcliffe N.M.*, (2014), A review of the volatiles from the healthy human body. J Breath Res, 8:014001
6. *Dormont L., Bessière J.M., Cohuet A.,* (2013), Human skin volatiles: A review. J Chem Ecol, 39:569-578
7. *Duffy E., Morrin A*., (2019), Endogenous and microbial volatile organic compounds in cutaneous health and disease, Trend Anal Chem, 111:163-172
8. *Filipiak W., Mochalski P., Troppmair J., Unterkofler K., Agapiou A., Davis C., Cumeras R., Ager C., Filipiak A.*, (2016), A compendium of volatile organic compounds (VOCs) released by human cell lines, Curr Med Chem, 23:2112–2131
9. *Gociman I.T., Marginean G.E., Baraitareanu S., Nicolae C.G.,Vidu L.,* (2019), Research on the evolution of the Aberdeen Angus breed in Romania. Scientific Papers-Series D-Animal Science, 62(2): 145-149
10. *Khalid T., Aggio R., White P., Costello B., Persad R., Al-Kateb H., Jones P., Probert C.S., Ratcliffe N.*, (2015), Urinary volatile organic compounds for the detection of prostate cancer. PLoS One 10: e0143283
11. *Khatib S., Finberg J.P.M., Artoul F., Lavner Y., Mahmood S., Tisch U., Haick H., Aluf Y., Vaya J.*, (2014), Analysis of volatile organic compounds in rats with dopaminergic lesion: Possible application for early detection of Parkinson's disease. Neurochem Int, 76:82-90
12. *Kusano M., Mendez E., Furton K.G.,* (2011), Development of headspace SPME method for analysis of volatile organic compounds present in human biological specimens. Anal Bioanal Chem 400: 1817-1826
13. *Roodta A.P., Naudéa Y., Stoltzb A., Rohwer E.*, (2018), Human skin volatiles: Passive sampling and GC×GC-ToFMS analysis as a tool to investigate the skin microbiome and interactions with anthropophilic mosquito disease vectors. J Chromatography B, 1097-1098:83-93
14. *Ruzsanyib V., Mochalskia P., Schmida A., Wiesenhofera H., Klieberb M., Hinterhubera H., Amanna A.*, (2012), Ion mobility spectrometry for detection of skin volatiles. J Chromatography B, 911:84-92.