TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2021) 45: 411-418

doi:10.3906/vet-2008-90

Comparison of different extraction solvents used in GC-MS analysis for detecting volatile odor compounds in heat cow sweat

Özgül ANİTAŞ*, Serap GÖNCÜ
Department of Animal Science, Faculty of Agriculture, Çukurova University, Adana, Turkey

Received: 25.08.2020 • **Accepted/Published Online:** 06.03.2021 • **Final Version:** 29.06.2021

Abstract: The accurate estrus detection is essential for successful reproduction. Many scientists reported the importance of pheromones for accurate detection of estrus. In addition, the choice of the appropriate device and the appropriate extraction solvent is important for obtaining quickly and more estrus-specific pheromones. In this study, sweat samples were collected in heat cows and volatile organic compounds (VOCs) in the gas chromatography mass spectrometry were evaluated using three different solvents (diethyl ether, dichloromethane, and hexane) for extraction. VOCs with different numbers and different ratios were identified in all three extraction solvents. As the solvent, 9 compounds were detected in diethyl ether, 8 compounds in dichloromethane, and 12 compounds in hexane. Volatile odor compounds common to all three extraction solvents were L-Proline, 1-[O-(1-oxohexyl)–N-[N-[N6-(1-oxohexyl)-N2-[N-(1-oxohexyl)–L-valyl]–L-lysyl]–L-valyl]–L-tyrosyl]-, methyl ester/Tetradecane; 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester; Phenol, 2,2'–methylenebis [6-(1,1-dimethylethyl)–4–methyl-; Palmitin,1,2–di-. According to the results of the analysis, the most volatile compounds were detected when using hexane as the extraction solvent.

Key words: GC-MS, estrus, solvent, sweat, volatile compounds

1. Introduction

Sweat is a biofluid produced by the eccrine and apocrine sweat glands of mammals [1]. It is a slightly acidic biofluid (pH range: 4.0–7.0) composed mainly of water (99%) containing electrolytes, small molecules, proteins, peptides, metal ions, and xenobiotics, among others. In addition to other compounds, there are also volatile organic compounds in sweat. Volatile organic compounds (VOCs) of sweat could be used as biomarkers for chemo signal [2,3]. Moreover, VOCs released by the cow during estrus have been proposed to have pheromonal properties. Considering that estrus-specific compounds may show pheromone activity in cows, correct detection of these compounds is important [4]. Therefore, determination of the extraction solvents to be used in the gas chromatography-mass spectrometry (GC-MS) device to expose the volatile compounds in sweat samples also plays an important role in the accuracy of analysis results.

The quality of sweat analysis depends on the efficiency of sample collection and the accuracy and sensitivity of analytical methods [1,5,6]. Different analytical approaches have been used for the analysis of volatile compounds in sweat; however, researchers need to focus more on determining which method to apply to get the right volatile compounds because even using different methods in the same device affects the number of volatile compounds. There are two steps in analysis for volatile compounds in GC-MS: extraction (steam distillation, hydrodistillation, simultaneous distillation–extraction) and analysis in GC-MS. In extraction time, losses of some volatile compounds, low extraction efficiency, degradation of compounds through thermal or hydrolytic effects, and toxic solvent residue in the extract may be encountered. These shortcomings have led to the consideration of various solvent extraction methods proposed for the analysis of volatile components [7,8].

Solvent extraction techniques, combined with separation and detection methods, are techniques used to effectively remove and capture volatile odor compounds from samples. Due to the polarity of the compounds and the wide range of volatility, using proper extraction solvent is a starting point for detection and identification of VOCs. It is an important specialty for the accuracy of the analysis to identify the important odor compounds in the sweat of the cows, especially during estrus period, by using suitable extraction solvent. The use of the appropriate solvent has greatly affected dissolution power and extraction efficiency [9,10].

* Correspondence: ozgulanitas01@gmail.com

In this study, we evaluated the volatile organic compounds present in sweat in GC-MS by using three different solvents for the extraction, separation, and analysis of the collected samples. The differences between the distributions of the chromatograms obtained as a result of the analysis were evaluated. However, previous reports of different solvents that affect the analysis result of volatiles in estrus animal sweat, causing the detection of different volatiles are insufficient; therefore, our study represents an original approach.

2. Materials and methods

The present study was conducted at the research and experimental farm located at Faculty of Agriculture, Çukurova University, Adana, Turkey. This study was approved by Çukurova University Animal Experiments Local Ethics Board (Approval no:7 26.02.2018 / 2). There were 160 dairy cows in the farm, of which 15 were selected from lactating herd depending on their age, weight (550–650 kg), and lactation number to organize a homogeneous experimental group and they were synchronized using the Ovsynch protocol. Sweat samples were taken from cows who had no reproductive problems and whose postpartum was between 45 and 60 days [11].

On any day of the estrus cycle (day 0), the cows were injected with 2 mL of intramuscular GnRH (100 μg as 2 mL of Cystorelin i.m., Merial, Athens, GA). On the 7th day, 5 mL of intramuscular PGF2α (5 mg/mL of the active substance dinoprost; Dinolytic, Pfizer Manufacturing Belgium Nv/SA, Puurs, Belgium) was injected. On the 9th day following the first GnRH injection, second injection of 2 mL of intramuscular GnRH was made [11]. Moreover, the time of ovulation was determined by means of an ultrasound technique using a 47–63 Hz 100LC scanner (Pie Medical, Maastricht, the Netherlands). The most suitable time for insemination was determined based on behavioral observations by monitoring the presence or absence of a standing reflex, movement intensity, and snuggling at 4-h intervals. It has been reported that the highest pregnancy rate of dairy cows occurs when inseminated 4 to 12 h after the onset of estrus [12]. According to the results of this research, sweat samples were taken in the first 8 h from the animals showing signs of heat by examining the ultrasound and observation findings.

Experimental group of cows were fed with total mix ration (TMR) (concentrated forage: roughage rate = 60:40). TMR is composed of corn silage, alfalfa, wheat straw, and concentrate (18% crude protein and 2650 kcal/metabolic energy (ME)/kg).

2.1. Sweat samples

In cattle, sweat glands are found in 21 different body regions varying in distribution and size. Most sweat glands are located in the neck area. There are also sweat glands in the nose of the animal. Nasal sweat is easily obtained from the outer surface of the nose of the Holstein cow, free of mucosa.

The animals were placed in a separate compartment to minimize the effects of other odors before taking the sweat from the nose [13]. The nose of the animal was cleaned. This procedure involved washing the nose with deionized water and wiping it with a clean cotton swab. The sweat sample was scraped from the nose of the cow with a swab into a 10-mL glass vial (the vials were 10 mL size magnetic screw top (with PTFE/silicon septum) clear glass vial (Agilent Technologies, Santa Clara, CA, USA)) [13]. The sweat was allowed to collect again on the nose and this process was repeated for each animal until 4 mL of sweat was collected in the vials [13]. The vials were stored in the freezer at –20 °C until analysis [14].

The day before the analysis, the samples were taken out of the freezer and placed in the refrigerator to thaw. Volatile fatty acid analyses of sweat samples were performed by gas chromatography-mass spectrometry at Fisheries Faculty Laboratories in Çukurova University.

2. 2. Extraction and identification of volatile compounds

The samples collected from the particular stage as per the experimental protocol were pooled to minimize the effect of individual variation [15]. Three different extraction solvents (diethyl ether, dichloromethane, and hexane) were used to extract volatile compounds in sweat samples from heat cows. Sweat and extraction solvents used for GC-MS analysis (2 mL of sweat + 2 mL of extraction solvents (diethyl ether, dichloromethane, and hexane)) were placed in the GC-MS vial.

2.3. Gas chromatography and mass spectrometry analysis of all samples

Volatile compounds in sweat were analyzed on an automatic HS-40 head space autosampler (Perkin Almer GC with split splitless inlet MSD system). Needle temperature was 120 °C, thermostat time and degree was 30 min and 35 °C during the extraction in the headspace auto sampler. HP-5 MS (30 m × 0.25 mm × 0.25 μm), fused-silica capillary column was used. Helium (1 mL/min) was used as a carrier gas. The injector temperature was 250 °C, set for splitless injection. The oven conditions were set to 50 °C for 1 min and then the temperature was increased to 200 °C at a rate of 4 °C/min. Thermal desorption was allowed for 1.5 min. The detector temperature was 280 °C. The components were identified by comparison of mass spectra and retention time data with those of authentic samples and complemented with identification by doing a NIST, Wiley, Flavor library search of the acquired mass spectral data.

3. Results

Analysis of volatile compounds in sweat, which is a biological sample, taken from heat cows is important

for determining the correct compounds. Rapid and high detection of sweat compounds is also necessary for the accuracy of the analysis method. Therefore, increasing the selectivity and rates of the target compounds in the analysis makes it mandatory to try different solvents. In this study, volatile compounds in sweat determined using diethyl ether, dichloromethane, hexane as extraction solvent were indicated in Tables 1–3, according to the peak order.

It is seen in Tables 1–3 that the number of volatile fatty acid compounds is different for all three extraction solvents. According to the analysis results shown in Table 1, when diethyl ether was used as extraction solvent, 28 compounds were obtained and 19 of these were unidentified compounds and 9 of them were known compounds.

In contrast to Table 1, less compounds were detect using dichloromethane as extraction solvent (Table 2); 11 compounds were obtained and 3 of these were unidentified compounds while 8 of them were known compounds.

When hexane was used as the extraction solvent, more compounds were detected compared to diethyl ether and dichloromethane (Table 3). It is seen in Table

Table 1. Volatile odor compounds for extraction solvent diethyl ether (n = 6).

Volatile fatty acid	Peak	Retention time (min)	Score (Lib)	Height	Area	%
Unidentified compound	1	6.0434	73.85	524,354	2,664,291	20.246
Unidentified compound	2	13.31897	69.53	24,786	52,515	0.399
Unidentified compound	3	16.91755	63.41	18,713	46,611	0.354
Unidentified compound	4	18.18995	72.89	21,217	51,489	0.391
Unidentified compound	5	18.5683	71.99	22,262	71,758	0.545
Unidentified compound	6	21.79977	66.21	26,078	58,096	0.441
Unidentified compound	7	23.42252	77.05	38,658	146,594	1.114
Unidentified compound	8	26.27845	76.78	28,850	131,282	0.998
Unidentified compound	9	26.59797	77.26	22,195	58,069	0.441
Unidentified compound	10	27.35468	75.87	25,867	64,907	0.493
L-Proline, 1-[O-(1-oxohexyl)-N-[N-[N6-(1-oxohexyl)-N2-[N-(1-oxohexyl)-L-valyl]-L-lysyl]-L-valyl]-L-tyrosyl]-, methyl ester/Tetradecane	11	30.8104	75.18	64,529	203,114	1.543
Unidentified compound	12	34.72858	74.44	21,724	53,183	0.404
Butylated hydroxytoluene	13	35.49652	84.48	915,730	4,386,932	33.336
Unidentified compound	14	37.71623	77.08	26,746	91,528	0.696
Unidentified compound	15	43.53747	68.78	45,799	155,228	1.180
Unidentified compound	16	43.899	78.64	12,299	64,713	0.492
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	17	46.02347	68.43	193,423	635,839	4.832
Unidentified compound	18	48.01618	72.84	23,338	46,465	0.353
Unidentified compound	19	48.84017	78.21	18,565	45,424	0.345
Unidentified compound	20	52.67978	68.75	22,697	45,660	0.347
Pterin-6-carboxylic acid	21	56.15778	54.6	62,644	77,615	0.590
Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	22	56.1718	54.56	64,104	119,534	0.908
Octadecane, 3-ethyl-5-(2-ethylbutyl)-	23	56.53612	63.05	33,618	201,200	1.529
Palmitin, 1,2-di-	24	57.57865	58.11	105,299	330,520	2.512
Unidentified compound	25	59.40025	57.98	27,539	64,766	0.492
17-Pentatriacontene	26	59.96075	58.43	27,649	93,646	0.712
Unidentified compound	27	61.21065	57.48	28,576	83,196	0.632
24,25-Dihydroxy cholecalciferol	28	63.56473	59.11	247,485	3,115,659	23.676

Table 2. Volatile odor compounds for extraction solvent dichloromethane (n = 6).

Volatile fatty acid	Peak	Retention time (min)	Score (Lib)	Height	Area	%
Unidentified compound	1	6.0406	61.04	507,029	1,912,233	18.051
Methylene chloride	2	9.85493	61.62	57,080	177,295	1.674
Unidentified compound	3	23.41968	67.98	33,443	82,409	0.778
L-Proline, 1-[O-(1-oxohexyl)-N-[N-[N6-(1-oxohexyl)-N2-[N-(1-oxohexyl)-L-valyl]-L-lysyl]-L-valyl]-L-tyrosyl]-, methyl ester/Tetradecane	4	30.80752	72.24	42,239	77,384	0.730
Unidentified compound	5	31.16065	72.37	23,216	85,251	0.805
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	6	46.029	60.13	148,439	544,508	5.140
Ethylene oxide	7	47.64337	80.75	20,358	113,699	1.073
Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	8	56.17183	52.51	58,793	180,509	1.704
17-Pentatriacontene	9	56.96775	74.91	484,928	7,124,852	67.257
Palmitin, 1,2-di-	10	57.58148	61.75	43,136	193,260	1.824
N-Desmethylta pentadol	11	62.214	56.49	42,643	102,127	0.964

3 that 21 compounds were obtained. According to the analysis results shown in Table 3, when hexane was used as extraction solvent, 21 compounds were obtained and 9 of these were unidentified compounds while 12 of them were known compounds.

When Tables 1–3 are examined, it can be seen that the volatile compounds with the highest % rate were different. Butylated hydroxytoluene 33.336% in diethyl ether, 17-pentatriacontene 67.257% in dichloromethane, and .beta.-Sitosterol 58,255% in hexane were volatile compounds detected in the sweat of the estrus cow with a maximum rate.

The % ratios of the unidentified compounds in diethyl ether, dichloromethane, and hexane differ from each other. The % ratios of unidentified compounds for each solvent (diethyl ether, dichloromethane, and hexane) are 30.361%, 19.634%, and 9.462%, respectively (Figure 1). In our study, it was seen that the unidentified compounds with the highest % ratios were detected when diethyl ether was used, while the lowest % ratios were detected when hexane was used.

When the chromatograms of the GC-MS analysis results are examined, the differences in the retention times of the volatile compounds between the solvents were seen more clearly and comparatively. Chromatograms of VOCs in sweat samples in different solvents are illustrated in Figure 2.

The low retention time of the 1st peak in which the first volatile odor compounds are detected in the solvents increases the extraction efficiency of these solvents. Figure 3 shows the peak order and retention times of the compounds in hexane, diethyl ether, and dichloromethane used as solvents.

Figure 3 shows that there were more peaks in hexane and more compounds were detected accordingly, but when the initial retention times of the volatile compounds in the solvents are examined, the first peak was formed in diethyl ether and dichloromethane in a shorter time.

4. Discussion

The quality of cow sweat analysis depends on the efficiency of sample collection and the accuracy and sensitivity of analytical methods [16]. In this study, three different extraction solvents (diethyl ether, dichloromethane, and hexane) were used to extract volatile compounds in sweat samples from heat cows.

Different numbers of unidentified compounds were obtained in different solvents used to extract volatile compounds in sweat (Tables 1–3). The most unidentified compound in the analysis result was detected when diethyl ether was used as solvent (Table 1). The low number of compounds in the solvent is probably due to its low polarity [17]. Table 3 shows that the solvent with the lowest number of unknown compounds as a result of the analysis is dichloromethane. This property of dichloromethane was due to its high solubility for various lipidic compounds and its high polarity to remove lipids in sweat from nonlipid compounds such as carbohydrates and proteins.

Table 3. Volatile odor compounds for extraction solvent hexane (n = 6).

Volatile fatty acid	Peak	Retention time (min)	Score (Lib)	Height	Area	%
3-Hexanone	1	11.53098	75.97	381,812	1,347,628	5.268
Butanal, 3-methyl-	2	12.00463	79.17	465,256	1,793,542	7.011
Unidentified compound	3	12.41942	73.99	68,301	218,729	0.855
Unidentified compound	4	20.4995	77.65	18,462	159,701	0.624
Methylpent-4-enylamine	5	23.4339	72.85	191,368	619,587	2.422
Unidentified compound	6	26.60093	72.55	83,254	294,885	1.153
Unidentified compound	7	27.34363	75.6	46,976	159,821	0.625
Unidentified compound	8	28.28533	73.24	56,468	181,706	0.710
L-Proline, 1-[O-(1-oxohexyl)-N-[N-[N6-(1-oxohexyl)-N2-[N-(1-oxohexyl)-L-valyl]-L-lysyl]-L-valyl]-L-tyrosyl]-, methyl ester/Tetradecane	9	30.81057	75.67	173,367	559,375	2.187
Unidentified compound	10	34.73153	71.52	51,864	240,886	0.942
Unidentified compound	11	43.34143	74.57	38,519	178,654	0.698
Unidentified compound	12	45.03147	83	15,358	765,054	2.991
1,2-Benzened icarboxylic acid, bis(2-methylpropyl) ester	13	46.02362	67.59	129,486	392,505	1.534
Unidentified compound	14	50.88895	62.63	69,451	220,913	0.864
Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	15	52.69937	66.4	74,184	285,741	1.117
E,E-1,9,17-Docasatriene	16	54.13983	62.06	68,613	190,338	0.744
Z,Z-10,12-Hexadecadien-1-ol acetate	17	55.71763	58.37	53,711	184,246	0.720
Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	18	56.17163	60.32	205,460	598,737	2.341
Palmitin, 1,2-di-	19	57.58127	64.17	268,619	994,997	3.890
.beta.-Sitosterol	20	58.83677	69.17	317,267	1,291,835	5.050
.beta.-Sitosterol	21	59.05533	74.22	732,216	14,902,636	58.255

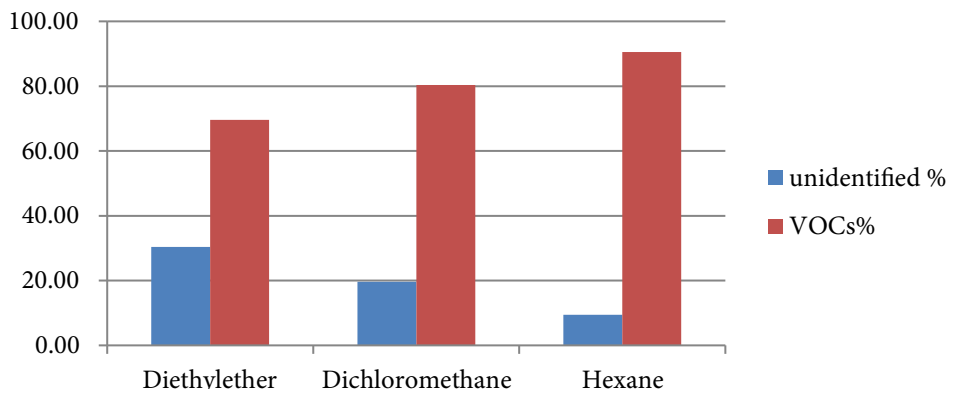

Figure 1. % ratios of VOCs and compounds in diethyl ether, dichloromethane, and hexane used as solvent.

In our study, unidentified compounds with the lowest % ratios were detected when hexane was used (Figure 1); this property of hexane was based on the wide variety in its ability to spread through the sample and its ability to dissolve different lipid classes.

Sankar and Archunan [15] and Mozūraitis et al. [18] used diethyl ether as solvent to extract volatile compounds from heat cow feces in their study. As a result of the analysis with the GC-MS device, they detected 3 and 31 (24 known, 7 unknown) volatile compounds, respectively. When Table

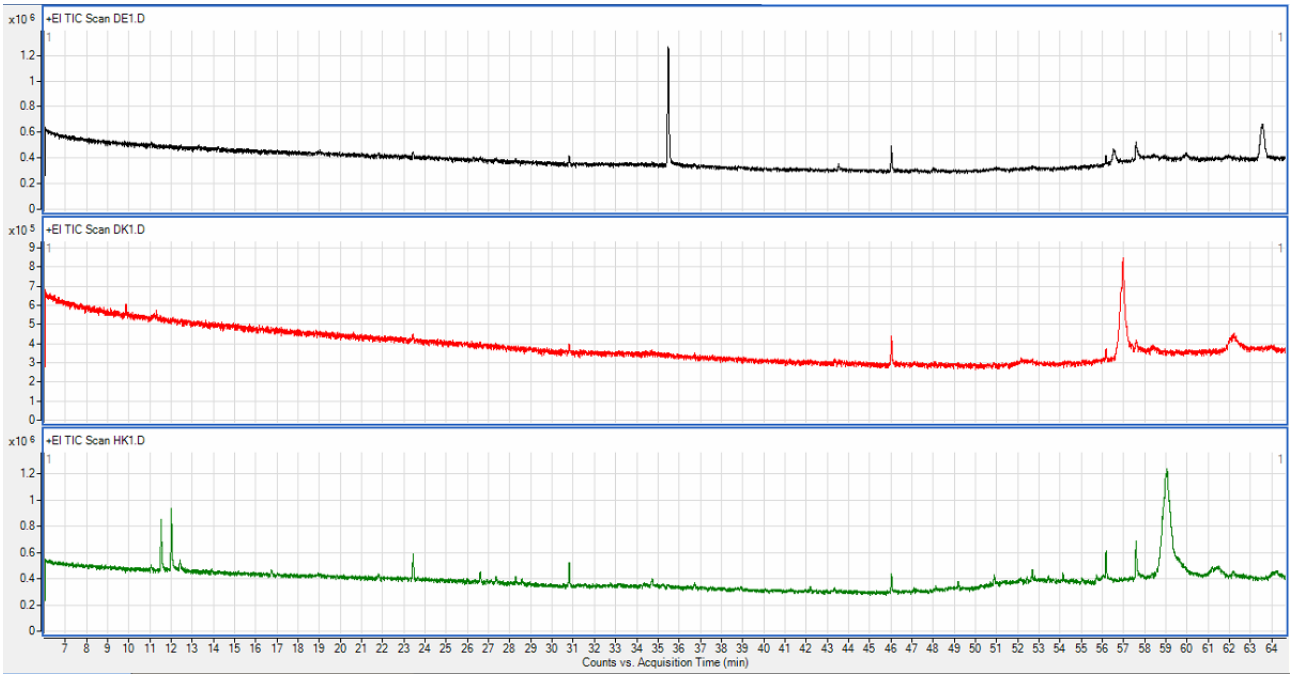

Figure 2. Chromatograms of VOC sweat samples obtained using three extraction solvents (a = diethyl ether, b = dichloromethane, c = hexane) in GC-MS.

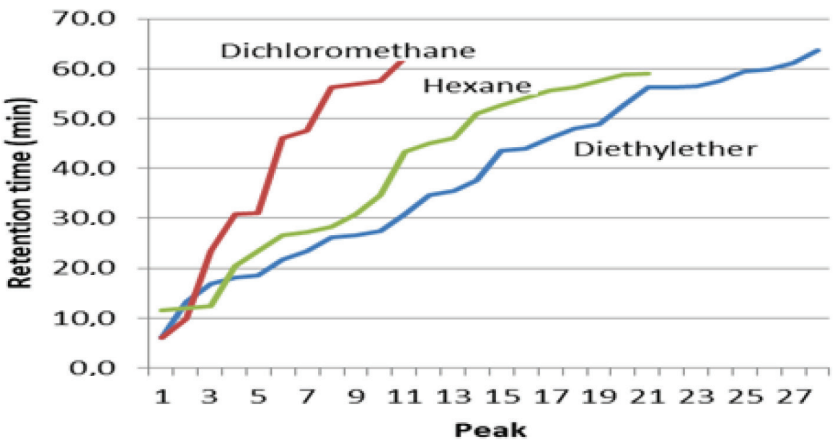

Figure 3. The peak order and retention times (min) of VOCs obtained in three solvents.

1 is examined, the reason for detecting different numbers of volatile compounds in sweat may be due to the solid state of the sample used by the researchers or due to less sensitive measurement methods.

Sankar and Archunan [19] used diethyl ether as solvent to extract volatile compounds found in the vaginal fluid of heat cows. As a result of the analysis, they detected 5 compounds during the heat period. Table 1 shows that more compounds were detected in sweat than in the study of Sankar and Archunan [19]. The reason for detecting different numbers of compounds despite using the same solvent may be due to the polarity (polar and apolar) and volatility (high or low) properties of the substance to be extracted, or the different properties of the device used.

Ramesh Kumar et al. [20] used dichloromethane as solvent to extract volatile compounds from heat cow urine in their study. As a result of the analysis, they detected 5 compounds. When Table 2 is examined, it can be seen that 8 compounds were obtained as a result of the analysis. Although the same solvent was used in our study, the reason for obtaining a large number of compounds

was the high polarity of sweat or the high molecular specificity and detection sensitivity of the device.

As a result of the analysis, the common compounds in all solvents were determined. Identification of compounds common to all three solvents suggests that these compounds may be metabolic products that can help determine estrus in the animal. Common volatile compounds for all methods are given below:

ü L-Proline, 1-[O-(1-oxohexyl)–N-[N-[N6-(1-oxohexyl)-N2-[N-(1-oxohexyl)–L-valyl]–L-lysyl]–L-valyl]–L-tyrosyl]-, methyl ester/Tetradecane;

ü 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester;

ü Phenol, 2,2'–methylenebis [6-(1,1-dimethylethyl)–4–methyl-;

ü Palmitin, 1,2–di-

However, the peak order, score (Lib), height, area, and % ratios of the common volatile compounds appear to be different in each extraction solvent. For example, L-Proline, 1-[O-(1-oxohexyl)–N-[N-[N6-(1-oxohexyl)-N2-[N-(1-oxohexyl)–L-valyl]-L-lysyl]–L-valyl]–L-tyrosyl]-, methyl ester/Tetradecane compound was determined in Table 1: peak order 11 and % rate 1.543; in Table 2: peak order, 4 and % rate 0.730; and in Table 3: peak order 9 and % rate 2.187.

In Figure 2, it is seen that 3 different extraction solvents showed various patterns. For example, it is seen that the retention time for determining the first volatile compound is 30th min in Figure 2, 9th min in Figure 3, and 11th min in Figure 4. Since the retention time is the time between injection and detection of the analyte, it can be said that the solvent that forms a peak in a short time by rapidly dissolving volatile compounds will give more accurate results. Although the highest peak number was seen in hexane in our study, it was seen that the first peak time (Figure 2) was determined in diethyl ether and dichloromethane. Although the number of peaks in hexanes was high, the number of unknown compounds was high and their ratio was high, and the initial retention time was long, maybe due to the fact that it does not have high polar properties to remove volatile compounds from the sample. In our study, the first peak time was obtained in a longer time than the researchers using both diethyl ether [15] and dichloromethane [20] as solvent. The reason for this difference was that the column was long, the temperature of the column was low, and the sweat has larger molecules than other samples.

Choi and Oh [21] detected sweat VOCs in humans in peak time intervals of 3–25 min and 25–42 min in two analyses. In Figure 2, volatile compounds in sweat are defined in peak time intervals of 30–63 min, in peak time intervals of 9–62 min in Figure 3, and in peak time intervals of 11–59 min in Figure 4. In our study, the recognition range of volatile compounds in chromatograms was similar to the results of Choi and Oh [21].

As a result of this study, more volatile compounds with the highest percentage were determined when hexane was used, but early results were obtained from diethyl ether and dichloromethane. Highly volatile compounds were detected in sweat when hexane was applied because it has properties such as quick recovery, nonpolar existence, low vaporizing latent heat (330 kJ/kg) and high solvent selectivity. In addition, the shorter initial retention time of diethyl ether and dichloromethane than hexane may be due to their high polar properties to remove volatile compounds.

Acknowledgments

We thank the Scientific Research Projects Coordination Unit of Çukurova University (Project Number: FDK-2018-10400) for financial support. We are also grateful to Prof Dr Yeşim ÖZOĞUL for her valuable help and contributions in the Laboratory of Seafood Processing Technology, Faculty of Fisheries, Çukurova University.

References

1. Baker LB. Sweating rate and sweat sodium concentration in athletes: a review of methodology and intra/interindividual variability. Sports Medicine 2017; 47 (1): 111-128. doi: 10.1007/s40279-017-0691-5

2. Phillips M, Cataneo RN, Ditkoff BA, Fisher P, Greenberg J et al. Prediction of breast cancer using volatile biomarkers in the breath. Breast Cancer Research and Treatment 2006; 99: 19-21. doi: 10.1007/s10549-006-9176-1

3. Monedeiro F, dos Reis RB, Peria FM, Sares CTG, De Martinis BS. Investigation of sweat VOC profiles in assessment of cancer biomarkers using HS-GC-MS. Journal of Breath Research 2019; 99 (1): 19-2. doi: 10.1088/1752-7163/ab5b3c

4. Nordéus K, Båge R, Gustafsson H, Glinwood R, Söderquist L. A small expose on bovine pheromones: with special reference to modifications of the reproductive cycle. Chemical Signals in Vertebrates. 2016; 13: 33-42 doi: 10.1007/978-3-319-22026-0_4

5. Gallardo E, Queiroz JA. The role of alternative specimens in toxicological analysis. Biomedical Chromatography 2008; 22 (8): 795-821. doi: 10.1002/bmc.1009

6. Ely MR, Ely BR, Chinevere TD, Lacher CP, Lukaski HC et al. Evaluation of the Megaduct sweat collector for mineral analysis. Physiological Measurement 2012; 33 (3): 385-394. doi: 10.1088/0967-3334/33/3/385

7. Ferhat MA, Tigrine-Kordjani N, Chemat S, Meklati BY, Chemat F. Rapid Extraction of volatile compounds using a new simultaneous microwave distillation: solvent extraction device. Chroma 2007; 65: 217-222. doi: 10.1365/s10337-006-0130-5

8. Zhao C, He X, Li C, Yang L, Fu Y et al. A microwave-assisted simultaneous distillation and extraction method for the separation of polysaccharides and essential oil from the leaves of Taxus chinensis Var. mairei. Applied Sciences 2016; 6 (2): 19. doi: 10.3390/app6020019

9. Wahl JH, Colburn HA. Solvent Extraction of Chemical Attribution Signature Compounds from Painted Wall Board: Final Report 2009; United States: N. p. doi: 10.2172/1008265

10. Cai Z, Qu Z, Lan Y, Zhao S, Ma X et al. Conventional, ultrasound-assisted, and accelerated-solvent extractions of anthocyanins from purple sweet potatoes. Food Chemistry 2016; 197: 266-272. doi: 10.1016/j.foodchem.2015.10.110

11. Aksu EH, Bozkurt T, Türk G. Farklı senkronizasyon uygulamalari ile senkronize edilen ineklerde üreme performansi üzerine vitamin E'nin etkisi. Foray Üniversitesi Sağlık Bilimleri Dergisi Veterinerlik Fakültesi Dergisi 2010; 24 (2): 71-76 (in Turkish).

12. Dransfield MBG, Nebel RL, Pearson RE, Warnick LD. Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system. Journal of Dairy Science 1998; 81: 1874-1882.

13. Kennedy GA. Detecting hypocalcemia using bovine nose sweat. United States Patent 2011; No: 7.964.409.

14. Kumar RK, Archunan G, Jeyaraman R, Narasimhan S. Chemical characterization of bovine urine with special reference to estrus. Veterinary Research Communications 2000; 24: 445-454. doi: 10.1023/A:1006495404407

15. Sankar R, Archunan G. Identification of putative pheromones in bovine (Bos taurus) faeces in relation to estrus detection. Animal Reproduction Science 2008; 103 (1-2): 149-153. doi: 10.1016/j.anireprosci.2007.04.014

16. Baker LB. Physiology of sweat gland function: The roles of sweating and sweat composition in human health. Temperature 2019. doi: 10.1080/23328940.2019.1632145

17. Bahrami N, Yonekura L, Linforth R, Carvalho da Silva M, Hill S et al. Comparison of ambient solvent extraction methods for the analysis of fatty acids in non-starch lipids of flour and starch. Journal of the Science of Food and Agriculture 2013; 94 (3): 415-423. doi: 10.1002/jsfa.6449

18. Mozūraitis R, Kutra J, Borg-Karlson A-K, Būda V. Dynamics of putative sex pheromone components during heat periods in estrus-induced cows. Journal of Dairy Science 2017; 100 (9): 7686-7695. doi: 10.3168/jds.2016-12376

19. Sankar R, Archunan G. Gas chromatographic/mass spectrometric analysis of volatile metabolites in bovine vaginal fluid and assessment of their bioactivity. International Journal of Analytical Chemistry 2011; 1-7. doi: 10.1155/2011/256106

20. Ramesh Kumar K, Archunan G, Jeyaraman R, Narasimhan S. Chemical characterization of bovine urine with special reference to oestrus. Veterinary Research Communications 2000; 24 (7): 445-454. doi: 10.1023/A:1006495404407

21. Choi MJ, Oh CH. 2nd dimensional GC-MS analysis of sweat volatile organic compounds prepared by solid phase micro-extraction. Technol Health Care 2014; 22 (3): 481-488. doi: 10.3233/THC-140807