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Differentiation of oleoresin capsicum sprays based on their capsaicinoid profiles

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ABSTRACT

Oleoresin capsicum (OC) sprays, often referred to as 'pepper sprays', contain a solution of active compounds, exerting an irritating effect on the human body. The active component of OC sprays are capsaicinoids, obtained by extraction from peppers. The profiles (quantitative relations) of natural capsaicinoids depend on the plant material, they were extracted from. Pepper spray is a non-lethal weapon that should only be used for self-defense but is often used by criminals to attack and incapacitate victims. Evidence related to these types of incidents, such as containers, clothes of victims or suspects, as well as traces of substances found at the scene, are submitted to the forensic laboratory. The purpose of the analysis is to identify the ingredients of the preparation (especially active components) and compare the traces found on objects from the victim or the scene of the incident with the preparation from the can or traces found on objects related to the suspect. The study aimed to investigate the possibility of differentiating OC gases based on capsaicinoid profiles recorded in GC-MS analyses. Sixty-four gases from 12 different manufacturers were purchased and tested.

The likelihood ratio (LR) approach was applied to the data expressing the relative capsaicinoids contents computed by integrating GC-MS signals. Two hypotheses were assumed that stated either common or different origins of the samples. Several LR models have been developed, and their performance has been controlled by the number of false positives and false negatives as well as empirical cross entropy.

The research results showed that differentiation was very successful, with more than 90% of correct responses. The results obtained show that OC sprays may be distinguished, even if they were produced by the same producer presumably if produced using different batches of pepper extract.

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1. Introduction

Self-defense sprays in the form of hand-held pressurized containers with nozzles containing a solution of irritating compounds are commonly called "pepper sprays" or "tear gases". In many countries, such devices can be legally purchased without permission. They should be utilized for self-defense only but are often used by criminals to attack and incapacitate their victims. Evidence connected with these kinds of cases, such as containers, the clothes of victims or suspects, or traces of substances found on the crime scene, are delivered to the forensic laboratories for analysis. The study aims to identify the preparation components, especially active

agents, and compare the traces found on items originating from the victim or the crime scene with the preparation from the can or traces found on items connected with the suspect.

The main ingredients of self-defense sprays are active compounds, solvents, and propellants. The active ingredients of the tear gases available on the market are most often "*oleoresin capsicum*" (OC), an extract obtained from peppers, containing capsaicinoids. More than 90% of the available sprays belong to this group. Other possible active ingredients found in some sprays are synthetic capsaicinoid pelargonic acid vanillylamide (PAVA), o-chlorobenzalmalononitrile (CS), chloroacetophenone (CN) and dibenz (b,f)-1,4-oxazepine (CR). 'Tear gases' manufactured in Russia and Ukraine may contain N-nonanoylmorpholine (MPK/MPA). This compound is not used in formulations produced in other countries, and very little can be found in the literature about its properties and toxicity [1].

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The sprays' active components are dissolved in organic solvents as in a pure form, and under normal conditions, they are solids [2]. Solvents commonly found in such formulations are ethanol, 2-propanol, ethyl acetate, 1,2-propanediol, and polyethylene glycol [2,3]. Propellants are added to the cans to pressurize the content and propel the formulation. Propane-butane, compressed CO₂, or nitrogen are most often used for this purpose [4].

Though different compounds used as active ingredients of self-defense sprays differ in physical and biochemical properties (e.g. volatility, toxicity), they have a similar influence on the human body. They are irritants, affecting peripheral sensors and causing local sensations of pain and burning of the eyes, mucous membranes, respiratory system, and sensitive parts of the skin. They also provoke involuntary reflexes like the closing of the eyes (blepharospasm), tearing, coughing and sneezing, secretion of mucus, and nausea. An individual exposed to these compounds is therefore incapacitated [2].

Oleo-resin capsicum, a waxlike resin produced by the solvent extraction of natural plant material, this is peppers (fruits of different species of genus *Capsicum*), contains capsaicinoids - a group of compounds of similar chemical structure and properties, responsible for its pungency. The capsaicinoids that are present in the greatest amount in OC sprays are dihydrocapsaicin (35.8–48%), capsaicin (33–47.8%), nordihydrocapsaicin (7.7–20%), homocapsaicin (1.3–7%), nonivamide (1.2–5.5%) and homodihydrocapsaicin (1.9–2.9) [5,6]. The total concentration of capsaicinoids in the peppers differs and depends upon the variety of the pepper, the growing conditions, and the time of harvest [5,7–10]. The same factors are probably responsible for the differences in the relative amounts of specific capsaicinoids observed in the plant materials [5,7–10]. Capsaicinoids present in the OC sprays should show the same differences in the profiles. This supposition was already confirmed by some studies [5], though in some other research, the opposite results were obtained; this is that the relative amounts of capsaicinoids in the tested OC sprays did not exhibit significant variability [11]. It should be noted that in both studies a very small number of sprays were tested, i.e. 7 and 5, respectively.

The data obtained from the analysis of pepper gases is not rich in information, e.g. the chromatograms presented in Fig. 1. Moreover, the differences between profiles of capsaicinoids are, in most cases, not noticeable to the naked eye. One of this publication aims is to check that the computational methods, especially the likelihood ratio, are useful for distinguishing pepper gases based on differences in profiles of capsaicinoids.

The presented research aimed to address the following problems:

- 1) What is the variability of the profiles of capsaicinoids in the OC sprays?
- 2) Is it possible to distinguish tested sprays based on the profiles of capsaicinoids (is the between-samples variability sufficiently higher than within-samples variability to facilitate the differentiation?). If not, which of them cannot be distinguished, and what is the probable cause (e.g., the same producer/distributor and possibly the same batch of OC used as an active ingredient)? How many groups of indistinguishable sprays can be created?
- 3) If two samples of OC-containing formulations are indistinguishable - how strongly this supports the hypothesis that the samples come from the same pepper spray? And, if the profiles of capsaicinoids in the two samples are different, how strongly this supports the hypothesis that the samples come from the different pepper sprays?

Solving these problems enables us to answer whether the variability of the capsaicinoid profile in the OC sprays can be utilized for forensic purposes, such as during the comparative analysis of samples from the suspect and the crime scene.

Further, the influence of some other factors on the capsaicinoid profiles must be investigated, such as solvent extraction or the storage time and conditions.

2. Materials and methods

2.1. Collection and characteristics of OC sprays

53 OC sprays (sprays No. 1–13 and 25–64 in Table 1), produced/distributed by 12 different companies, were purchased in 7 different shops, from November 13, 2018, to March 3, 2019. The details are listed in Table 1. From February 9, 2021, to February 16, 2021, an additional 11 OC sprays, produced by F.W Klever GmbH, were purchased in 4 shops (sprays No. 14–24 in Table 1). For some of the sprays, only information about the distributor was available because no information about the producer was printed on the cans. Some gases (the same producer/distributor, name, appearance, and volume of the can) were bought in duplicates or triplicates, but each of the specimens in a different shop. Most of the specimens bought in duplicates/triplicates (6 of 11) have different expiration dates, suggesting different production batches.

For some of the sprays produced/distributed by the same company, the only difference between specimens was the can's volume. Name, the appearance of the packaging, and form of the preparation (aerosol, jet, gel, or foam), were the same.

The market of OC sprays is dominated by two companies. From 53 sprays collected randomly from November 13, 2018, to March 3, 2019, 17 were produced by Security Equipment Corporation, USA, and 12 were produced by F.W. Klever GmbH, Germany. The remaining 24 sprays represent ten brands, and some of these brands are represented by only one specimen (Table 1).

2.2. GC-MS analysis of the OC preparations

Samples were obtained by spraying the gases into the beaker. 10% dilutions in methanol (LiChrosolv for liquid chromatography, Merck KGaA, Darmstadt, Germany) were prepared, placed in an ultrasonic bath for 15 min (Sonorex RK100H, Bandelin Electronics GmbH, Berlin, Germany) and cleaned of solid particles by centrifugation (5 min 1320 rpm, microcentrifuge: 5415D, Eppendorf, Hamburg, Germany). Each of the obtained supernatants was analyzed three times, using Agilent analytical system: GC 7890B equipped with an autosampler for liquid samples G415A and coupled with MS 5977B. 1 µl of the samples were injected via split/splitless injector (300 °C; 1:20), and compounds were separated on the DB5-MS UI column (30 m, i.d. 0.25 mm, film 0.5 µm; J&W Scientific, Folsom, CA, USA). The temperature program of chromatographic analysis was as follows: 100 °C kept for 1 min; increase 20 °C/min to 300 °C; 300 °C kept for 10 min. Molecules were ionized by electron impact (70 eV) and mass spectra were recorded in full-scan mode, with a scan range of 35–350 *m/z*.

2.3. Data analysis

The Total Ion Chromatograms (TIC) were recorded in triplicate for each of the samples. The Extracted Ions Chromatograms (EIC) for a sum of the ions characteristic for capsaicinoids, this is *m/z* = 122, 137, 152, 195, 293, 305, 307, 319, 321, were displayed, and peaks of the four most abundant capsaicinoids were integrated: nordihydrocapsaicin (NDHC), nonivamide (NI), capsaicin and dihydrocapsaicin (DHC). The area of its peak described each capsaicinoid. The areas were normalized to be independent of the concentration or amount of samples subjected to the analysis. The peak areas for NDHC, NI, and DHC were divided by the peak area for capsaicin to normalize the data. In this way, the number of variables was reduced from four to three, i.e., NDHC, NI, and DHC. Homocapsaicin and

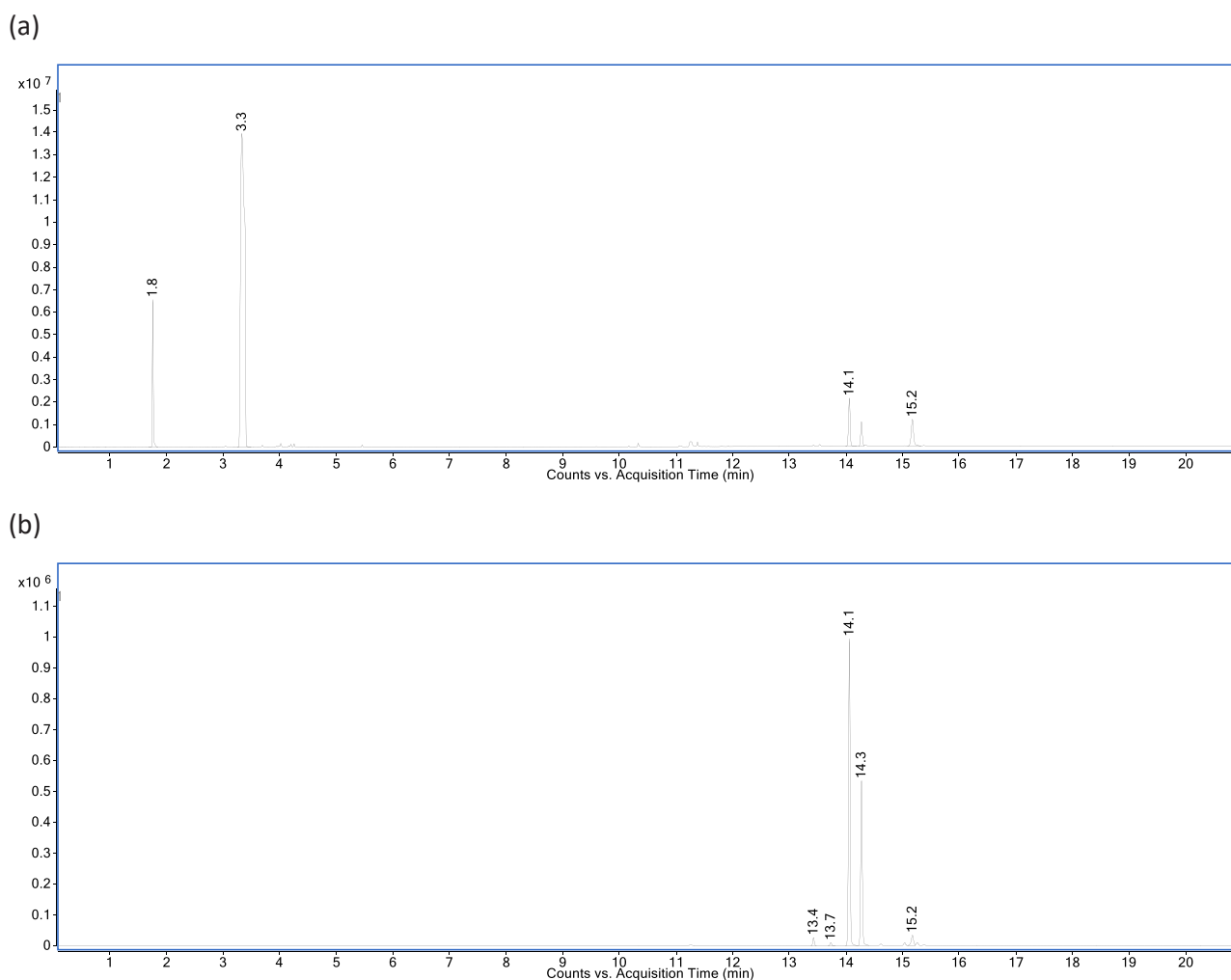


Fig. 1. An exemplary result of the GC-MS analysis for one of the OC sprays analyzed. (a) Total Ion Chromatogram (TIC), scan range 35–350 m/z . (b) Extracted Ion Chromatogram (EIC), for sum of ions characteristic for capsaicinoids ($m/z = 122, 137, 152, 195, 293, 305, 307, 319, 321$). Signals of the following capsaicinoids are visible on the EIC: 13.4 min: nordihydrocapsaicin; 13.7 min: nonivamide; 14.1 min: capsaicin, 14.3 min: dihydrocapsaicin.

homodihydrocapsaicin were not considered due to their small amount in the analyzed samples.

Eventually, the ratios of the three areas were logarithmized (base 10). This step aims to make the generated ratios variance independent of whether the original data is a divisor or a dividend. The logarithmic transform's key advantage is that the data ranges are significantly reduced, and the distribution becomes less deviated from normality, which makes them in line with the requirements of subsequent statistical/chemometric modeling.

2.3.1. Likelihood ratio

Likelihood ratio expressed as $LR=f(E|H_1)/f(E|H_2)$ is a well-documented and most adequate tool for determining how many times the hypothesis that two compared samples are of common origins (H_1) is more likely than the hypothesis assuming their separate sources (H_2). The question is not only about the similarity of the data (E) for compared samples, but also the probability of observing these data is relevant. This is because the similarity may be coincidental, especially when the matching features are widespread in a population, and it becomes highly likely that they will occur by chance in two compared samples, which actually have nothing in common. The strength of the evidence increases as the probability of observing the considered characteristics, assessed based on the population, decreases. Therefore, the conclusions are more persuasive if the similarity is observed between rare features than when detected

between typical features. LR values above unit threshold support H_1 , while those below one support H_2 . The further the LR value from one, the stronger the support for the corresponding hypothesis.

The mathematics governing LR calculations as well as the proper formulas for estimating LR are detailed elsewhere [12–14].

The LR models' performance is assessed by checking that a correct hypothesis is indicated and focusing on this indication's strength. The former assessment controls only if the LR is below or above one and reports false positive answers when $LR > 1$, but the samples are from two different sources (true- H_2), or false negative response when $LR < 1$ but the samples are of common origin (true- H_1). These metrics, however, do not exploit the potential of the LR as its magnitude provides a lot more valuable information about the strength of support for each of the hypotheses under consideration. It is expected to receive $LR \gg 1$ when H_1 correct and $LR \ll 1$ if H_2 correct, and LR near one if it points towards the incorrect hypothesis. Empirical cross entropy (ECE) [14–17] is then the most appropriate tool that provides a much more comprehensive description of the LR model performance.

In the ECE approach, a penalty is assigned to each LR value that increases as support for the wrong hypothesis magnifies. The resultant penalty for all LR values yielded in the considered LR model is used for estimating how well the LR model behaves in relation to the indecisive LR values equal to one. The ECE results are illustrated in the diagrams shown in Fig. 2. Typical ECE plots are constructed by

Table 1
OC sprays characteristics.

Spray No.	Name and information from the label	Expiration date	Shop No.	Form of preparation
Group 1. Distributed by: www.x-effects-shop.com (Producer unknown: no information on the can). 1 spray				
1	Pfeffer Spray Black Eagle fog zur Tierabwehr 40 ml	05/2019	6	aerosol
Group 2. Producer: F.W. Klever GmbH , Germany. 12 sprays				
2	KO Pieprz Strumień, 50 ml (1 of 2)	08/2021	2	jet
3	KO Pfeffer Fog, 50 ml	11/2021	4	aerosol
4	KO Pfeffer Fog 100 ml	08/2020	4	aerosol
5	KO Pieprz Stożek 50 ml (1 of 2)	no info	5	aerosol
6	KO Pieprz Stożek 40 ml (1 of 2)	08/2021	5	aerosol
7	Police Guard 100 ml	09/2021	5	aerosol
8	Anti-hund, 50 ml (1 of 2)	11/2021	5	aerosol
9	KO Pieprz Strumień 40 ml (1 of 2)	03/2022	6	jet
10	KO Pieprz Stożek 50 ml (2 of 2)	08/2021	6	aerosol
11	KO Pieprz Strumień 50 ml (2 of 2)	08/2021	6	jet
12	KO Pieprz Stożek 40 ml (2 of 2)	04/2021	6	aerosol
13	Anti-hund, 50 ml (2 of 2)	11/2021	6	aerosol
Group 2'. Producer: F.W. Klever GmbH , Germany. 11 sprays, purchased in the period from February 9, 2021, to February 16, 2021				
14	KO Pieprz Strumień 40 ml (1 of 2)	11/2021	7	jet
15	KO Pieprz Strumień, 50 ml (1 of 3)	09/2024	7	jet
16	Anti-hund, 50 ml (1 of 2)	08/2024	6	aerosol
17	KO Pieprz Strumień, 50 ml (2 of 3)	09/2024	6	jet
18	KO Pieprz Strumień, 50 ml (3 of 3)	11/2024	5	jet
19	Anti-hund, 50 ml (2 of 2)	08/2024	5	aerosol
20	KO Pieprz Strumień 40 ml (2 of 2)	11/2024	5	jet
21	KO Pfeffer Fog, 50 ml	08/2024	4	aerosol
22	KO Pfeffer Fog, 100 ml	11/2024	4	aerosol
23	KO Pfeffer Spray, 15 ml	03/2023	4	aerosol
24	KO Pfeffer Jet 50 ml	12/2024	4	jet
Group 3. Producer: Hoernecke Chemie , Germany. 4 sprays				
25	Original TW 1000 pepper-jet 63 ml	06/2019	4	jet
26	Original TW 1000 pepper-jet 40 ml	06/2019	4	jet
27	Original TW 1000 pepper-gel 50 ml	06/2019	4	gel
28	Anti-Dog Tierabwehr 63 ml	12/2021	7	aerosol
Group 4. Producer: KKS GmbH , Germany. 1 spray				
29	protect Pfeffer- Spray anti dog, 40 ml	31/12/2019	4	aerosol
Group 5. Producer: Mace Security International Inc. , USA. 2 sprays				
30	Mace brand hot pink pepper spray, 24 g	10/2022	7	aerosol
31	Mace brand pepper gel Magnum 3, 45 g	10/2022	7	gel
Group 6. Producer: Mace Personal Defence & Security Inc. , USA. 1 spray				
32	Mace pepper spray Pepper Foam Magnum 4, 113 g/3.99 oz	no info	7	foam
Group 7. Producer: Mace Tactical Solutions Inc. , USA. 1 spray				
33	Take Down Extreme, 40 g/1.4 oz	10/2022	7	aerosol
Group 8. Distributor: Militaria.pl , Poland. 5 sprays (Producer unknown: no information on the cans).				
34	Policyjny Gaz, 50 ml (1 of 2)	22/10/2021	1	aerosol
35	Anti Dog Gaz Obroenny, 50 ml	11/09/2021	1	aerosol
36	Gaz Obroenny, 50 ml	06/10/2020	1	aerosol
37	Policyjny Gaz, 50 ml (1 of 2)	13/09/2021	2	aerosol
38	Gaz Obroenny, 15 ml	03/10/2021	7	aerosol
Group 9. Producer: Security Equipment Corporation , USA. 17 sprays				
39	Sabre Red Crossfire Tierabwehrspray, 47.9 ml	05/2022	1	aerosol
40	Sabre Red Foam, NET 1.6 OZS (1 of 2)	05/2022	6	foam
41	Sabre Red Law Enforcement Unit Cone Delivery, NET 4.0 OZS.	02/2022	6	aerosol
42	Sabre Red Crossfire Technology, Stream, Net. 1.4 OZS.	11/2020	6	jet
43	Sabre Red Foam NET 1.8 OZ.	04/2021	6	foam
44	Frontiersman Bear Attack Deterrent, Net Contents: 7.9 OZ.	10/2020	6	aerosol
45	Sabre Red, Law Enforcement Strength, Model # M-120FT-OC, Net 110 Grams	02/2021	6	aerosol
46	Sabre Red Protector Dog Deterrent Pepper 1.8 OZ (1 of 2)	02/2021	6	aerosol
47	Sabre Red For Law Enforcement Only 16.0 OZS.	03/2023	6	aerosol
48	Phantom OC by Sabre For Law Enforcement & Corrections Only, 16.0 OZ	03/2023	6	aerosol
49	Sabre Red Tierabwehrspray, 52.2 ml	05/2022	7	aerosol
50	Sabre Red Tierabwehrspray Cyclist, 31.3 ml	05/2022	7	aerosol
51	Sabre Red Protector Dog Deterrent Pepper 1.8 OZ (2 of 2)	08/2022	7	aerosol
52	Sabre Red Tierabwehrspray Runner, 19.8 ml	05/2022	7	aerosol
53	Sabre Red Tierabwehrspray 22.2 ml	05/2022	7	aerosol
54	Sabre Red Foam 2.2 OZS	09/2023	7	foam
55	Sabre Red Foam, NET 1.6 OZS (2 of 2)	09/2023	7	foam
Group 10. Distributor: Sharg , Poland. 3 sprays. (Producer unknown. Information on the can - made in Germany).				
56	Red Pepper Gel Defence, 63 ml	12/2019	2	gel
57	Police RSG 2 million Scoville Heat Units Gel Foam, 63 ml	12/2014	3	foam
58	Defence NATO Red Pepper Gel 2000000	12/2022	7	gel
Group 11. Producer: UMAREX GmbH & Co.KG , Germany. 5 sprays				
59	Walther Military Gas 40 ml	07/2023	1	aerosol
60	Perfecta Pfefferspray Stop Attack, 40 ml (1 of 2)	03/2023	1	aerosol
61	Perfecta Pfefferspray Stop Attack, 40 ml (2 of 2)	03/2023	2	aerosol
62	Perfecta Pfefferspray, jet, Stop Attack Xtreme, 50 ml	12/2023	7	jet
63	Perfecta 110 Animal Stop 10% 15 ml	03/2021	7	aerosol

(continued on next page)

Table 1 (continued)

Spray No.	Name and information from the label	Expiration date	Shop No.	Form of preparation
Group 12. 64	Producer/distributor: UZI . The USA. 1 spray Pepper Shield Pepper Spray1/2 oz. net	no info	3	aerosol

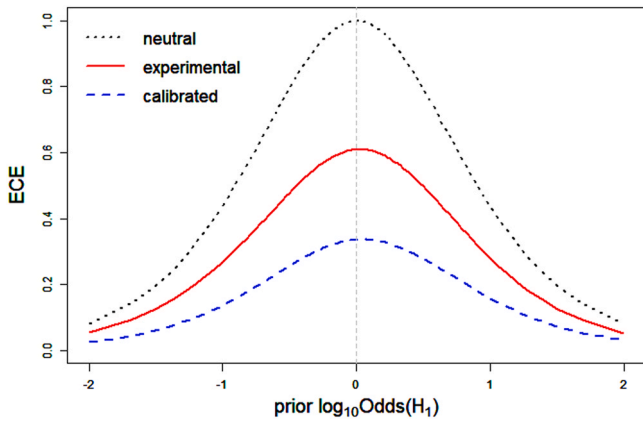


Fig. 2. Empirical cross entropy diagram (description in the text).

connecting neighboring points of ECE values, each computed for a set of LR values and specified prior odds (i.e., the log-ratio of beliefs about the evidence before it is examined). The red line shows the performance of the considered LR model in relation to the other two,

which designate the best (dashed blue line drawn for the calibrated LR values [14–17]) and neutral (dotted black line drawn for the model in which LR = 1) models. The LR model's performance is doomed satisfactory if the red line falls between the other two, particularly when it approaches the blue line. If we set the information loss as 100% for the model with LR = 1, then it can be concluded that the considered LR model performance is better than that and reduces this information loss. The performance, however, cannot be better than the performance observed for the calibrated LR values. If the red line happens to have higher values than the black line, the model introduces more confusing information than if indecisive LR = 1 was assumed and increases information loss.

2.3.1.1. LR models validation protocol. When constructing the LR model, it is assumed that each sample collected in the database constitutes an individual source. The LR models' performance was first controlled by stating whether the test samples share common origins or not according to the LR value of their comparison. The two test samples were drawn from the database of $m = 64$ sprays $m(m-1)/2 = 2016$ times. For each comparison, $m-2$ remaining samples were used for training the LR model (establishing all the relevant

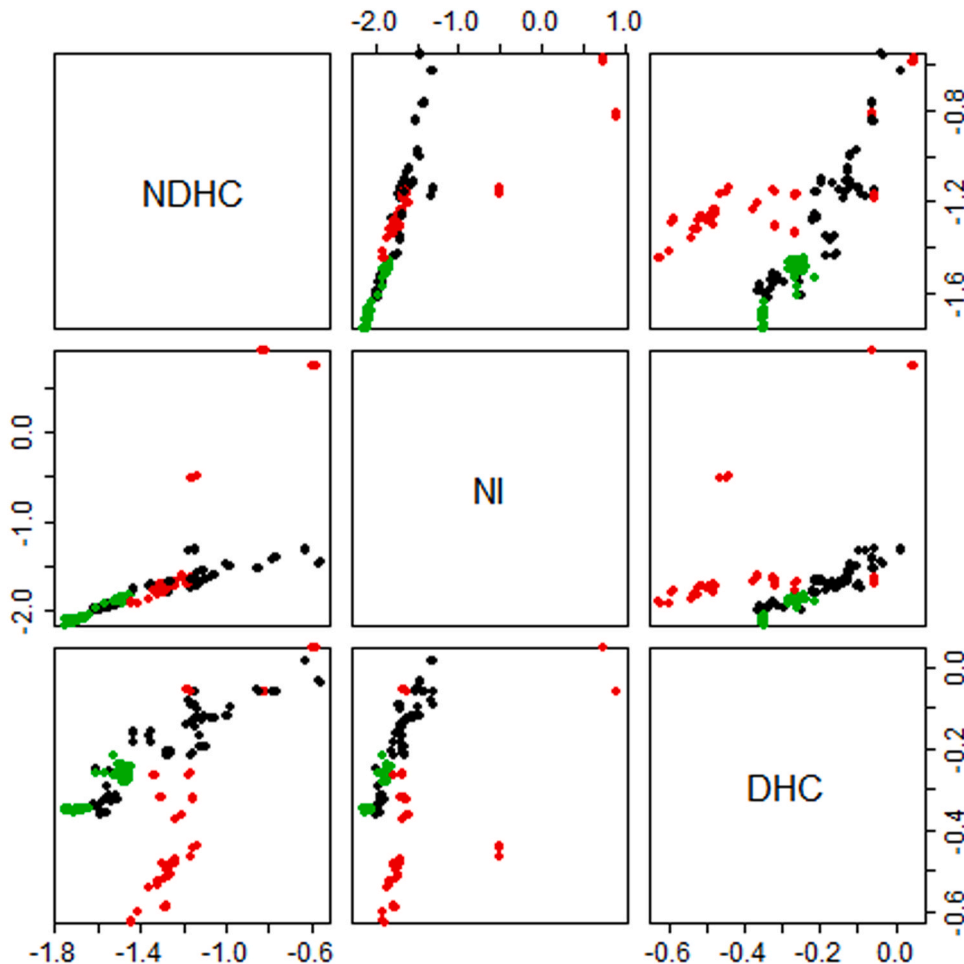


Fig. 3. Logarithms (base 10) of three studied capsaicinoids' normalized content (NDHC - nordihydrocapsaicin, NI - nonivamide, DHC - dihydrocapsaicin). Green and red colors refer to samples produced by F.W. Klever GmbH and Security Equipment Corporation, respectively. Black points correspond to the products of other producers.

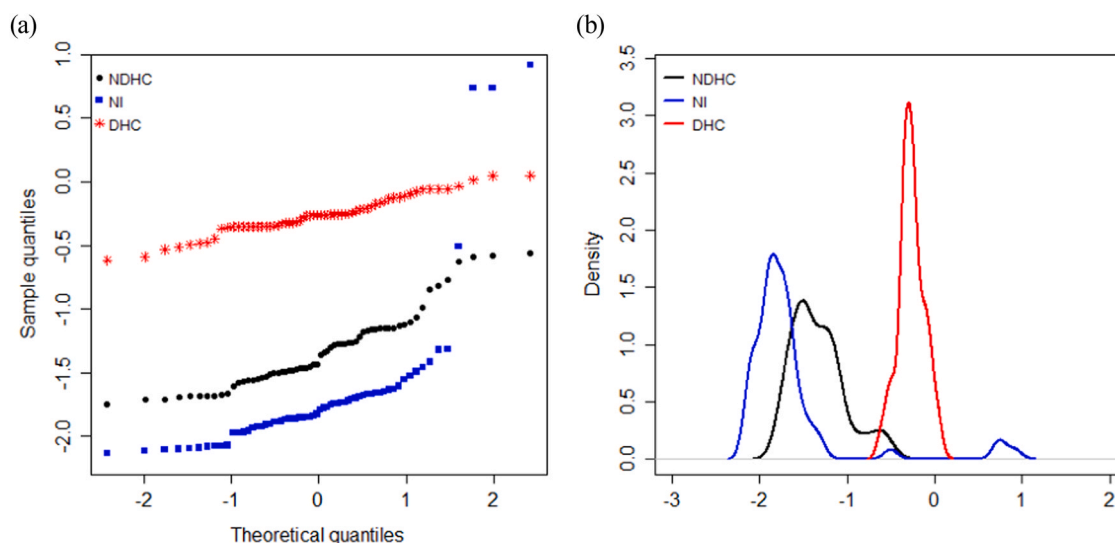


Fig. 4. Quantile-quantile plots and distributions of the three studied capsaicinoids (NDHC - nordihydrocapsaicin, NI - nonivamide, DHC - dihydrocapsaicin).

distributions [13,14]). LR values for comparing samples with different origins should be lower than one. Otherwise, it delivers support for the incorrect H_1 hypothesis and generates a false positive answer.

Pairs of test samples were also simulated to imitate samples of same-source origins. There were $m = 64$ such pairs generated by splitting three replicate measurements into two: one measurement and two measurements. These two new samples were compared using the LR model trained on the remaining $m-1$ samples. Then the anticipated LR values should exceed one. Values below one deliver support for the incorrect H_2 hypothesis and constitute a false negative answer. Since there were three replicates, three ways of splitting the sample into two new ones were possible. These three ways resulted in three different, but hopefully similar, LR values for each same-source comparison.

2.3.2. Software

All the calculations were performed in R software [18] using scripts written by the authors.

3. Results and discussion

3.1. Descriptive statistics

The graph in Fig. 3 clearly shows that the grouping of samples largely coincides with the two leading producers, i.e., F.W. Klever GmbH and Security Equipment Corporation (Table 1), likely due to the fact that their offer is most represented in our database. The number of samples for other manufacturers is far too low to draw credible conclusions. However, the clustering is only partial, and despite some tendency for separation, most of the samples mix up between producers. For instance, F.W. Klever GmbH sprays (green points in Fig. 3) demonstrate two very tiny but condensed groups, which despite their apparent inner similarity, also reveal a high resemblance to samples of other producers. It makes F.W. Klever GmbH sprays uncharacteristic in terms of the capsaicinoids content. We can also observe that four (two of which overlap) samples are isolated from the main group since they significantly differ in NI content from the remaining samples. If there is a similarity between any of these samples and the potential evidence samples, the conclusion about their common origins is much more reliable than if a similarity is found to any of the samples from the main group.

The diagram in Fig. 3 clearly points that the three measurements recorded for each of the samples reveal high similarity, as each sample forms a separate small cluster of three points. Thus the variation of the data within each sample is lower than the variation between the samples' means. These findings are a prerequisite for effective discrimination between samples. There is no clear visually-based indication towards any of the variables regarding their discrimination potential from the plots.

Fig. 4a compares the three studied variables' distributions to the normal distribution by plotting the theoretical vs. experimental samples quantiles. The distribution for DHC is most normal, while NI's distribution demonstrates the deviations from normality arising from much more varied content in the studied samples. Ambiguity in determining whether a distribution is normal or not as well as deviations from normality manifested in Fig. 4b, support the concept to model the distributions for the studied variables using the kernel density estimation instead of assuming normality.

3.2. Differentiation of the gases using LR

The average false positive and false negative rates are largely acceptable (Fig. 5). Despite high efficiency in differentiating between different-source samples (resulting in low levels of false positives), the models, fortunately, do not indicate the samples with common source as having separate origins. This is due to very low variation of the replicate measurements for each sample, and, as anticipated, it leads to remarkably lower false negative rates than the false positive rates. The correlation between the false response rate and the model complexity (number of variables included in the model) is noteworthy. The univariate models seem most misleading, and the most complex trivariate model, which incorporates all three variables, yields the lowest errors. Thus only occasional misassignments of the sources of the samples (with an exception for the gases produced by F.W. Klever GmbH) prove that LR models are capable of differentiating between OC gases produced by different manufacturers and also between gases produced by the same producers, but under some circumstances which are presumed below.

ECE diagrams shown in Fig. 6 correlate fairly well with the observations from Fig. 5 and confirm that the trivariate model not only supports the correct hypothesis about the (un)common samples origins, but also yields very low LR for different-source comparisons and very high LR for same-source comparisons. The support towards the incorrect hypothesis, if any, is then very weak. It additionally

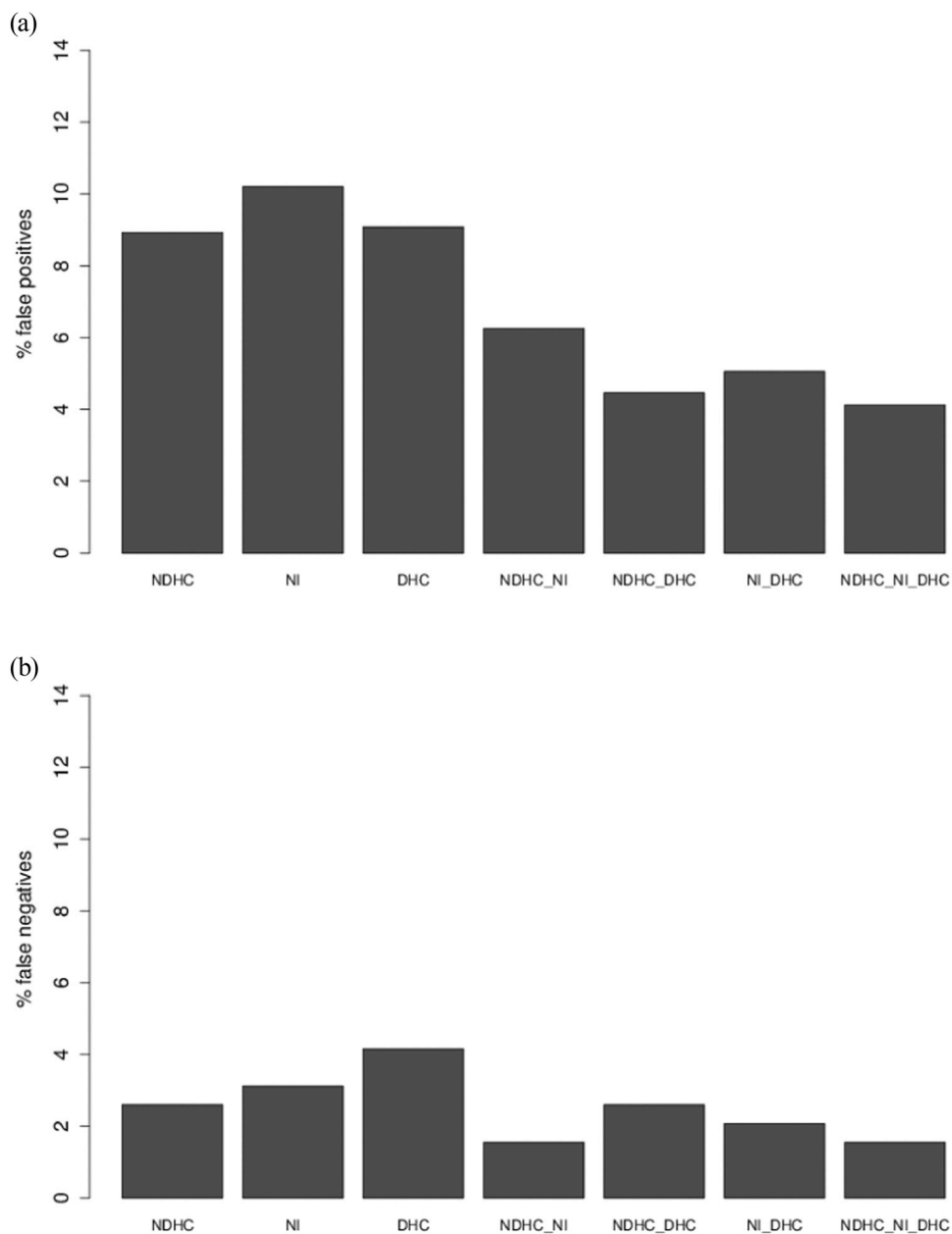


Fig. 5. (a) False positive and (b) false negative rates for all studied models (NDHC-nordihydrocapsaicin, NI-nonivamide, DHC-dihydrocapsaicin).

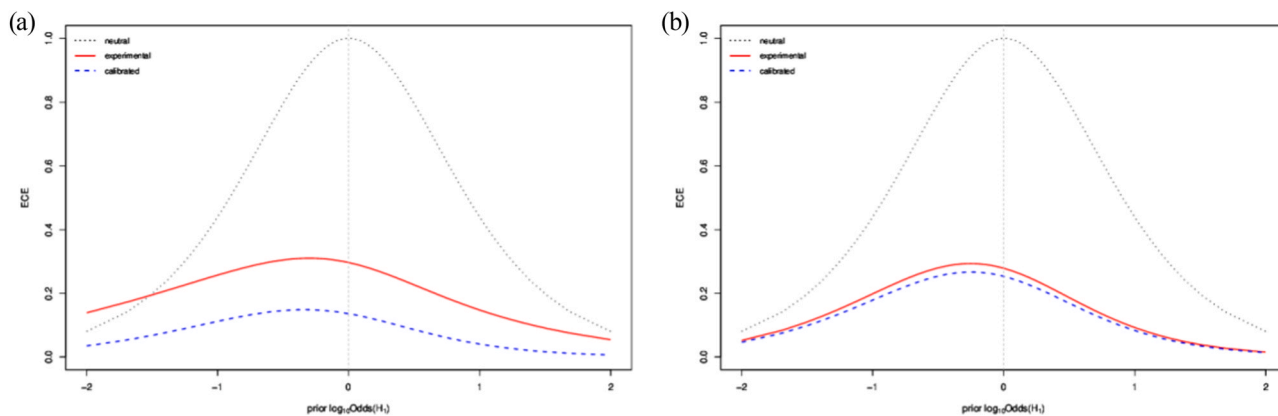


Fig. 6. Empirical cross entropy diagrams for (a) trivariate model (NDHC_NI_DHC) and (b) univariate model based on NI (NDHC-nordihydrocapsaicin, NI-nonivamide, DHC-dihydrocapsaicin).

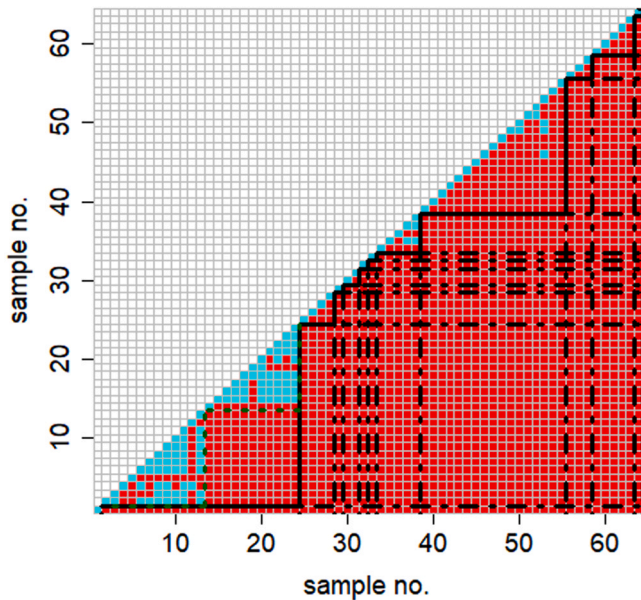


Fig. 7. Confusion table for trivariate model (NDHC_NI_DHC; NDHC-nordihydrocapsaicin, NI-nonivamide, DHC-dihydrocapsaicin). Blue boxes show LR values exceeding one, while red ones correspond to LR values below one. Bolded lines separate different producers in the order provided in Table 1. Green lines mark two groups of samples from F.W. Klever GmbH collected in two time periods. The numbering of samples is in accordance with the information given in Table 1.

reinforces the usefulness and reliability of this LR model in real cases. In other words, if we assume that no information gain is provided with $LR = 1$ (i.e., 100% of information loss), the trivariate model reduces the information loss (increases information gain) by at least 70%, practically regardless of the assumed prior odds. ECE diagrams for other models (e.g., Fig. 6b and Figs. SM1–6 in Supplementary Materials) offer vital evidence that the remaining models are not inferior in effectiveness to the most complex trivariate model. It leads us to the conclusion that the differences between the models' performance are rather negligible, and any of them may be utilized in practice.

Fig. 7 presents the results of pairwise comparisons between gases using the trivariate model in the form of a confusion table. Results of pairwise comparisons between gases marked with numbers 1–13 and 25–65 (Table 1), which were bought within the period November 13, 2018, to March 3, 2019, show that most of the gases are distinguishable both between groups for specific producers and within those groups. The only exceptions are the gases produced by F.W. Klever GmbH, constituting the second largest group (gases 2–13) which are mostly indistinguishable. Based on these results, we can readily conclude that OC gases may be easily distinguished in practice if produced by different manufacturers. Also, we hypothesize that the gases are distinguishable even if they were produced by the same company as long as they were produced from different batches of OC extracts.

We will deeply inspect this statement using an interesting trend, easily visible in the confusion table for the trivariate model in Fig. 7 for the products of F.W. Klever GmbH. The vast majority of the false positive responses in this group arise from significant similarity of the gases, very likely produced from the same batch of OC extract. One of the factors responsible for much lower variability within the group of gases produced by F.W. Klever GmbH compared to, e.g. Security Equipment Corporation, may be their price. Gases made by F.W. Klever GmbH are about three times cheaper, and therefore they are bought more often. The time between buying them by the shop from the wholesaler/producer and reselling them to the individual customers is relatively short. Therefore the probability that such

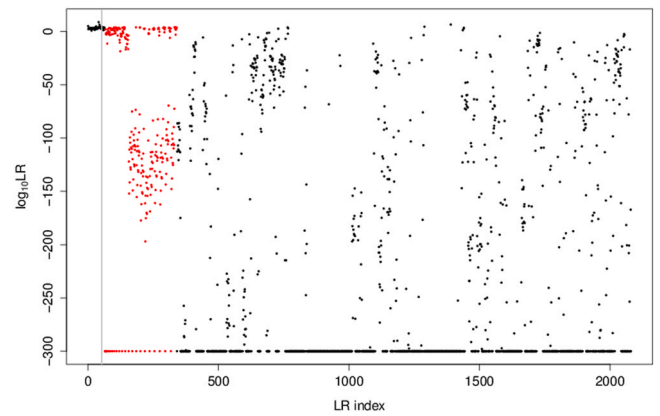


Fig. 8. $\log_{10}LR$ values received for same- and different-source comparisons using trivariate model (NDHC_NI_DHC; NDHC-nordihydrocapsaicin, NI-nonivamide, DHC-dihydrocapsaicin). Grayline separates 64 same-source LR values expected to be higher than (log higher than 0) one and 2016 different-source comparisons assumed to be lower than one (log lower than 0). Red points refer to the comparisons between different samples produced by F.W. Klever.

gases available in different shops in a specific period were produced from the same batch of OC extract is higher than such probability for more expensive gases. More expensive gases are bought rarely, therefore specimens available in various shops at a particular time most probably were produced from different OC extract batches.

To verify this hypothesis, two years later, from February 9, 2021, to February 16, 2021, 11 OC gases produced by F.W. Klever GmbH were bought in four different shops and analyzed (gases 14–24 in Table 1). These new gases proved to be, in most cases, indistinguishable from each other but distinguishable from previously bought items, also those produced by F.W. Klever GmbH (see Fig. 7 and Figs. SM1–6 in Supplementary Materials). This conclusion is easy to explain as both groups gather samples collected in two separate periods. Thus within the groups of samples collected in short periods, the samples are much more similar than between these periods since the gases are probably produced from the same batches of OC extracts. This observation supports our hypothesis about the influence of the price of gases on the presence of their various batches on the market, and thus on their differentiation. Fig. 8 demonstrates that the majority of the misleading LR values yielded for comparisons between F.W. Klever products (red dots) are ideally within the range of values for comparisons from the same source. Hence, F.W. Klever's samples, most likely from the same batches of OC extracts, exhibit the similarity on the level typical for the samples truly originating from the same source.

It is notable that gas no. 14 purchased in the second period from F.W. Klever GmbH is relatively easily differentiated from the other samples of this manufacturer bought within the same period. However, some similarities are observed in its DHC content to Klever gases purchased in the first period. Their close expiry dates (Table 1) suggest that gas no. 14 may have been produced from the same OC extract batch as gases purchased in the first period. These findings remain in agreement with our hypothesis, explaining the limitations in differentiating gases produced from the same batches of OC extracts.

Gas no. 14 also displays some similarity to gases distributed by Militaria.pl, when the LR models are based on NI and NDHC combined (see Fig. SM4 in Supplementary Materials). This bivariate model also demonstrates that there is one more gas distributed by Militaria.pl, which is indistinguishable from the F.W. Klever GmbH gases. It is possible that they have the same producer, but unfortunately, information about the producer is unavailable for the samples distributed by Militaria.pl.

It is worth emphasizing that the samples purchased in duplicates/triplicates (apart from F.W. Klever products) are usually easily distinguished and thus regarded as constituting different sources despite their high and foreseeable similarity. Univariate models (see Figs. SM1–3 in Supplementary Materials) occasionally happen to have more problems with differentiating the samples.

4. Conclusions

The conducted research showed that OC sprays may be distinguished based on differences in profiles of the four most abundant capsaicinoids from the OC extracts presumably if they were produced from different batches of OC extract. This is evidenced by the fact that out of 53 samples collected for testing in the first period, coming from 12 different manufacturers, only 12 gases produced by one, the most popular manufacturer, turned out to be indistinguishable. The result of an additional experiment supported the hypothesis that only OC sprays produced from the same batch of OC extract cannot be distinguished: 11 new gases from this manufacturer, purchased two years after buying the "old" ones, were analyzed and turned out to be indistinguishable, for most of the items, but distinguishable from the 12 gases purchased previously.

If the pepper sprays produced from different OC extract batches are distinguishable, then obtaining the same result (indistinguishable profiles of capsaicinoids) for samples coming from victim/crime scene and suspect, supports the hypothesis of the suspect's involvement in the event. This is because it is unlikely that two unrelated pepper spray gases (from the suspect and the actual perpetrator) accidentally come from the same production batch (the same company and production time).

It is impossible to calculate how strongly this hypothesis is supported because there is no way to get information, what percentage of people with OC gases own gases produced by a specific company, nor how often people having OC gases change them, i.e. they are buying new ones.

Direct comparison of the results obtained for sprays with the results obtained for the samples secured from the crime scene or the victim would be possible only if the storage of these samples and the subsequent solvent extraction have not significantly affected the profile of capsaicinoids. Therefore, how the different ways of packaging samples, their storage time, and extraction process influence the profile of capsaicinoids require further research.

CRedit authorship contribution statement

Rafał Borusiewicz: Conceptualization, Methodology, Resources, Investigation, Project administration, Writing – original draft preparation, Writing – reviewing & editing. **Agnieszka Martyna:** Software, Validation, Formal analysis, Data curation, Writing – original draft, Visualization, Writing – reviewing & editing. **Grzegorz Zadora:** Conceptualization, Writing – reviewing & editing; Supervision. **Anastasiia Zahrebelna:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2021.111031.

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