Sensors & Actuators: B. Chemical 308 (2020) 127697

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Piezoelectric peptide-hpDNA based electronic nose for the detection of terpenes; Evaluation of the aroma profile in different *Cannabis sativa L.* (hemp) samples

Sara Gaggiotti[a], Sara Palmieri[a], Flavio Della Pelle[a], Manuel Sergi[a], Angelo Cichelli[b], Marcello Mascini[a,*], Dario Compagnone[a,*]

[a] Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, 64100, Teramo, Italy
[b] Department of Medical, Oral and Biotechnological Sciences, "G. d'Annunzio" University Chieti-Pescara, Via dei Vestini 31, 66100 Chieti, Italy

ARTICLE INFO

Keywords:
Terpenes
Electronic nose
hpDNA
Peptides
SPME/GC–MS
Artificial neural network
Gas sensors
Hemp

ABSTRACT

A piezoelectric peptide-hpDNA based gas sensor array has been used for the detection of terpenes coming from *Cannabis sativa* samples. The array consisted in 11 sensors, 6 having pentapeptides and 5 having hairpin DNA as binding elements. The volatile composition of 28 *Cannabis sativa* samples, assessed by GC–MS analysis, allowed their classification into 2 groups having as monoterpenes and sesquiterpenes in different amounts. The response of the gas sensor array to the same samples demonstrated that both type of sensors are sensitive to the terpenes and contribute to classification. A satisfactory classification (79 % of correctly identified samples) was found using a PLS-DA approach. Using the same dataset and a simple ANN approach the headspace analytical profile of the two different groups was predicted with an average prediction error ≤1 %.

1. Introduction

Olfactory indicators in plants include volatile organic compounds (VOCs) that are mainly represented by the terpenoid fraction. Terpenes are an important class of plant constituents deriving from different combinations of C5 isoprene subunits. They are known to possess various medicinal and pharmacological properties [1]. The volatile and semi-volatile fractions of terpenoids can be divided into two different classes based on the number of carbon atoms in their structure, specifically monoterpenes (C10) and sesquiterpenes (C15). Larger terpenes exist as waxes and resins, as well as oxygenated terpenoids. Terpenoids are quite potent and affect animal and even human behavior when inhaled from ambient air [2].

Cannabis sativa L. (family Cannabaceae) has been widely used in the past for different purposes, such as the production of tissues or in the medical/pharmacological field since it is considered a valuable medicinal plant with a variety of therapeutic benefits [3–8]. A recent cannabis use survey revealed that 60 % of cannabis users rely on smelling the flower to select their cannabis [9]. Among the chemical constituents of *C. sativa* terpenes play a major role. The most prevalent monoterpenes found were: a) α-pinene and β-pinene which are characterized by pine fragrance and antiseptic effect; they are acetylcholinesterase inhibitor aiding memory and may counteract THC intoxication side effects; b) myrcene with a musky fragrance and anti-oxidant and anti-carcinogenic properties; c) limonene having a citrus fragrance and antifungal and anti-carcinogenic activity; d) linalool potentially effective for anxiety and convulsions with a floral fragrance [2,10,11]. The sesquiterpene β-caryophyllene, the most predominant sesquiterpene found in cannabis, has been reported to interact with cannabinoid receptors type 2, and be responsible for the anti-inflammatory effects of some cannabis preparations. Interestingly, caryophyllene oxide has been reported as the main component for cannabis identification by drug-sniffing dogs [12,13]. Casano and colleagues in 2010 [14] found that in *Cannabis* the relationship between monoterpenes and sesquiterpenes in leaves and inflorescences is different. The monoterpenes have higher volatility and dominate in the inflorescences to repel insects, the more bitter sesquiterpenes dominate in the leaves acting as anti-herbivores for grazing animals. Moreover, different content in terpenes pattern can be associated to different cultivars of Cannabis such as Futura 75, Antal, Carmagnola, and Kompolti [15–18].

Gas chromatography coupled with flame ionization (GC/FID) or mass spectrometric detector (GC/MS) are most frequently used approaches for the analysis of volatile terpenes are [14,19,20]. However, other techniques such as headspace-GC/FID, headspace-GC/MS, two-

* Corresponding authors.
E-mail addresses: mmascini@unite.it (M. Mascini), dcompagnone@unite.it (D. Compagnone).

https://doi.org/10.1016/j.snb.2020.127697
Received 26 November 2019; Received in revised form 7 January 2020; Accepted 8 January 2020
Available online 10 January 2020
0925-4005/

dimensional (GC × GC/qMS), solid phase headspace micro-extraction (HS-SPME) GC–MS and GC × GC/MS have been also used for analysis of the volatile fraction of cannabis and hashish samples [21,22]. Very few gas sensors have been reported in the literature for the detection of terpenes. Attempts to measure pure compounds were made using C-MOS [23] based sensors while QCMs coupled with molecularly imprinted polymers [24] were used to differentiate fresh and dried herbs.

In this work the terpenes fraction of different hemp samples purchased from 3 Italian regions were detected. A QCMs based electronic nose (e-nose) equipped with two different types of sensors was used for the purpose. A set of penta-peptides and hairpin DNA (hpDNA) with different loops were used to modify QCMs and used as binding elements. The peptides and hpDNA were selected via molecular modelling in previous works [25,26]; and were tested giving satisfactory results in the assay different food commodities from olive oil [27], chocolate [28], candies [29], fruit juices [30], carrots [31,32], and pasta [33].

The use of the mixed peptide-hpDNA array resulted in the discrimination of different cultivars of Cannabis sativa. The results, obtained on 28 cannabis samples showed that both peptides and hp-DNA possess, to different extent, the ability to interact with different terpenes, allowing the discrimination of the cultivars because of the different aromatic profiles.

2. Materials and methods

2.1. Standards reagents and samples preparation

The 28 hemp samples used in this study were bought in different shops from Italian regions (Emilia Romagna, Lazio, Lombardia) as whole flowers in small zip-lock bags and were stored at room temperature until use. Samples were ground using a Kenwood mixer chopper (De Longhi Appliances s.r.l., Treviso, Italy) and sifted with a 1 μm sieve. After grinding, they were stored in hermetically sealed plastic bags (see Fig. S1.).

2.2. SPME-GC–MS analysis

A Clarus 580 Gas Chromatography (GC) coupled to a Clarus SQ 8 Mass Spectrometer (MS) (PerkinElmer - Waltham, Massachusetts, USA) was used. 1 g of sample was inserted in a 20 ml-vial closed with crimp top caps and rubber septa. The samples were kept 20 min at 50 °C and then exposed to the fiber (Divinylbenzene/Carboxen/Polydimethylsiloxane, DVB/CAR/PDMS, 50/30 μm, Supelco, Bellefonte, PA) for 10 min at a fixed temperature (40 °C). The fiber was then inserted in the desorption chamber and GC analysis was carried out using the following temperature gradient: start at 50 °C (1 min), ramp 7 °C/min to 145 °C (hold 5 min), ramp 4 °C/min to 175 °C and ramp 7 °C/min to 250 °C (hold 5 min). Helium at flow rate of 1 mL/min was used as carrier; Split of the injector was set to 1:50 and injector transfer line temperature were at 250 °C. A fused silica Zebron- ZB-Semi-Volatile column (30 m × 250 μm × 0.25 μm – Phenomenex, Torrance, California, USA) was used according to [34]. The compounds were identified by matching the obtained spectra with the NIST Mass Spectral Library 2.0 (NIST - Gaithersburg, Maryland, USA) and confirmed by retention index (R_{Index}) as proposed in a previous works [17].

2.3. Gas sensors array procedure

A UTV E-nose developed by Sensors group, University of Rome Tor Vergata (Italy) equipped with 11 QCM (20 MHz) sensor array was used. QCMs were from KVG GmbH (Germany). Six QCMs were functionalized with different pentapeptides (IHRIC, KSDSC, LAWHC, LGFDC, TGKFC and WHVSC) that were purchased from Espikem (Italy, purity > 85 %). 5 QCMs were functionalized with different sequences of hpDNA (see Fig. S2.). The tetrameric loop hpDNA (CGGG) were from Thermo Fischer Scientifics (Italy), pentameric loops (TAAGT and CCCGA) and hexameric loops (CATCTG and ATAATC) from Integrated DNA Technologies (USA). The loops were extended with the same double helix stem of four base pair DNA (GAAG to 5′ end and CTTC to 3′ end). The peptides and hpDNA were functionalized respectively with zinc oxide nanoparticles (ZnONPs) and gold nanoparticles (AuNPs); the structures of the biomolecules and preparation of the sensors were reported in previous works [25,26]. Nitrogen (N_2) was used as gas carrier. The analysis of the samples was carried out using 1 g of dry hemp in glass lab bottles (100 mL) heated at 40 °C for 10 min. This time was selected as optimal to sample the VOCs of hemp samples headspace. The gas carrier enriched with all VOCs was directed to the E-nose chamber and measured for 5 min. The signal obtained was expressed in terms of Frequency shift (ΔF in Hz) that represented the maximum of interaction between sensors and VOCs.

2.4. Statistical analysis

Univariate analysis was performed using XLSTAT software (Addinsoft, USA). Experimental results were expressed as means ± standard deviations. Statistical significance was assessed using analysis of variance (ANOVA) with the Tukey HSD (honestly significant difference) multiple comparison analysis and Persons correlation test. The criterion for statistical significance of differences was p-value < 0.05 for all comparisons.

Multivariate statistical analysis was performed using three different approaches, principal component analysis (PCA), hierarchical cluster analysis (HCA) and partial least square discriminant analysis (PLS-DA) using MatLab R2011b (MathWorks, Natick, MA, USA) integrated with two toolboxes for MatLab obtained from Milano Chemometrics and Quantitative structure activity relationship (QSAR) Research Group [35,36]. PLS-DA was run on the dataset with a cross validation of the model using a 'venetian blinds' approach with number of cv groups equal to 3. The dataset of gas sensors array, or GC−MS were auto scaled (zero mean and unitary variance) before statistical procedures. Artificial Neural Network (ANN) was run using JustNN software (www.justnn.com).

3. Results and discussion

3.1. Hemp samples classification using SPME /GC–MS

28 different VOCs were identified in the headspace of the hemp samples. The relative amount of these compounds expressed as peak area vs. total area of the chromatogram is reported in Table S1 (supplementary material). High relative amount of the sesquiterpene β-caryophyllene and the monoterpene β-myrcene were found having respectively an average concentration of 25.7 % and 19.8 %. Only other three VOCs (D-limonene, α-pinene and humulene) were found above 5 %; the average amount of the other 23 VOCs was below 5 % with 9 VOCs below of 1 %.

The majority of the 28 VOCs detected were monoterpenes (19 in total); among these 5 had hydroxylic group, one had epoxide group, one with thiol-ketone group and three aromatics. The 9 sesquiterpenes included one alcohol, one epoxide and three aromatics. The structural and physicochemical properties of the 28 VOCs found are reported in Table S2.

The relative amount of the 28 VOCs found in the 28 hemp samples were analyzed with the unsupervised multivariate agglomerative hierarchical clustering (AHC) algorithm; Ward's method using as dissimilarity parameter the Euclidean distance was applied. As shown in Fig.1, the AHC algorithm classified the 28 hemp samples in two groups containing 12 (group A) and 16 (group B) samples. According to the ANOVA test (Tukey HSD multiple comparison analysis; P < 0.05) reported in Table 1, 13 VOCs were significant in classifying the 28 hemp samples in two groups.

High relative amounts of the sesquiterpenes β-caryophyllene,

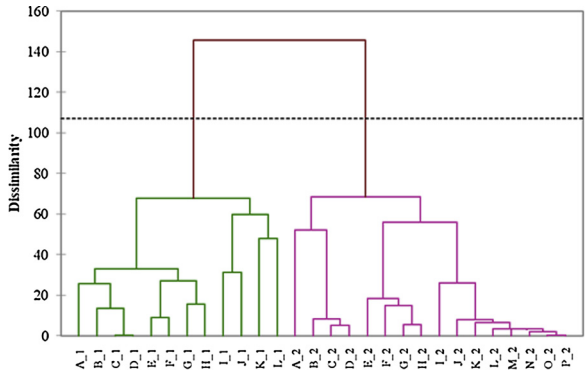

Fig. 1. AHC dendrogram of the 28 VOCs detected by SPME/GC–MS in the 28 hemp samples. The data were auto scaled (zero mean and unitary variance) before AHC in order to remove differences in the concentration range.

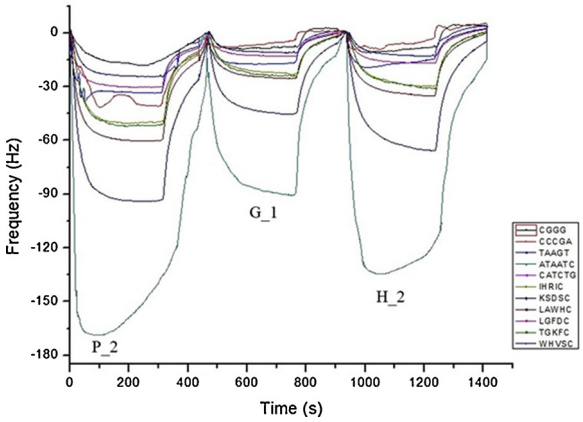

Fig. 2. Frequency shift recorded with hpDNA and peptides using 3 different hemp samples (P_2, G_1 and H_2).

humulene, α-selinene, caryophyllene oxide, α-bergamotene and β-selinene was crucial to classify group A samples; according to literature data these aromatic profiles are peculiar of some cultivars (Carmagnola CS, Antal, Finola, Futura 75, KC Zuzana). Group B had a higher concentration of the monoterpenes β-myrcene and D-limonene; the samples volatile profile can be associated to different cultivars (Fibrant, Tiborszallasi, Carmagnola, Ferimon, and Kompolti) [34,37,38].

3.1.1. HpDNA and peptides gas sensors array response vs hemp samples

Previous works have demonstrated that these set of sensors based on peptides functionalized with ZnONPs or hpDNA functionalized with AuNPs can be used for the analysis of VOCs (other than terpenes) present in food matrices [30–33]. In order to carry out analysis on different hemp samples it was necessary to find the optimal conditions for the gas sensor array analysis. Three different temperatures (25 °C; 40 °C and 50 °C) and four different amounts (0.10 g; 0.50 g; 1 g and 3 g) of samples were tested. From the data reported in Fig.S3 it is possible to observe that the optimal temperature was 40 °C. Increasing the temperature to 50 °C there was a significant drift of the signal, and in particular, the AuNP-hpDNA sensor with CATCTG as loop gave a very low response. The response at 25 °C was much lower indicating that the headspace was not yet saturated. The amount selected for the analysis was 1 g of sample, no significant increase of the frequency shift was observed using 3 g.

Fig. 2. reports a typical measurement of 3 hemp samples from both groups. Both hpDNA and peptides sensors gave similar kinetic behavior. The sensor having higher response was hpDNA with ATAATC loop followed by the peptide KSDSC, while the smallest signal was given by hpDNA with CGGG loop. A similar trend was observed also using pure terpenes (data not shown). The inter-day RSDs were in all case lower than 15 %. It is important to notice that the sensors were used for hundreds of measurements for 3 consecutive months and no significant drift of the signal was observed proving the robustness of the gas sensor

Table 1
Statistical significance of single VOCs detected in the 28 hemp samples to classify the hemp samples by using analysis of variance (ANOVA) with the Tukey HSD (honestly significant difference) multiple comparison analysis. The criterion for statistical significance of differences was $P < 0.05$ for all comparisons. The parameter F was used to sort in descending order the VOCs.

compound	R^2	F	Pr > F	Group A Mean	Group B Mean	Significant
β-caryophyllene	0.859	158.316	0.000	1.051 a	−0.788 b	Yes
humulene	0.564	33.671	0.000	0.852 a	−0.639 b	Yes
β-myrcene	0.536	30.085	0.000	−0.830 b	0.623 a	Yes
D-limonene	0.451	21.369	0.000	−0.762 b	0.571 a	Yes
α-selinene	0.440	20.445	0.000	0.752 a	−0.564 b	Yes
caryophyllene oxide	0.414	18.397	0.000	0.730 a	−0.547 b	Yes
α-bergamotene	0.321	12.292	0.002	0.642 a	−0.482 b	Yes
eucalyptol	0.309	11.652	0.002	0.631 a	−0.473 b	Yes
β-selinene	0.306	11.460	0.002	0.627 a	−0.470 b	Yes
p-Mentha-8-thiol-3-one	0.193	6.202	0.019	0.498 a	−0.373 b	Yes
L-borneol	0.189	6.057	0.021	0.493 a	−0.370 b	Yes
p-cymene-8-ol	0.184	5.867	0.023	0.487 a	−0.365 b	Yes
α-terpineol	0.184	5.858	0.023	0.486 a	−0.365 b	Yes
β-pinene	0.084	2.370	0.136	−0.328 a	0.246 a	No
α-pinene	0.069	1.923	0.177	−0.298 a	0.223 a	No
β-phellandrene	0.068	1.895	0.180	−0.296 a	0.222 a	No
γ-terpinene	0.048	1.320	0.261	−0.249 a	0.187 a	No
α-phellandrene	0.047	1.287	0.267	0.246 a	−0.185 a	No
β-bisabolol	0.042	1.140	0.296	0.232 a	−0.174 a	No
3-carene	0.029	0.768	0.389	−0.192 a	0.144 a	No
linalool	0.024	0.640	0.431	−0.176 a	0.132 a	No
β-Farnesene	0.019	0.512	0.481	0.158 a	−0.118 a	No
o-cymene	0.005	0.143	0.708	0.084 a	−0.063 a	No
camphene	0.005	0.124	0.727	−0.078 a	0.059 a	No
p-Cymene	0.001	0.017	0.897	−0.029 a	0.022 a	No
β-guaiene	0.000	0.008	0.931	−0.020 a	0.015 a	No
fenchol	0.000	0.008	0.931	0.019 a	−0.015 a	No
terpinolene	0.000	0.001	0.980	0.006 a	−0.004 a	No

array. The whole lot of samples were then analyzed to test the discrimination ability of the sensor arrays. The ΔF (Hz) response of hpDNA and peptide modified sensors array for the 28 hemp samples are reported in Table S3. This dataset was used to analyze the correlation among gas sensors array and GC−MS response by computing the Pearson coefficients. The correlation matrix between the 28 VOCs and the 11 sensors is reported in Table S4. The correlation coefficients, calculated using the response to the 28 hemp samples of both sensors array and GC−MS, evaluate the degree of linear correlation among variables. The hpDNA had higher correlation than peptides toward the VOCs significant to classify the hemp samples. The hpDNA loops CCCGA and TAAGT were significantly anticorrelated with β-caryophyllene, L-borneol and α-terpineol. A positive correlation was observed for hpDNA loop ATAATC and p-Mentha-8-thiol-3-one and for the loop CATCTG with both L-borneol and α-terpineol. These two alcohols correlated also with the peptide KSDSC that was the only peptide showing a significant correlation with the VOCs significant to classify the hemp samples. All sensors correlated with β-pinene, α-pinene and camphene. It should be highlighted that peptides binds the VOCs particularly via electrostatic interactions (hydrogen bond, van der Waals forces) and the 28 terpenes found in the hemp headspace differs each other in structural conformation but not in electrostatic molecular surface. On the other hand, hpDNA showed more variability in terms of correlation towards the VOCs tested, unlike the results obtained in previous work [39] where this correlation was greater in peptides than in hpDNAs. These results indicate that hpDNA and peptides have different interaction with volatiles confirming that a mixed set of hpDNA and peptides can provide a synergistic response in the detection of VOCs in real samples. The sensors dataset was, then, processed by PCA to find every possible combination between the aromatic compounds present in the samples analyzed and the 11 sensors used. PCA algorithm was carried out after rows normalization of ΔF signals and then autoscaling (zero mean and unitary variance) in order to remove differences in concentration range. Fig.3 reports the score (A) and loading (B) plots. The loadings represents the contribution of each sensor to the principal components. As shown in Fig.3B, the PC 1 axis highlighted the differences between hpDNA and peptides. Peptides had very similar pattern recognition performance contributing only in separating hemp samples on PC 1. All hpDNA loops played an important role in separation of the hemp samples. The sequence CGGG didn't give a significant contribution to separate the groups, while TAAGT and CCCGA along the PC2 contributes to the discrimination for the A group. Finally, the ATAATC along the PC3 seems to strongly contribute to the separation of group A as well as the CATCTG along PC1 to group B The score plot in Fig. 3A exhibited, as expected using an explorative analysis as PCA only a partial discrimination of the 2 groups.

A supervised multivariate discriminant analysis was then applied.

Table 2
PLS-DA confusion matrices using all the 5 hp DNA and 6 peptides sensors. True groups are read along the columns and estimated groups along the % correct. The total accuracy was also reported.

6 pentapeptides				
real/pred	group 1	group 2	Total	% correct
group 1	8	4	12	67%
group 2	3	13	16	81 %
Total			28	74 %
5 hpDNA				
real/pred	**group 1**	**group 2**	**Total**	**% correct**
group 1	9	3	12	75 %
group 2	3	13	16	81 %
Total			28	78 %
	5 hpDNA and the 6 pentapeptides			
real/pred	**group 1**	**group 2**	**Total**	**% correct**
group 1	10	2	12	83%
group 2	4	12	16	75 %
Total			28	79 %

Data are reported in Table S3. A numerical evaluation of the classification properties was obtained using the specificity, sensitivity and precision of the two groups along with the real-predicted samples reported using the confusion matrix format. The statistical summary results of the PLS-DA algorithm is reported in Table 2. The results showed partial discrimination between the two groups with a sensitivity that contributed to significant classification error. The percentage of correctness using all sensors or only hpDNA and peptides was satisfactory but not enough to correctly classify the whole set of samples. Considering all the sensors together the correct classification was achieved in 79 % of the samples; this was higher than using only peptides (74 %) or hpDNA (78 %).

A more complex statistical approach to implement class recognition was then attempted. To this aim, an artificial neural network (ANN) was developed (Fig. 4). The ANN was carried out using as inputs the frequency shift of the 11 sensors and as outputs, the two hemp groups. The network structure was composed of one hidden layer, where the information is automatically processed in a blind manner, connecting nodes as in Fig. 4A. The network had a growth rate of 10 cycles or 5 s, 1 hidden layer, and a learning rate of 0.7. The target error was fixed at < 0.01, one hundred cycles before the validating cycle and 100 cycles per validating cycle were used, and the learning process was stopped when all the validating examples were within the 10 % as validating error. The system has been trained, through the back-propagation error algorithm [40]. The dataset obtained from 28 hemp samples was used as training examples, and the output value was generated after only 506 learning cycles, with a progressive end of the

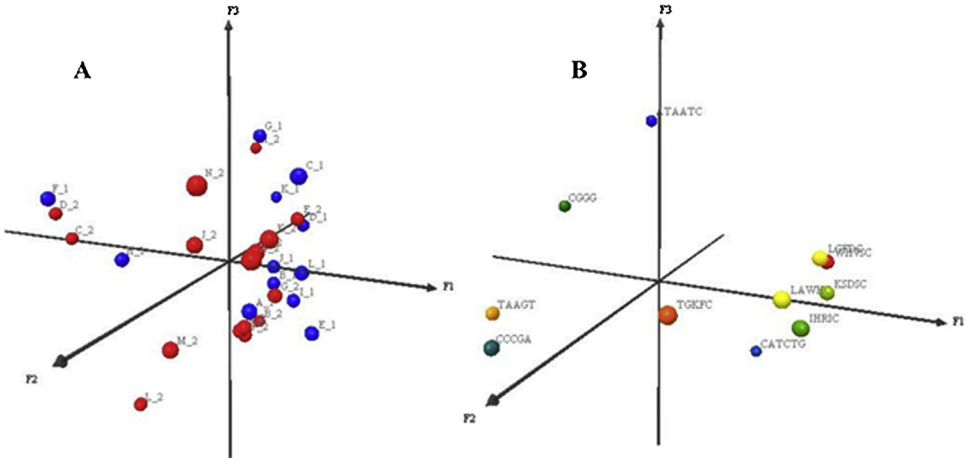

Fig. 3. Scores plot (A) and loadings plot (B) obtained from the PCA on the 28 hemp samples and the 5 hpDNA and the 6 pentapeptides sensors after rows normalization of ΔF signals. Plots of the first three components (explained variance: PC1 = 56.0 %; PC2 = 25.4 %; PC3 = 9.7 %; total = 91.1 %). Data have been auto scaled (zero mean and unitary variance) before PCA. In score plot (A) the two groups of hemp found using the relative concentration in VOCs detected by SPME/GC−MS analysis was highlighted with different colors (blue for group 1 and red for group 2).

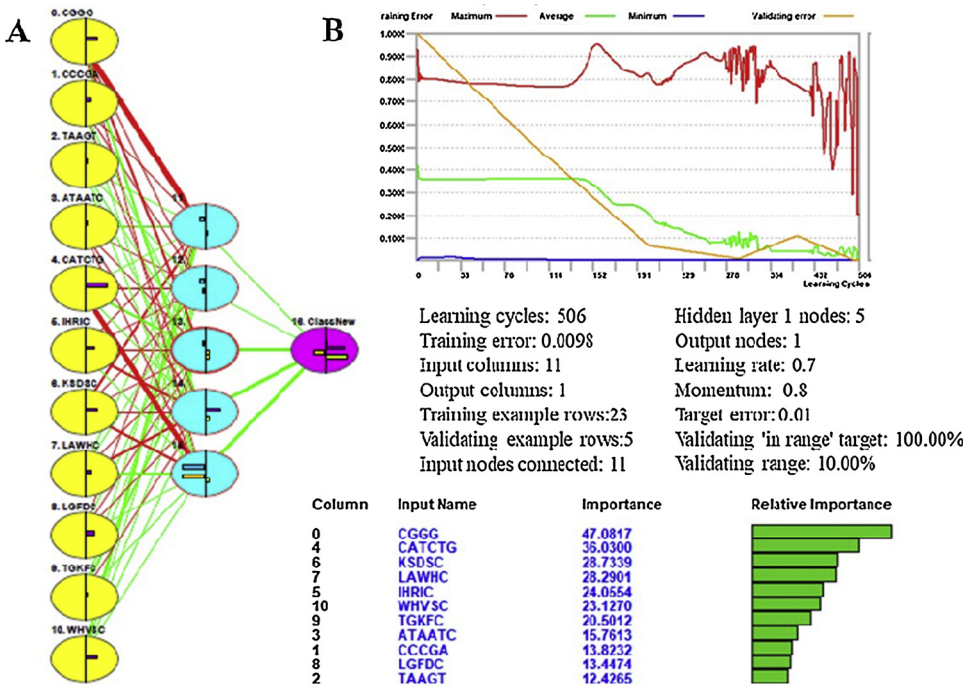

Fig. 4. Artificial Neural Network (ANN). (A) Diagram showing the structure of the ANN made using the sensors array response. Connections with a positive weight in the network are showed by the green lines, the red lines are of negative weights, and the dashed lines indicate insignificant one. The thickness of the lines is directly proportional to the weights of the edges in the network architecture. (B) ANN learning process after the introduction of response of the sensors (inputs) and the head space analytical profile groups (outputs). Learning cycles parameters were reported along with the relative importance for each sensor.

learning process, 0.0000, 0.2047, and 0.0098 (Fig. 4B). As a quality control, validating cycles were performed, in which five randomly selected datasets of the ANN were used as examples to test, at the end of the validating procedure, the error value. The network successfully completed the validating step, as indicated by the decline of the validating error which drops down below 10 % (Orange line in Fig. 4B). The relative importance of each input could be estimated by considering the weights automatically attributed to each of them by the system. Noteworthy, the ANN designed in this study successfully provided a tool capable of predicting the headspace analytical profile of the two different groups of hemp with a very high predictivity (average prediction error ≤ 1 %).

4. Conclusions

This study demonstrate, for the first time, that an electronic nose consisting of pentapeptides and hpDNA can be used for the detection of aroma patterns rich in terpenes. These molecules are present in plants, are of utmost importance in different fields and have been rarely detected using gas sensors. The gas sensor array reported in here can represent a valid tool for the traceability, rapid quality and process control for plants or plant derived extracts or products containing high amount of terpenes.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.snb.2020.127697.

References

[1] R. Brenneisen, Chemistry and Analysis of Phytocannabinoids and Other Cannabis constituents, Marijuana and the Cannabinoids, Springer, 2007, pp. 17–49.
[2] E.B. Russo, T. THC, potential cannabis synergy and phytocannabinoid-terpenoid entourage effects, Br. J. Pharmacol. 163 (2011) 1344–1364.
[3] P.F. Whiting, R.F. Wolff, S. Deshpande, M. Di Nisio, S. Duffy, A.V. Hernandez, et al., Cannabinoids for medical use: a systematic review and meta-analysis, JAMA 313 (2015) 2456–2473.
[4] D.R. Blake, P. Robson, M. Ho, R.W. Jubb, C.S. McCabe, Preliminary assessment of the efficacy, tolerability and safety of a cannabis-based medicine (Sativex) in the treatment of pain caused by rheumatoid arthritis, Rheumatology 45 (2006) 50–52.
[5] M.P. Davis, Cannabinoids for symptom management and cancer therapy: the evidence, J. Natl. Compr. Cancer Netw. 14 (2016) 915–922.
[6] M.E. Gerich, R.W. Isfort, B. Brimhall, C.A. Siegel, Medical marijuana for digestive disorders: high time to prescribe? Am. J. Gastroenterol. 110 (2015) 208.
[7] L. Amato, S. Minozzi, Z. Mitrova, E. Parmelli, R. Saulle, F. Cruciani, et al., Systematic review of safeness and therapeutic efficacy of cannabis in patients with multiple sclerosis, neuropathic pain, and in oncological patients treated with chemotherapy, Epidemiol. Prev. 41 (2017) 279–293.
[8] T. Rudroff, J.M. Honce, Cannabis and multiple sclerosis—the way forward, Front. Neurol. 8 (2017) 299.
[9] S. Rice, J.A. Koziel, Characterizing the smell of marijuana by odor impact of volatile compounds: an application of simultaneous chemical and sensory analysis, PLoS One 10 (2015) e0144160.
[10] J. Gertsch, M. Leonti, S. Raduner, I. Racz, J.-Z. Chen, X.-Q. Xie, et al., Beta-caryophyllene is a dietary cannabinoid, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 9099–9104.
[11] P.G. Fine, M.J. Rosenfeld, The endocannabinoid system, cannabinoids, and pain, Rambam Maimonides Med. J. 4 (2013) e0022.
[12] A.-L. Klauke, I. Racz, B. Pradier, A. Markert, A. Zimmer, J. Gertsch, et al., The cannabinoid CB2 receptor-selective phytocannabinoid beta-caryophyllene exerts analgesic effects in mouse models of inflammatory and neuropathic pain, Eur. Neuropsychopharmacol. 24 (2014) 608–620.
[13] S. Elzinga, J. Fischedick, R. Podkolinski, J. Raber, Cannabinoids and terpenes as chemotaxonomic markers in cannabis, Nat. Prod. Chem. Res. 3 (2015) 1000181.
[14] S. Casano, G. Grassi, V. Martini, M. Michelozzi, Variations in Terpene Profiles of Different Strains of Cannabis sativa L, XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): A New Look at Medicinal and 9252010, pp. 115-21.
[15] M.W. Giese, M.A. Lewis, L. Giese, A reliable and robust method for the analysis of cannabinoids and terpenes in cannabis, Google Patents 2018.
[16] S. Arnoldi, G. Roda, E. Casagni, L. Acqua, M. Dei Cas, F. Fare, et al., Characterization of the volatile components of cannabis preparations by solid-phase microextraction coupled to headspace-gas chromatography with mass detector (SPME-HSGC/MS), J. Chromatogr. Sep. Tech. 8 (2017) 1–6.
[17] G. Zengin, L. Menghini, A. Di Sotto, R. Mancinelli, F. Sisto, S. Carradori, et al., Chromatographic analyses, in vitro biological activities, and cytotoxicity of cannabis sativa l. Essential oil: a multidisciplinary study, Molecules 23 (2018) 3266.
[18] L. Nissen, A. Zatta, I. Stefanini, S. Grandi, B. Sgorbati, B. Biavati, et al., Characterization and antimicrobial activity of essential oils of industrial hemp varieties (Cannabis sativa L.), Fitoterapia 81 (2010) 413–419.
[19] M.W. Giese, M.A. Lewis, L. Giese, K.M. Smith, Method for the analysis of cannabinoids and terpenes in Cannabis, J. AOAC Int. 98 (2015) 1503–1522.
[20] J.T. Fischedick, A. Hazekamp, T. Erkelens, Y.H. Choi, R. Verpoorte, Metabolic fingerprinting of Cannabis sativa L., cannabinoids and terpenoids for chemotaxonomic and drug standardization purposes, Phytochemistry 71 (2010) 2058–2073.
[21] C. Da Porto, D. Decorti, A. Natolino, Separation of aroma compounds from industrial hemp inflorescences (Cannabis sativa L.) by supercritical CO2 extraction and on-line fractionation, Ind. Crops Prod. 58 (2014) 99–103.
[22] J. Omar, M. Olivares, J.M. Amigo, N. Etxebarria, Resolution of co-eluting compounds of Cannabis sativa in comprehensive two-dimensional gas chromatography/mass spectrometry detection with multivariate curve resolution-alternating least squares, Talanta 121 (2014) 273–280.

[23] S.P. Lee, Terpene sensor array with bridge-type resistors by CMOS technology, IOP Conference Series: Materials Science and Engineering (2015) 012065 IOP Publishing.
[24] N. Iqbal, G. Mustafa, A. Rehman, A. Biedermann, B. Najafi, P.A. Lieberzeit, et al., QCM-arrays for sensing terpenes in fresh and dried herbs via bio-mimetic MIP layers, Sensors 10 (2010) 6361–6376.
[25] M. Mascini, S. Gaggiotti, F. Della Pelle, J. Wang, J.M. Pingarrón, D. Compagnone, Hairpin DNA-AuNPs as molecular binding elements for the detection of volatile organic compounds, Biosens. Bioelectron. 123 (2019) 124–130.
[26] M. Mascini, D. Pizzoni, G. Perez, E. Chiarappa, C. Di Natale, P. Pittia, et al., Tailoring gas sensor arrays via the design of short peptides sequences as binding elements, Biosens. Bioelectron. 93 (2017) 161–169.
[27] M. Del Carlo, G. Fusella, A. Pepe, M. Sergi, M. Di Martino, M. Mascini, et al., Novel oligopeptides based e-nose for food quality control: application to extra-virgin olive samples, Qual. Assur. Saf. Crop. Foods 6 (2014) 309–317.
[28] D. Compagnone, M. Faieta, D. Pizzoni, C. Di Natale, R. Paolesse, T. Van Caelenberg, et al., Quartz crystal microbalance gas sensor arrays for the quality control of chocolate, Sens. Actuators B Chem. 207 (2015) 1114–1120.
[29] D. Pizzoni, D. Compagnone, C. Di Natale, N. D'Alessandro, P. Pittia, Evaluation of aroma release of gummy candies added with strawberry flavours by gas-chromatography/mass-spectrometry and gas sensors arrays, J. Food Eng. 167 (2015) 77–86.
[30] M. Mascini, S. Gaggiotti, F. Della Pelle, C. Di Natale, S. Qakala, E. Iwuoha, et al., Peptide modified ZnO nanoparticles as gas sensors array for volatile organic compounds (VOCs), Front. Chem. 6 (2018) 105.
[31] S. Gaggiotti, F. Della Pelle, V. Masciulli, C. Di Natale, D. Compagnone, Monitoring Shelf Life of Carrots With a Peptides Based Electronic Nose, Convegno Nazionale Sensori, Springer, 2018, pp. 69–74.
[32] S. Gaggiotti, M. Mascini, P. Pittia, F. Della Pelle, D. Compagnone, Headspace volatile evaluation of carrot samples—comparison of GC/MS and AuNPs-hpDNA-Based E-Nose, Foods 8 (2019) 293.
[33] S. Gaggiotti, B. Shkembi, G. Sacchetti, D. Compagnone, Study on volatile markers of pasta quality using GC-MS and a peptide based gas sensor array, LWT 114 (2019) 108364.
[34] E.A. Ibrahim, M. Wang, M.M. Radwan, A.S. Wanas, C.G. Majumdar, B. Avula, et al., Analysis of terpenes in Cannabis sativa L. Using GC/MS: method development, validation, and application, Planta Med. 85 (2019) 431–438.
[35] D. Ballabio, A MATLAB toolbox for principal component analysis and unsupervised exploration of data structure, Chemom. Intell. Lab. Syst. 149 (2015) 1–9.
[36] D. Ballabio, V. Consonni, Classification tools in chemistry. Part 1: linear models. PLS-DA, Anal Methods 5 (2013) 3790–3798.
[37] F. Pellati, V. Brighenti, J. Sperlea, L. Marchetti, D. Bertelli, S. Benvenuti, New methods for the comprehensive analysis of bioactive compounds in Cannabis sativa L.(hemp), Molecules 23 (2018) 2639.
[38] R. Iseppi, V. Brighenti, M. Licata, A. Lambertini, C. Sabia, P. Messi, et al., Chemical characterization and evaluation of the antibacterial activity of essential oils from fibre-type Cannabis sativa L.(Hemp), Molecules 24 (2019) 2302.
[39] S. Gaggiotti, C. Hurot, J.S. Weerakkody, R. Mathey, A. Buhot, M. Mascini, et al., Development of an optoelectronic nose based on surface plasmon resonance imaging with peptide and hairpin DNA for sensing volatile organic compounds, Sens. Actuators B Chem. (2019) 127188.
[40] D.E. Rumelhart, G.E. Hinton, R.J. Williams, Learning representations by back-propagating errors, Cognit. Model. 5 (1988) 1.

Sara Gaggiotti attempted both bachelor and master's degree in Food Science & Technology at the University of Teramo. Nowadays, she is a last-year Ph.D. student in Food Sciences at the University of Teramo, and she is the author of 4 in international journals. Her research field is focused on the development of rapid diagnostic tools for food quality and safety control.

Sara Palmieri has completed the five-year degree in Pharmacy at the University G. D'Annunzio Chieti-Pescara in 2016. Today, she is finishing the last year of PhD in Food Science at the Faculty of Biosciences at the University of Teramo. Her doctoral project is based on the extraction and characterization of Bioactive Compounds present in plants and evaluating their application in the field of Bioscience. She is the author of a work in an international journal.

Flavio Della Pelle is currently a researcher at the University of Teramo (Italy). Flavio Della Pelle holds the PhD in Food science and analytical chemistry at the University of Teramo and Alcalá de Henares (Madrid, Spain), respectively. He is author of over 35 scientific publications in the analytical chemistry field. The Flavio Della Pelle principal research line is focused on the development of robust and simple alternative analytical methodologies and devices based on nano and micro-structured materials, for the 'assessment of the food quality and safety'

Manuel Sergi is responsible for MS-Lab in University of Teramo and manages different projects financed both by public or institutional bodies and private companies. His scientific activity is focused on development and validation of analytical methods based on LC–MS for both food and forensic purposes. The study of analytical performances of MS based techniques also compared with screening methods is one of the focuses of research activity; the application on real cases facing the issues arising from the different matrices represented also a key task. Author of 78 papers on international peer-reviewed journals with a H index = 21 with over 1300 citations (source Scopus, September 2019)

Angelo Cichelli is full professor of Food Science Technology, coordinator of Food Sciences and Health course at Univ. D'Annunzio (CH-PE, Italy). Director (from 2004) of G. D'Annunzio School of Advanced Studies (PhD programs and technological transfer). He is Italian expert (contaminants) of the International Organization of Vine and Wine (O.I.V.-Paris). His fields of interest are mainly concentrated in the chemistry and technology of different foods (olive oil, wine) and has published over 160 publications.

Marcello Mascini is assistant professor in analytical chemistry. His research area was focused on the development of screening methods for fast and real time detection of analytes important for food, environmental and health analysis. The research interests were with a particular focus on new methods to develop bio-inspired and bio-mimetic systems in analytical application using molecular modeling and multivariate analysis.

Dario Compagnone has coordinated in the last 10 years the analytical chemistry group of the Faculty of Biosciences of the University of Teramo. He is author of over 170 papers on international scientific journals. His research interests are focussed on the development of sensing and biosensing strategies for the rapid detection of quality and safety markers of food.