

# Determination of volatile characteristics of Greek and Turkish pine honey samples and their classification by using Kohonen self organising maps <sup>☆</sup>

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## Abstract

Forty-four samples of pine honey were analysed, 22 from Greece and 22 from Turkey. A purge & trap–gas chromatograph–mass spectrometer system was used for the extraction, separation and identification of volatile and semi-volatile compounds. In all, 77 compounds were extracted and identified. Two of them, the 3-carene, and one unidentified compound ( $m/z$  55, 79, 91, 107, 123, 165) were found to be specific to Turkish honeys and may be considered as markers characterising the Turkish origin of samples. Beyond this result, and by using the Kohonen self-organizing map (KSOM), a clear differentiation was obtained between the Turkish and Greek pine honey samples from a global consideration of all the analysed volatile constituents.

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**Keywords:** Purge & trap; GC–MS; Pine honey; KSOM

## 1. Introduction

Pine honey is produced from honeydew secreted by the insect *Marchalina hellenica* (Gennadius) which is restricted to *Pinus brutia* Ten and *Pinus halepensis* Miller (Bodenheimer, 1953; Nikolopoulos, 1965; Kailidis, 1965). This type of honey is produced only in Greece and Turkey. The annual Greek honey production is estimated to be 12,000 tons, 60–65% of which is honeydew honey from pine trees.

Until the year 1983 there had been a local prejudice against honey from pine trees, arising mainly from the Greek regulations and official perception that pine honey is inferior to delicately-flavoured blossom honey (Greek Circular for food and drinks 10/1971). Honeydew honey was regarded as insect excrement by consumers. Beekeepers made no effort to market the quality of pine honey, nor to inform their customers about the real value of this honeydew honey. Most of them sold pine honey as a mix-

ture with blossom honey or as “honey” without any indication of its honeydew origins.

Thrasyvoulou and Bladenopoulou (1984) published a comparative analysis of Greek pine and blossom honeys. The results indicated that pine honey has high nutritional value because of its great content of minerals. In addition, a broad explanation was given why honeydew, secreted by plant-sucking insects, is not an excrement in the accepted sense of the term. Plant sap is not digested in the insect stomach. It passes through special filter chambers in the insect, is enriched by secretions, and reappears as honeydew.

Pine honey became gradually accepted as honey of good quality. It has no incisive taste or aroma, a very low tendency to crystallize (Manikis & Thrasyvoulou, 2001), is very thick, stores well, and is produced from pine forests far away from any environmental pollution. It is less sensitive to heating because of its low rate of forming HMF (Thrasyvoulou, 1986). These characteristics are very desirable in monofloral honeys and also in blends. Nowadays, pine honey is considered as one of the most attractive

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monofloral honeys in the Greek market and its price is higher than blossom honey.

Pine honey is also produced in Turkey from the same insect (*Marchalina hellenica*). Comparative analytical studies of Greek and Turkish honeys of the same floral source can vary due to the different geographical origins. Since the broad variation of physicochemical parameters within each honey cannot differentiate the two types of honeys, pine honey samples from both countries were analysed to characterise volatile compounds. In order to ordinate the whole matrix of the obtained results, an application of the Kohonen self organising maps (KSOM) was used as an artificial neural network (ANN) algorithm in the unsupervised learning category (Kohonen, 1995). This method has recently been successfully applied for the ordination of volatile analysis data characterising strawberry samples (Urruty, Giraudel, Lek, Roudeillac, & Montury, 2002; De Boishebert, Urruty, Giraudel, & Montury, 2004). More recently, the very same approach, based on an objective calculation of chemical distances between samples, allowed clear differentiation between fresh and frozen strawberry samples, independently of their variety (De Boishebert, Giraudel, & Montury, 2006). So, the aim of this work was to (relatively) characterise Greek and Turkish pine honey samples by determining the compositions of their volatile constituents in order to differentiate between the two origins. Beyond the obvious compositional differences observed between the two types of samples, the KSOM algorithm was applied to exemplify how this type of separation could also be achieved by considering the whole volatile chemical fingerprint (non specific constituents), or a part of it with just the molecules found in all the samples of both origins. Meanwhile, the components revealed to be the most determinant of this discrimination were easily identified.

## 2. Material and methods

### 2.1. Sampling

Fresh samples were collected from beekeepers from the Halikidiki area in northern Greece and from Mugla in south-west Turkey. The classification as pine honey was based on the information from the beekeepers and the organoleptic, microscopic (honeydew elements), electrical conductivity ( $>0.9 \text{ mS cm}^{-1}$ ), pH ( $>4.5$ ) and the sum of glucose and fructose measurement ( $>45\%$ ). HMF and Diastase activity analysis confirmed the freshness of the samples. Samples that were doubtful in origin were excluded from further analysis. Twenty-two samples of Greek and 22 of Turkish pine honeys were selected for this study. All samples were stored at  $-18^\circ\text{C}$  until chemical analysis.

### 2.2. Sample analysis

All samples were analysed with the following procedures:

#### 2.2.1. Isolation

A purge & trap system (O.I. Analytical, 4500) was used for the isolation of the volatile and semi-volatile compounds from the honey samples. Ten grammes of the honey sample were diluted with 5 ml of water and 15  $\mu\text{l}$  of internal standard were added. The purge vessel was heated at  $40^\circ\text{C}$  and directly purged with helium gas (20 ml/min). The volatile compounds were collected on a preconditioned glass-lined stainless steel desorption tube (GLT), containing the porous polymer Tenax TM TA. Desorption was performed by raising the trap temperature at  $180^\circ\text{C}$  for 7 min and the analytes were transferred to the gas chromatograph. Styrene (Aldrich  $>99\%$ ) in acetone (90  $\mu\text{g/ml}$ ) was added as internal standard. The water for the dilution of samples was from Reidel-de Haën.

#### 2.2.2. Gas chromatography–mass spectrometry

The isolated compounds were separated with an Agilent Model 6980 gas chromatograph, coupled with an Agilent 5973 mass detector. The interface and source temperatures were  $280^\circ\text{C}$  and  $230^\circ\text{C}$ , respectively. Electron impact mass spectra were recorded at 70 eV. Separation was performed on a fused silica capillary column SGE BPX5 (30 m  $\times$  0.25 mm,  $df = 0.25 \mu\text{m}$ ). The oven temperature was programmed at  $40^\circ\text{C}$  for 5 min, then to  $55^\circ\text{C}$  at  $1^\circ\text{C/min}$ , to  $120^\circ\text{C}$  at  $3^\circ\text{C/min}$ , to  $230^\circ\text{C}$  at  $10^\circ\text{C/min}$  and to  $280^\circ\text{C}$  at  $20^\circ\text{C/min}$ . This temperature was held for 5 min. Helium was used as the carrier gas at a flow of 1 ml/min and the injector temperature was  $220^\circ\text{C}$ . The identification of isolated volatile compounds was achieved by comparing mass spectra of unknown peaks with those stored in the National Institute of Standards and Technology (NIST) and Wiley electronic libraries.

#### 2.3. Data treatment by using KSOM

Used like other data mining methods, the Kohonen network consists of two layers of neurons: the first one (input layer) is connected to each of the  $m$  vectors of the data set (i.e. one vector is a real honey sample composition with  $n$  components), and the second one (output layer) is the Kohonen map itself with a two-dimensional array of neurons. Each neuron of the output layer stores a virtual honey sample with a chemical composition ( $n$  components) initially randomly affected and then iteratively computed. During the training, the virtual samples are modified in order to approximate the probability density function of the input data. At the end of the training, meaning after thousands of iterations, the map is organised with a defined chemical composition in each cell of the grid, and the real honey samples, with their measured compositions, can be projected onto it. The main interest of this approach is to preserve the topology of the data set and this means that samples positioned in the same area on the map are considered as chemically close and vice versa. This algorithm uses a *non supervised* training procedure where no constraints relative to the results

are applied to the learning system and where statistics (large number of samples) are pointless because no averaged values are used. Actually, only chemical distances between virtual and real samples are calculated and compared for the training and finally, the projection of real samples onto the trained map is performed according to the shortest distances, whatever their number. Formerly, this algorithm was proved relevant in the case of relatively small data sets (Goodacre, Kaderbhai, McGovern, & Goodacre, 1999) with relatively small sample panels. Moreover, the size selected for the map allows the chemist to choose the resolution strength of the data treatment. As already explained in previously cited papers, the larger the grid constituting the second layer of the neural network, the thinner are the differences between the cells and then between the samples projected into these cells. In fact, the resolution effect of the method increases with the number of cells selected for the map to be trained.

In a first step, each map was trained from the 44 pine honey samples analysis results (77 measured constituents), by using exactly the procedure outlined above and very precisely described by Urruty et al. (2002) in a study relative to classification of strawberry samples according to varieties. In a second step, the same samples were projected onto the trained maps, providing topological classification features for the whole sample panel (codes were from G1 to G22 for Greek samples and T1 to T22 for Turkish ones) The obtained corresponding maps are presented and discussed in the following paragraphs.

### 3. Results

After extraction and separation, 77 different volatile compounds were found in samples of Greek and Turkish honeys. Chromatograms of two different samples, one of each country, are presented in Fig. 1. Table 1 lists the compounds identified by their GC retention time (R.T.), the percentage of their appearance in the samples, and their average and standard deviations.

- Fifteen compounds were found in all samples (100%) of the Greek and Turkish honeys (codes ch2, ch3, ch4, ch7, ch11, ch16, ch18, ch27, ch32, ch38, ch48, ch51, ch63, ch68, ch74).
- Nine compounds were found exclusively in Turkish honeys (codes ch5, ch17, ch28, ch29, ch47, ch54, ch69, ch72, ch76). Of these, 3-carene (ch29) and the unknown (ch72) were present in all the Turkish samples examined.
- Two compounds were found only in Greek honeys (ch30 in all samples, ch40 in 65% of samples). The compound ch30 (1,4 dichloro-benzene) is exogenous in origin due to chemicals that beekeepers use to protect their combs from the wax moth (*Galleria mellonella* L). Another compound of the same origin is ch62 (naphthalene) which was found in samples from both countries.
- Fifty one compounds (ch62 included) were found in only some of the honey samples from both countries (different frequencies), as shown in Table 1.

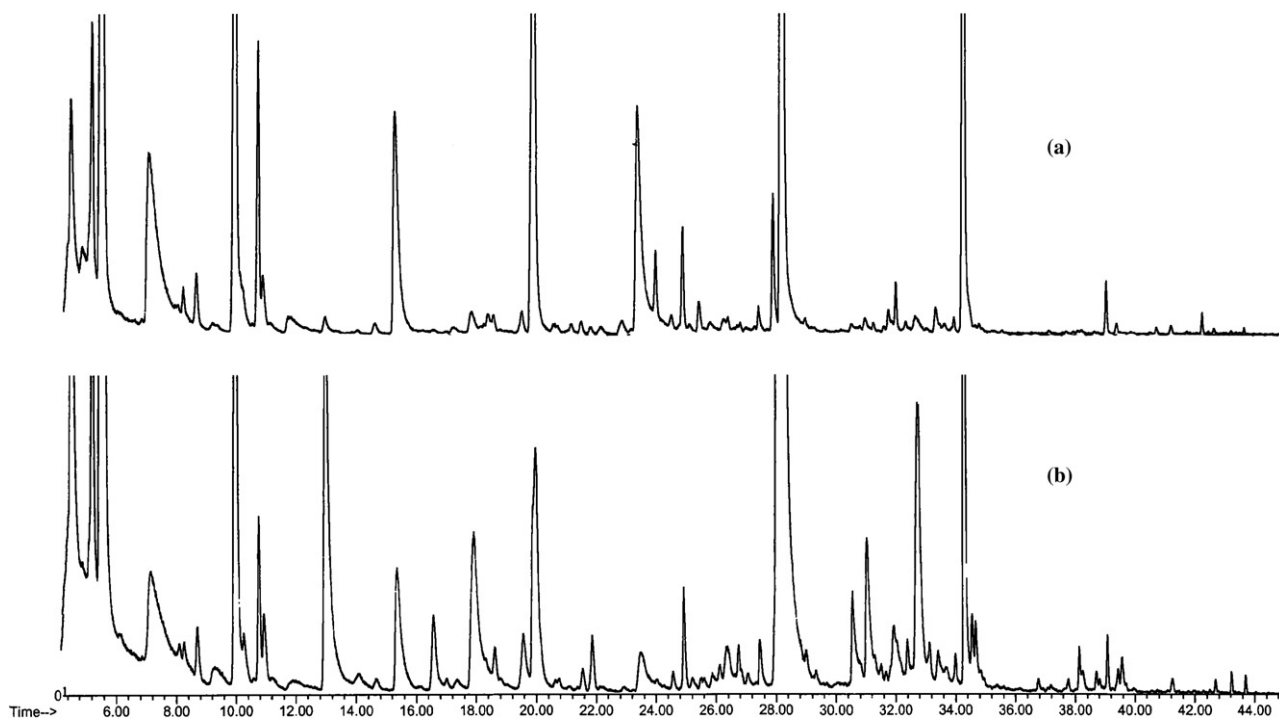


Fig. 1. Gas chromatograms obtained from one Greek (a) and another Turkish (b) pine honey sample.

Table 1  
Volatile compounds of Greek and Turkish pine honey

Code	R.T.	Compounds	Samples having the compounds (%)		Average $\pm$ SD <sup>a</sup>	
			Turkey	Greece	Turkey	Greece
ch1	4.539	Toluene	100.00	95.65	2.557 $\pm$ 2.101	0.663 $\pm$ 0.435
ch2	5.236	1-Octene	100.00	100.00	1.489 $\pm$ 0.354	1.237 $\pm$ 0.498
ch3	5.545	Octane	100.00	100.00	20.614 $\pm$ 4.475	12.183 $\pm$ 4.562
ch4	7.341	Furfural	100.00	100.00	1.397 $\pm$ 0.878	2.403 $\pm$ 2.292
ch5	8.071	3,5,5-Trimethyl-2-cyclopent-1-one	63.64	0.00	0.088 $\pm$ 0.020	0.000 $\pm$ 0.000
ch6	8.254	Ethylbenzene	68.18	91.30	0.106 $\pm$ 0.038	0.143 $\pm$ 0.083
ch7	8.688	p-Xylene	100.00	100.00	0.256 $\pm$ 0.110	0.267 $\pm$ 0.100
ch9	10.191	1-Nonene	9.09	65.22	0.320 $\pm$ 0.411	0.285 $\pm$ 0.107
ch10	10.248	2-Heptanone	95.45	91.30	0.182 $\pm$ 0.060	0.278 $\pm$ 0.225
ch11	10.757	Nonane	100.00	100.00	0.986 $\pm$ 0.454	1.781 $\pm$ 0.723
ch12	10.917	Heptanal	95.45	100.00	0.264 $\pm$ 0.051	0.258 $\pm$ 0.093
ch13	11.214	2-Heptanol	63.64	95.65	0.051 $\pm$ 0.020	0.125 $\pm$ 0.107
ch14	11.831	1-(2-Furyl)-Eethanone	90.91	78.26	0.140 $\pm$ 0.061	0.197 $\pm$ 0.093
ch16	12.986	$\alpha$ -Pinene	100.00	100.00	3.599 $\pm$ 0.905	0.216 $\pm$ 0.128
ch17	14.050	Camphene	81.82	0.00	0.071 $\pm$ 0.020	0.000 $\pm$ 0.000
ch18	14.643	Unknown (m/z 65, 77, 91, 119, 189)	90.91	100.00	0.073 $\pm$ 0.036	0.123 $\pm$ 0.055
ch19	15.346	Benzaldehyde	100.00	100.00	2.787 $\pm$ 1.548	4.264 $\pm$ 3.434
ch20	16.129	3C Benzene (m/z 57, 77, 91, 15, 120)	13.64	4.35	0.068 $\pm$ 0.015	0.430 $\pm$ 0.000
ch21	16.564	$\beta$ -Pinene	100.00	8.70	0.449 $\pm$ 0.169	0.050 $\pm$ 0.029
ch22	17.335	1-Heptanal	9.09	8.70	0.068 $\pm$ 0.010	0.167 $\pm$ 0.132
ch23	17.935	1-Octen-3-ol	36.36	30.43	0.786 $\pm$ 0.664	0.539 $\pm$ 0.482
ch24	18.032	3C Benzene	4.55	8.70	0.034 $\pm$ 0.000	0.068 $\pm$ 0.010
ch25	18.312	6-Methyl-5-hepten-2-one	54.55	86.96	0.144 $\pm$ 0.086	0.088 $\pm$ 0.035
ch26	18.604	2-Pentyl-furan	95.45	95.65	0.135 $\pm$ 0.037	1.169 $\pm$ 4.995
ch27	19.558	Decane	100.00	100.00	0.201 $\pm$ 0.112	0.198 $\pm$ 0.103
ch28	19.901	Octanal	95.45	0.00	0.486 $\pm$ 0.135	0.000 $\pm$ 0.000
ch29	19.976	3-Carene	100.00	0.00	1.230 $\pm$ 0.316	0.000 $\pm$ 0.000
ch30	20.000	1,4-Dichloro-benzene	0.00	100.00	0.000 $\pm$ 0.000	21.855 $\pm$ 19.077
ch31	20.713	$\alpha$ -Terpinene	22.73	13.04	0.080 $\pm$ 0.039	0.084 $\pm$ 0.029
ch32	21.536	$\pi$ -Cymene	100.00	100.00	0.151 $\pm$ 0.064	0.100 $\pm$ 0.031
ch33	21.856	Limonene	100.00	65.22	0.419 $\pm$ 1.059	0.082 $\pm$ 0.035
ch34	22.896	1-Ethyl-hexanol	22.73	65.22	0.146 $\pm$ 0.169	0.114 $\pm$ 0.042
ch35	23.485	Benzacetalddehyde	95.45	95.65	0.787 $\pm$ 0.586	2.646 $\pm$ 2.140
ch36	23.999	Ocimene	13.64	26.09	0.115 $\pm$ 0.053	0.614 $\pm$ 0.473
ch37	24.559	$\gamma$ -Terpinene	100.00	86.96	0.107 $\pm$ 0.059	0.119 $\pm$ 0.049
ch38	24.931	1-Chloro-octane	100.00	100.00	0.499 $\pm$ 0.229	0.483 $\pm$ 0.259
ch39	25.279	Acetophenone	45.45	26.09	0.064 $\pm$ 0.021	0.164 $\pm$ 0.103
ch40	25.491	Cycloheptanemethanol	0.00	65.22	0.000 $\pm$ 0.000	0.184 $\pm$ 0.116
ch41	25.891	cis-linalool-oxide	77.27	52.17	1.085 $\pm$ 1.059	0.200 $\pm$ 0.092
ch42	26.125	1,2-Dibromo-3-chloro-propane	31.82	4.35	0.233 $\pm$ 0.157	0.000 $\pm$ 0.000
ch43	26.348	1-Octanol	18.18	26.09	0.268 $\pm$ 0.079	0.241 $\pm$ 0.178
ch45	26.748	$\alpha$ -Terpinolene	100.00	21.74	0.171 $\pm$ 0.085	0.117 $\pm$ 0.089
ch46	26.834	1-Methyl-4-(1-methylethenyl)-benzene	50.00	65.22	0.102 $\pm$ 0.040	0.139 $\pm$ 0.060
ch47	27.040	trans-linalool-oxide	59.09	0.00	0.279 $\pm$ 0.111	0.000 $\pm$ 0.000
ch48	27.451	2-Nonanone	100.00	100.00	0.211 $\pm$ 0.059	0.236 $\pm$ 0.142
ch49	27.931	Undecane	90.91	91.30	0.321 $\pm$ 0.228	0.493 $\pm$ 0.271
ch50	28.046	Linalool	100.00	39.13	0.847 $\pm$ 1.129	1.469 $\pm$ 1.582
ch51	28.309	Nonanal	100.00	100.00	41.594 $\pm$ 8.071	21.823 $\pm$ 9.057
ch52	29.006	Isophorone	63.64	17.39	0.171 $\pm$ 0.064	8.908 $\pm$ 13.926
ch53	29.303	a-Camphene-aldehyde	45.45	4.35	0.089 $\pm$ 0.017	0.176 $\pm$ 0.000
ch54	30.195	2,6-Dimethyl-1,3,5,7-octatetraene	27.27	0.00	0.120 $\pm$ 0.023	0.000 $\pm$ 0.000
ch55	30.572	Lilac aldehyde (A, B, D)	86.36	39.13	0.369 $\pm$ 0.201	0.375 $\pm$ 0.310
ch56	30.806	1-(1,4-Dimethyl-3-cyclohexanyl)-ethanone)	81.82	21.74	0.155 $\pm$ 0.083	0.741 $\pm$ 0.912
ch57	31.058	Lilac aldehyde (C, A, B)	86.36	56.52	0.553 $\pm$ 0.324	0.487 $\pm$ 0.526
ch58	31.292	Neroloxide	59.09	26.09	0.125 $\pm$ 0.062	0.180 $\pm$ 0.111
ch59	31.526	1,2,3-Tribromo-propane	45.45	8.70	0.158 $\pm$ 0.125	0.099 $\pm$ 0.035
ch60	31.944	Borneol	81.82	95.65	0.202 $\pm$ 0.098	0.232 $\pm$ 0.138
ch62	32.389	Napthalene	100.00	95.65	0.515 $\pm$ 0.495	3.668 $\pm$ 10.880
ch63	32.778	Nonanol	100.00	100.00	2.941 $\pm$ 1.838	1.273 $\pm$ 1.409
ch64	33.144	1,2,3-Trimethylindene	100.00	13.04	0.242 $\pm$ 0.050	0.241 $\pm$ 0.147
ch65	33.412	Methyl-salicylate	100.00	78.26	0.210 $\pm$ 0.088	0.529 $\pm$ 0.947
ch66	33.675	a-Thujenal	27.27	17.39	0.115 $\pm$ 0.043	0.125 $\pm$ 0.061

Table 1 (continued)

Code	R.T.	Compounds	Samples having the compounds (%)		Average $\pm$ SD <sup>a</sup>	
			Turkey	Greece	Turkey	Greece
ch67	33.990	Undecane	95.45	86.96	0.121 $\pm$ 0.053	0.084 $\pm$ 0.030
ch68	34.304	Decanal	100.00	100.00	3.539 $\pm$ 0.653	9.393 $\pm$ 2.725
ch69	34.555	Santene	54.55	0.00	0.162 $\pm$ 0.046	0.000 $\pm$ 0.000
ch70	34.675	p-1-Meht-en-9-al	63.64	8.70	0.144 $\pm$ 0.061	0.067 $\pm$ 0.002
ch71	34.847	3-Phenyl-furan	59.09	52.17	0.283 $\pm$ 0.163	0.189 $\pm$ 0.106
ch72	38.139	Unknown (m/z 55. 79. 91. 107. 123. 165)	100.00	0.00	0.187 $\pm$ 0.045	0.000 $\pm$ 0.000
ch73	38.236	Anethole	86.36	43.48	0.090 $\pm$ 0.024	0.201 $\pm$ 0.140
ch74	39.082	Tridecane	100.00	100.00	0.194 $\pm$ 0.078	0.244 $\pm$ 0.145
ch75	39.442	Tetradecanal	100.00	73.91	0.079 $\pm$ 0.024	0.077 $\pm$ 0.027
ch76	39.568	Unknown (m/z 55. 82. 96. 138. 177)	54.55	0.00	0.086 $\pm$ 0.038	0.000 $\pm$ 0.000
ch78	41.254	1,2-Dihydro-1,1,6-trimethyl-naphthalene	90.91	73.91	0.092 $\pm$ 0.041	0.134 $\pm$ 0.099
ch79	42.700	Damascenone	31.82	8.70	0.155 $\pm$ 0.071	0.076 $\pm$ 0.007
ch80	43.226	Jupinene	77.27	4.35	0.082 $\pm$ 0.074	0.052 $\pm$ 0.000
ch81	43.706	<i>trans</i> -caryophyllene	90.91	17.39	0.054 $\pm$ 0.017	0.198 $\pm$ 0.173
ch82	44.043	1a. 2. 3. 5. 6. 7. 7a. Tetra-1H-cyclopropa[a]naphthalene	18.18	4.35	0.202 $\pm$ 0.079	0.105 $\pm$ 0.000

<sup>a</sup> The value of each compound corresponds to percent values of the total volatile compound of honey (%  $\pm$ SD).

Although the aim of this research was not the quantitative determination of volatile compounds, relative amounts of separated compounds indicated differences (Table 1). For example,  $\alpha$ -pinene (ch16) was found to be 16 times higher in Turkish honey while isophorone (ch52) was 52 times higher in Greek honeys.

Moreover, independently of the two exogenous chemicals used by beekeepers (ch30 and ch62), two compounds (ch29 and ch72) were found in 100% of Turkish samples and were absent in 100% of Greek samples, in the panel concerned for this study. Therefore, in a first approach, these two compounds may be considered as markers characterising the Turkish origin of samples.

Beyond this first obvious result, the Kohonen algorithm has been used to exemplify the efficacy of this method for performing the same type of data mining from complex multivariate matrices in the case where no specific constituents were found and the separation into clusters (only due to the different composition patterns of the same compounds present in both types of samples). So, the chemical result data base was reduced to the 4 lines corresponding to the different values of ch29 and ch72 specific to Turkish samples and ch30 and ch62 as exogenous compounds. Thus, the resulting 44 column matrix only contained 73 lines, indicating the composition of each sample, of both origins, in these 73 volatile compounds. The two cell map trained from this matrix, and indicated in Fig. 2, provided a separation of the 44 samples into 2 clusters that exactly fitted the two types of origins, exactly as in the case where the full matrix (77 lines) was used (Fig. 2). In this way, the discrimination between the two origins is obtained by globally considering all the analysed volatile constituents and also with the specific ones put aside.

Moreover, the same type of two-cell map was also trained from analysis results relative to the 15 constituents listed in paragraph (a) and found in 100% of both types of

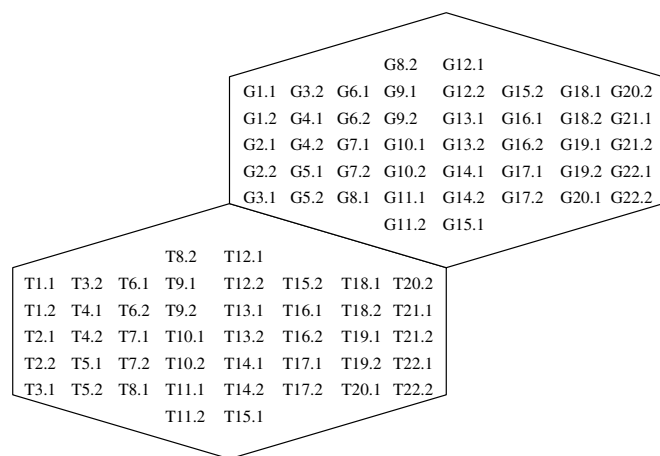


Fig. 2. Distribution of the 44 pine honey samples into a 2-unit map whatever the selected matrix (77, 73, 15 or 9 constituents selected). G and T letters refer to Greek and Turkish origins, respectively. First index refers to the sample number and the second one, to the replication.

honey samples. The projection of the 44 samples afforded exactly the same discrimination effect and the same map as in the first case (Fig. 2). In terms of resolution effect, this last result indicated that part of the information contained in the matrix with the 73 volatile compounds was redundant and not necessary for achieving the discrimination effect between the two origins of honey. As long as a clear differentiation was obtained when the map was computed from the 15 compounds found in all the samples (a), it appeared interesting to determine, among these 15 analytes, those that were the most relevant to the discrimination. As already published (Giraudel & Lek, 2001), by using grey shades for displaying the contribution of each of the selected compounds in the trained map (the darker the unit, the more concentrated was the virtual sample), the efficacy of each compound for structuring the map was clearly indicated (the higher the contrast, the more

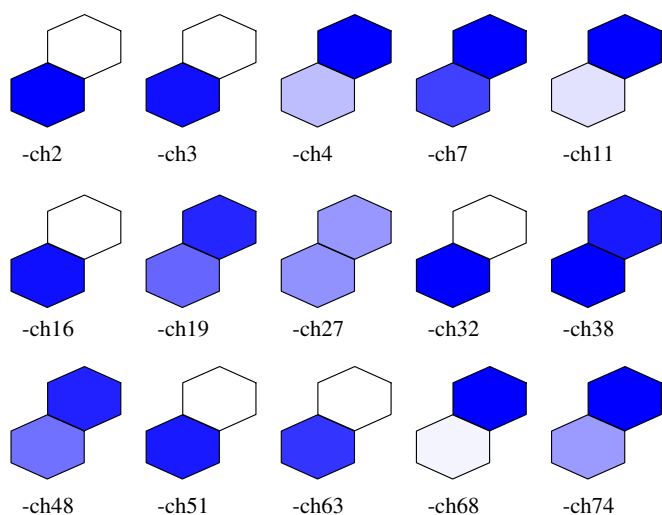


Fig. 3. Contribution of each constituent in the 2 cell map trained from the matrix with the 15 components present in 100% of the Greek and Turkish honeys. The darker the unit, the more concentrated is the virtual sample in this constituent.

structuring was the selected compound). Corresponding grey shade maps are indicated in Fig. 3, where 9 of the 15 compounds (ch2, ch3, ch4, ch11, ch16, ch32, ch51, ch63 and ch68) revealed much more structuring than the 6 others. So, a new two hexagon map was trained by using analysis data relative to these 9 analytes. Projections of the 44 samples onto this map gave the very same result as the two previously made ones (Fig. 2), indicating that the analysis of these 9 volatile components could be sufficient to allow the separation, in the case of this selected panel of samples. Moreover, as shown in Table 1, significant differences in the average contributions of these 9 compounds between the two areas could be noted. On the other hand, an additional map trained from the analysis results relative to the 6 less structuring components (ch7, ch19, ch27, ch38, ch48 and ch74), afforded the complete mixing of the

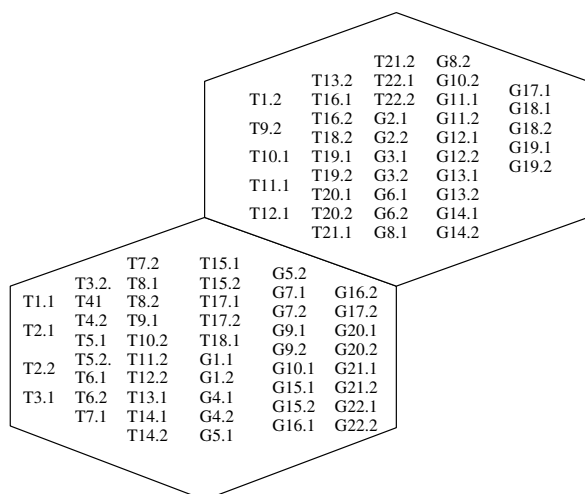


Fig. 4. Distribution of the 44 Greek and Turkish samples into a 2-unit map trained from the matrix with the 6 less structuring constituents.

same samples, as represented in Fig. 4. Actually and beyond the use of the 2 volatile compounds observed as specific constituents of Turkish honey samples, the KSOM algorithm was efficient. It allows the chemist to extract from the analytical data matrix which of the selected constituents are determinant for discriminating between these Greek and Turkish honey samples. In the case of more complex differentiation, the procedure could be repeated in order to determine the minimum number of compounds necessary for achieving the observed result and this approach should be of major interest.

#### 4. Discussion

Honeys of the same floral source can vary due to seasonal climatic variation or to different geographical origins. Honeydew secretions from pine trees are produced by the same insect (*Marchalina hellenica* L) in both Greece and Turkey but secondary plants may contribute to different volatile profiles.

A total of 77 volatile compounds were identified in samples from both countries. Their discrimination was made possible by using either specific compounds characteristic

Table 2  
Volatile compounds found in all Greek and Turkish pine honey and their presence in other honeys

Code	Compound in pine honey	Other honeys with the same compound (number indicates the reference)
ch2	1-Octene	Eucalyptus <sup>a</sup>
ch3	Octane	Lime <sup>b</sup> , eucalyptus <sup>c,d</sup> , blossom <sup>c</sup> , citrus <sup>e</sup> , tupelo <sup>f</sup> , alfalfa <sup>f</sup> , chestnut <sup>b</sup>
ch4	Furfural	Blossom <sup>a,g,h</sup> , sulla <sup>h</sup> , orange <sup>h,f</sup> , chestnut <sup>h</sup> .
ch7	<i>p</i> -Xylene	eucalyptus <sup>e</sup> , citrus <sup>e</sup>
ch11	Nonane	Lavender <sup>i</sup> , eucalyptus <sup>d</sup> , orange <sup>f</sup> , clover <sup>f</sup> , tupelo <sup>f</sup> , alfalfa <sup>f</sup> , chestnut <sup>b</sup> , lime <sup>b</sup>
ch16	$\alpha$ -Pinene	Chestnut <sup>h</sup> , helianthus <sup>a</sup> , lime <sup>a,b</sup>
ch18	Benzaldehyde	Chestnut <sup>a,h</sup> , blossom <sup>h</sup> , clover <sup>f</sup> , tupelo <sup>f</sup> , alfalfa <sup>f</sup> , erica <sup>j</sup>
ch27	Decane	Clover <sup>f</sup>
ch32	$\pi$ -Cymene	Chestnut <sup>k,b</sup> , clover <sup>f</sup> , tupelo <sup>f</sup> , alfalfa <sup>f</sup> , apple <sup>f</sup> , lime <sup>b</sup>
ch38	1-Chloro-octane	
ch48	2-Nonanone	Eucalyptus <sup>h</sup> , chestnut <sup>h</sup>
ch51	Nonanal	Eucalyptus <sup>h</sup> , sulla <sup>h</sup> , orange <sup>h</sup> , chestnut <sup>h</sup> , blossom <sup>h</sup>
ch63	Nonanol	Honeydew honey <sup>g</sup> , eucalyptus <sup>h</sup> , chestnut <sup>h</sup>
ch68	Decanal	Blossom <sup>a,h</sup> , chestnut <sup>k,h</sup> , sulla <sup>h</sup> , orange <sup>h</sup> , eucalyptus <sup>h</sup>
ch74	Tridecane	

<sup>a</sup> Radovic et al. (2001).

<sup>b</sup> Guyot et al. (1998).

<sup>c</sup> Bouseta et al. (1992).

<sup>d</sup> Bouseta et al. (1996).

<sup>e</sup> Alissandrakis et al. (2003).

<sup>f</sup> Overton and Manura (1994).

<sup>g</sup> Soria et al. (2004).

<sup>h</sup> Verzera et al. (2001).

<sup>i</sup> Guyot-Declerk et al. (2002).

<sup>j</sup> Guyot et al., 1999.

<sup>k</sup> Bonaga and Giumanini (1986).

of one of the origins, or by considering the others and their relative proportions: octanal, 3-carene, camphene, octane, nonanal, decanal,  $\alpha$ -pinene,  $\beta$ -pinene, toluene, 1,2,3-trimethylindene and the unknown ( $m/z$  55, 79, 91, 107, 123, 165) appeared to be most determinant in this separation.

Literature was investigated to determine whether the 15 common compounds found in these Greek and Turkish pine honey samples could be considered as suitable markers. References relative to honeydew honey are limited but many of these compounds can be found in nectar honeys, mainly chestnut, eucalyptus, clover and orange. Some of these were indicated by other authors as reliable markers for a specific flora honey (Table 2). Only 1-chloro-octane (ch38) and tridecane (ch74) were not found in other honeys in the literature that was searched.

In conclusion, it has been demonstrated in this study, that KSOM was a valuable tool for elucidating the origin of honey samples according to their volatile constituents, especially when no specific markers could be detected. Even by considering only the 15 compounds that were found in 100% of all Greek and Turkish samples, the differentiation was made obvious and the method allowed highlighting of the most determinant of these compounds.

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