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PRELIMINARY RESULTS OF A RECOGNITION METHOD VISUALIZING THE AROMA AND FRAGRANCE FEATURES

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The lack of interpretation methods capable of examining the aroma-profiles of spicy and medicinal plants and other samples of food origin (wines, honeys, fruits, fruit-distillates) makes necessary a thorough investigation of the relating evaluation procedures. By adding three appropriate hydrocarbon standards to all sample extracts, and measuring the programmed temperature retention indices of the components and normalizing the peak areas to that of the compound corresponding to the most intense chromatographic peak, a visualization of the aroma characteristics could be achieved. The relationship or identity of aroma patterns could be deduced from the presence or absence of similar polygons in the "constellation-maps" of the components.

Keywords: GC-MS identification, relative chromatograms, aroma-map, polygonal-method

The aroma profile of wines, fruits, spicy and medicinal herbs is generally considered an important quality determining factor. While aroma profiles are highly characteristic and can be easily recognised by the expert, there are practically no general methods for their objective measurement. It is plausible that the taste/aroma of a product must be defined by a number of key components and their ratios (SHAATH & GRIFFIN, 1988), on the other hand it is not a trivial task to determine which of the several hundred or thousand components, identifiable by GC or GC-MS, are crucial for the characteristic taste or aroma. The second type of difficulty comes from the fact that the ratio of crucial components may change upon storage or handling to such an extent that the product will in fact loose its characteristic sensory profile. Also, the extraction of the aroma materials will introduce an inevitable bias. The third source of problem is that of the analytical measurement, in this case extraction and gas chromatographic analysis.

Fortunately, if the aroma structures of the spicy and medicinal plants are genetically coded and determined, as is supposed and are therefore a fixed characteristic

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of each plant (EVANS, 1986), a method similar to the relative mass spectrum construction procedure might help to overcome the recognition problems of the chromatographic aroma patterns as well.

Most of the computerized GC equipment calculate the area percent of the peaks in a chromatographic run with ease and automatically, but the result depends on the sensitivity of the integration, namely on the integration parameters to too high an extent. The problem is analogous to the spectral interpretation difficulties in mass-spectrometry (KAMEOKA, 1986) and the correct solution is absolutely similar as well. Transforming the absolute chromatograms (retention time vs. peak areas) into relative ones can ensure nearly distortion-free measurement of the compound ratios in the linearity range of the instrument and can also compensate for any effect of sample preparation to an extent not worse than the internal standard method. A considerable advantage of this conversion is that the normalizing reference compound behaves like a natural internal standard so that variations due to sample preparation and gas chromatography conditions are largely overcome (RAPP, 1988). These relative chromatograms have the same advantages as relative mass spectra compared to the corresponding absolute ones and can be handled identically from mathematical point of view.

Another difficulty is the creation of an "absolute x-axis", since the retention time is highly dependent on the gas chromatographic parameters and is not a unique function of structure (KOBAYASHI & KAWAKAMI, 1991). Totally different compounds may have the same retention time on the same column under different conditions. In gas chromatography at constant speed column heating the members of homologous series (n-alkanes, olefins...etc.) elute equidistantly, their reduced retention times define a straight line as a function of carbon number. For expansion of the horizontal axis scale not the carbon numbers themselves, but their hundredfold values (1000, 1100, ... 2000) are used. The parameters (slope and offset) are characteristic of the stationary phase and are constant if relative retention times (RRT = reduced retention time divided by that of the longest n-alkane) are used for calculation. In practice three properly chosen n-hydrocarbons (no coincidence of peaks) are enough to determine the equation of the linear function. Since RRTs can be calculated for all compounds eluting with the alkanes in the same chromatographic run and all of them lie on the straight line of the n-hydrocarbons, their "x"-co-ordinates in other words their places can be determined by the equation. The method described is called programmed temperature retention index (PTRI) measurement.

1. Materials and methods

Chemical substances, standards and solvents used in our work were of "analytical", "HPLC" or "GC" grade, and were purchased from Merck (Darmstadt, Germany), Carlo Erba (Milan, Italy) and Carl Roth (Karlsruhe, Germany). Although

spectral transparency is not equivalent to chemical purity, transmittance values of 90% at 200 nm wavelength do indicate the high quality of solvents.

1.1. Solvents and chemicals

n-Pentane, iso-Octane, bidistilled water, ethanol, normal-hydrocarbon standards .

1.2. Glassware and tools

The glassware was of thermoresistant Pyrex quality. Distillation equipment and other glass tubes were teflon-valve equipped.

Round bottom flasks (1 dm^3), distillation equipment with condenser, teflon-capped sample containers.

1.3. Instrumentation

Hewlett Packard 5890/II GC - 5971/A MSD (Palo Alto, CA, USA).

1.4. Samples

Honey (acacia), herbs (lavender), must of known origin and provenance, provided by courtesy of farmers and primary producers, were examined. They are as follows:

- Muscat Ottonel must from Gyöngyös region,
- acacia (*Robinia pseudoacacia*) honeys from the western border, Visegrád, Cserhát, and Alföld regions of Hungary,
- lavender (*Lavandula angustifolia* Mill.) herbs from the experimental farm of the University of Horticulture and Food Industry.

The herb samples were kept at 5 °C in aroma-tight bags excluding light. Before taking the quantity to be ground, the whole sample was homogenized by mixing. Grinding of the 250 g plant sample was performed by a laboratory mill, Lab. Mill-1 QC-114 (Labor MIM., Budapest, Hungary). Distillation was carried out immediately after grinding to minimise the loss of the most volatile compounds.

For steam distillation of all plants the same method was used. To gain results most representative of the samples, 3×75 g of the herbs were distilled separately and each distillate was measured by GC in 3 parallel injections, having diluted the collected essential oils with iso-octane containing the C_{10}, C_{14} and C_{20} n-hydrocarbon standards. The average of the 9 runs was calculated.

The preparation of the wine samples needed a combination of distillation and extraction. In the first step the volatiles together with the alcohol of 500 cm^3 wine were distilled resulting in 80 cm^3 of condensate, i.e. a volume representing more than 150% of the ethanol content. Prior to the distillation 100 g NaCl was added to the sample to increase the

volatility of the aroma compounds. Distillates of 3×500 cm^3 of the same wine were combined and extracted by 3×80 cm^3 n-pentane. Then the pentane extract was evaporated to 0.5 cm^3 in a cold N_2 stream and made up to 1 cm^3 with iso-octane containing the C_{10}, C_{14} and C_{20} n-hydrocarbon standards. The samples were analyzed by gas chromatography in 5 parallel injections and the average of the 5 runs was calculated.

In the case of honey samples a preparatory method similar to that of the wines was applied. A 600 g sample of the honeys was dissolved and made up to 1 dm^3 in bidistilled water. One third of the solution was diluted to 500 cm^3 with 50 cm^3 of ethanol (acting as scavenger substance) and the remainder by water. Then the volatiles together with the alcohol were distilled resulting in 80 cm^3 of condensate. Subsequent treatment and measurement used the same methods that were discussed in the previous paragraph (wine samples).

The GC-MS measurements were performed under the following conditions:

Instrument	:	Hewlett Packard 5890/ II GC - 5971A MSD
Column	:	60 m×0.25 mm Supelcowax 10 (fused silica)
Film thickness	:	0.25 μm
Initial temperature	:	T_1 = 60 °C
Temperature progr.	:	v_{heat} = 4.0 °C min^{-1}
Final temperature	:	T_2 = 280 °C
Det.temp (tf.line)	:	T_{det} = 280 °C
Carrier	:	He, 155 kPa, const. flow. mode, v_{lin} = 29.6 cm s^{-1}
Injector	:	split/splitless 155 kPa, T_{inj} = 250 °C
Injector mode	:	split mode, splitless 0.35 min
Mass range	:	*m/z* = 25–350 D
Scan speed	:	390 D s^{-1}

2. Results

In developing the *aroma-map* construction method, the retention times were converted into PTRIs. The reproducibility of the PRTI values is ±3 index units with respect to the mean. The peak areas were transformed into relative intensity data dividing the individual areas by that of the largest peak (ethyl-hexadecanoate (honeys), linalool (Lavender), l-alpha-terpineol (Muscat Ottonel-musts)). For minor components (under 3 rel. %) the reproducibility of relative intensities is approximately ±10%, for medium components (3–8 rel. %) it is between ±5–8% and for major constituents (above 8 rel. %) it is lower than ±5% (all values are given in rel. % of the mean value determined for a compound in question).

Table 1

List of identified compounds with their programmed temperature retention indices

Index	Compound	Index	Compound
945	Ethane, 1,1-diethoxy-	1355	3-Hexen-1-ol
967	2,4,5-trimethyl-1,3-dioxolane	1382	Octene-1-ol-acetate
988	Butane, 1,1-diethoxy	1391	3-Octanol
1020	alpha-Pinene	1422	Butanoic ac.hexyl ester
1057	Camphene	1433	hexyl-2-Me-Butyrate
1095	beta-Pinene	1436	Octanoic acid, ethyl ester
1030	Butanoic acid, ethyl ester	1440	p-Mentha-1,5,8,-triene
1062	1-Propanol, 2-methyl-	1443	Hexadecane, 2,6,10,14-tetramethyl-
1091	Pentane, 1-(1-ethoxyethoxy)-	1446	Linalool-oxide (2)
1108	1-Butanol, 3-methyl-, acetate	1447	Benzene, 1-isopropenyl-?-methyl-
1136	delta-3-Carene	1452	Linalool oxide (2,deriv.)
1138	alpha-Terpinene	1452	l-alpha-Terpineol deriv.
1149	beta-Myrcene	1453	Naphtalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-
1166	alpha-Terpinolene	1453	p-Mentha-1,5,8-triene
1187	Dodecane	1456	1,2,3,4-thydr-1,1,6-triMe-Naphthalenene
1189	dl-Limonene	1470	trans-Sabinenehydrate
1190	1-Butanol, 3-methyl- (impure)	1472	Linalooloxide (deriv.)
1199	1,8-Cineole	1473	Neroloxide (deriv.)
1213	7,7-diMe-2-Methoxy-norborn-2-ene	1477	Geraniol (deriv.)
1222	Hexanoic acid, ethyl ester	1479	Neroloxide (deriv.)
1226	1,3,6-Octatriene.....	1480	Linalool oxide (deriv.)
1232	1-Dodecene	1506	Pentadecane
1238	gamma-Terpinene	1509	Geraniol (deriv.B)
1245	1,3,7-Octatriene,3,7-dimethyl-	1537	Benzaldehyde
1250	3-Octanone	1537	Camphor
1262	Acetic acid, hexyl ester	1545	Nonanoic acid, ethyl ester
1267	Benzene, 1-methyl-4-(1-methylethyl)-	1553	Linalool
1267	Hexylacetate	1560	1-Octanol
1280	alpha-Terpinolene	1568	Linalyl acetate
1293	Tridecane	1574	1,3-diMe-bicyclo[3.3.0.]....
1297	Cyclohexane, 1,2,4-tris(methylene)-	1597	Bornyl formate
1314	Linalool (deriv.)	1611	3-Cyclohexene-1-ol..
1337	Propanoic acid, 2-hydroxy-, ethyl ester	1612	Hotrienol

Table 1. contd.

Index	Compound	Index	Compound
1339	Propanoic acid hexyl ester	1616	trans-Caryophyllene
1342	Propanoic ac.,2-Me-octyl ester	1629	1-Me-4-(1-MeEthenyl)-Cyclohexanol
1348	1-Hexanol	1632	Decanoic acid, ethyl ester
1640	5,7-Octadien-2-ol, 2,6-dimethyl-	1896	3-Octadecene, (E)-
1642	Decanoic acid, ethyl ester	1906	N-Acetyl-N'-phenylhydrazine
1645	Tricyclene(deriv.)	2009	Lavender-(Z)
1649	1-Hexadecene	1933	.beta.-Terpinene
1657	1-Nonanol	2014	2-Tridecanone
1663	.gamma.-Terpinene	2049	Tetradecanoic acid, ethyl ester
1666	(Z)-beta-Farnesene	2117	Heneicosane
1668	Tricyclene	2150	2-Pentadecanone, 6,10,14-trimethyl-
1675	(-)-Lavandulol	2265	Docosane
1685	1H-Pyrazole,3,5-diMe...	2304	(+-)-15-Hexadecanolide
1696	3-Cyclohexene-1-methanol...	2351	Hexadecanoic acid, thyl ester
1771	Ethanone, 1-(methylphenyl)-	1697	l-alpha-Terpineol
1786	Benzaldehyde,4-(1-MeEt)-	1701	endo-Borneol
1790	Benzene, 1-(1,1-dimethylethyl)-3-meth	1715	Bicyclo[4.4.0]dec-1-en, 2-isopropyl-
1799	Acetic acid, 2-phenylethyl ester	1721	p-Mentha-1(7),2-dien-8-ol
1811	.beta.-Damascenone	1721	Epoxylinalol
1816	trans-2-Caren-4-ol	1721	Nerylacetate
1824	cis-Carveol	1740	2-Cyclohexene-1-one
1826	Dodecanoic acid, ethyl ester	1740	.beta.-Citronellol
1826	Hexanoic acid	1747	Geranylacetate
1830	trans-Geraniol	1748	Naphthalene
1880	Nonadecane	1755	Nerol
1890	Benzeneethanol	1762	Naphthalene,1,2,3,4-..octahydro...

For reasons of space, no GC/MS data are shown. The list of identified compounds is shown in Table 1, which also contains the PTRIs measurable under the experimental conditions.

The diagrams constructed by the above process can be interpreted rather in a "star-map" than a spectrum-like manner. For example three representative aroma-maps are shown in Figs. 1, 2 and 3.

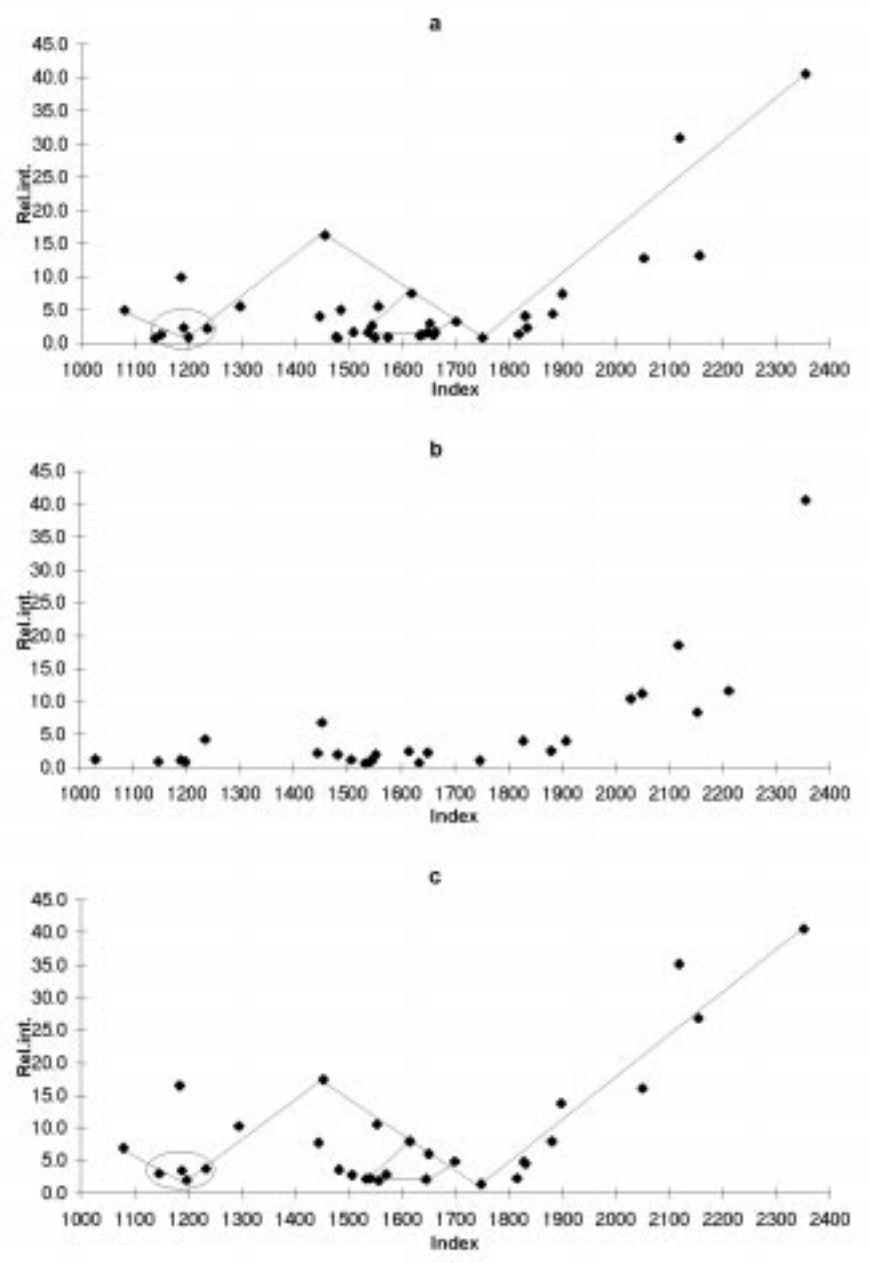

Fig. 1. The constellation map of acacia honeys. a. Acacia honey (western border of Hungary); b. acacia honey (Kecskemét region) (non characteristic of acacia); c. acacia honey (Cserhát region)

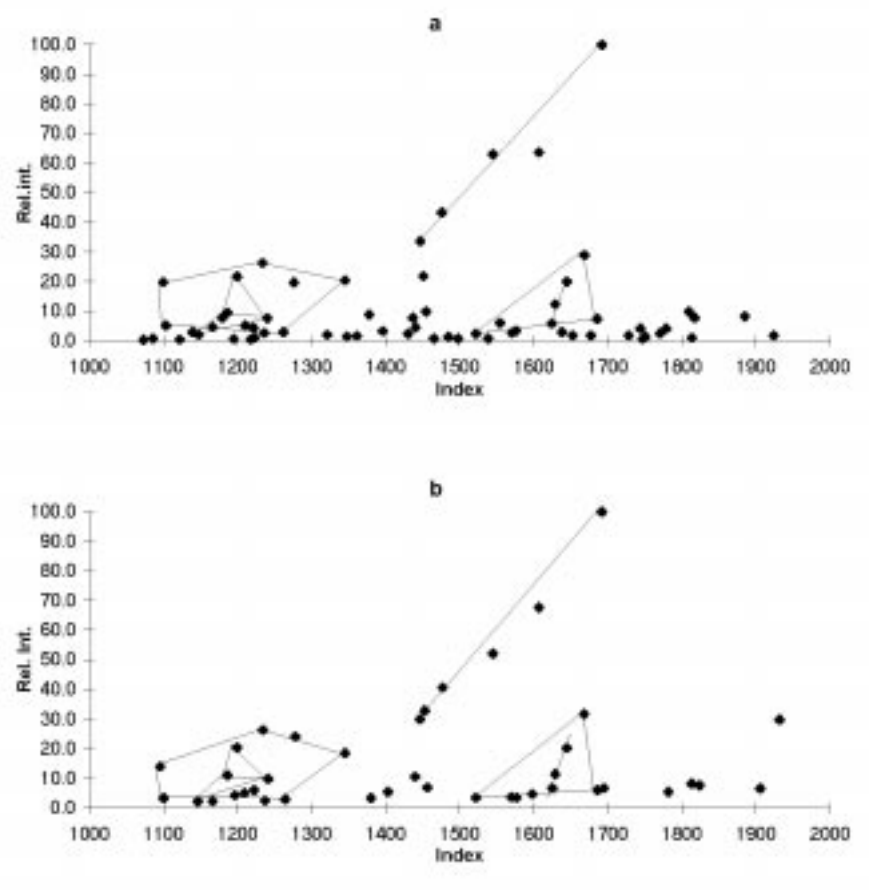

Fig. 2. The constellation map of Muscat Ottonel musts. a. Muscat Ottonel must '96; b. Muscat Ottonel must '97

The aroma-maps of Fig. 1 show the comparison of three acacia honeys. The characteristic ones "a" and "c" derive from Nagykanizsa (western border of Hungary) and Cserhát (North-West Hungary) regions. They were produced by a primary producer collaborating with us on our research. The third honey ("b") was purchased from an unknown producer and was told to originate from Kecskemét (Plains of Hungary) region. We found it to be non characteristic of other acacia honeys.

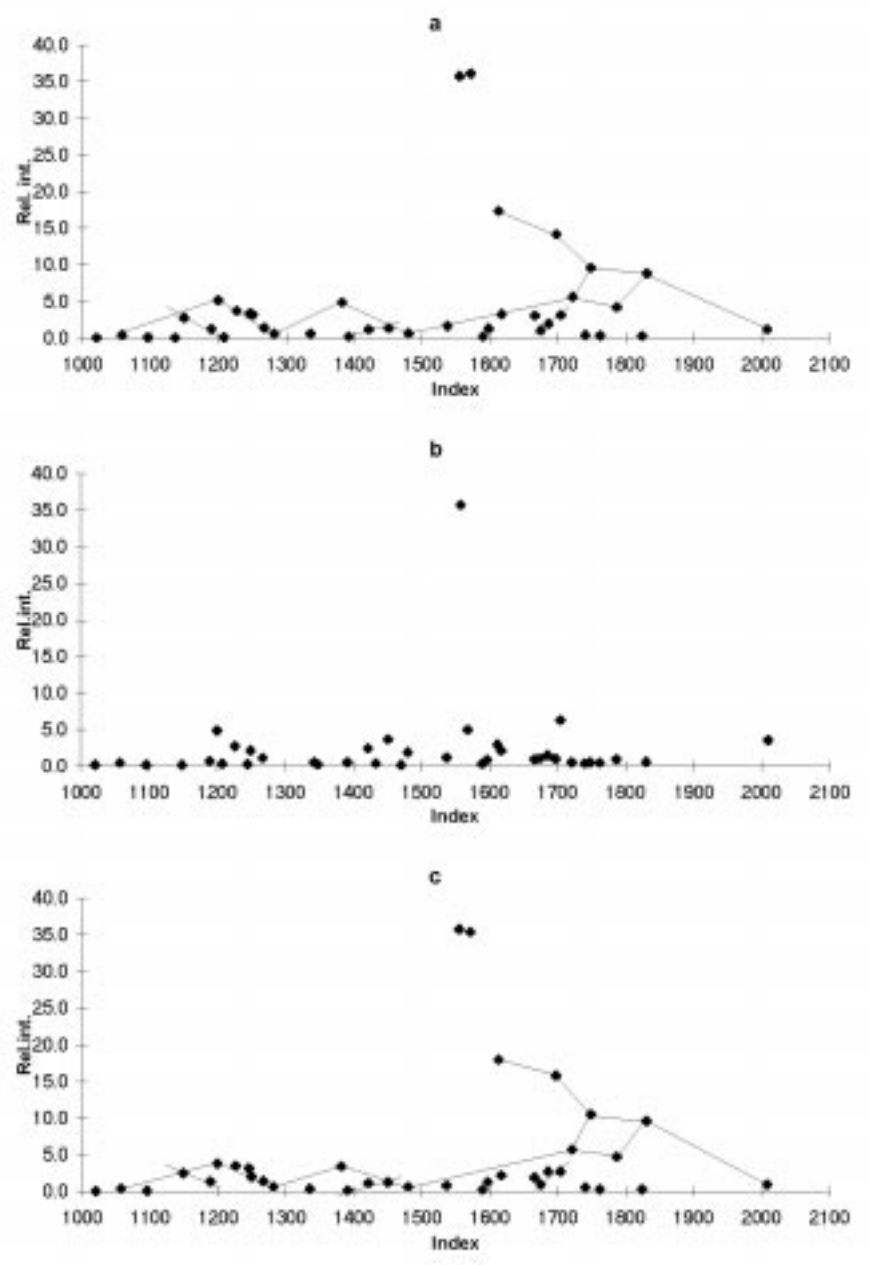

Fig. 3. The constellation map of lavenders. a. Lavender 1102; b. lavender 1107; c. lavender 1105

Relative chromatograms on Fig. 2 depict the aroma maps of Muscat Ottonel musts deriving from vintages 1996 ("a") and 1997 ("b"). Appart from observable differences the great similarity is obvious. The must samples were produced by the same primary producer in Gyöngyös (traditional wine producing district of North-East Hungary) region in both years.

Aroma-maps on Fig. 3 show the comparison of lavenders. In an experiment among six samples investigated 4 were members of the same cultivated variety (relative herbs) grown under slightly different conditions. Two of them were non-relatives. Relatives showed a special, "Ursa Major"-like structure whilst non-relatives did not. The figure depicts the aroma map of a non relative lavender ("b") between two relative ("a" and "c") ones. Samples derived from the pilot farm of the University of Horticulture and Food Industry.

3. Discussion

The thorough study on the honey, grape-must and lavender records discovered characteristic differences in every region of the chromatograms, but the detailed chromatograms would require too much room to be published. These studies demonstrated that a safe distinction can be made between any two same-type samples by visual evaluation of the relevant chromatograms, a conclusion that is not new, however.

The greatest disadvantage of relative retention time *versus* relative intensity diagrams used in our previous pepper-aroma research work (KORÁNY & AMTMANN, 1997) was that the meaning of the "x"-axis changed from sample type to sample type. As the retention time reference compounds were different in the pepper, wine, paprika, honey, medicinal herb, etc. samples, the relative retention time values had chemical information only within the diagram series of the same type of samples, and the aroma-spectra of the different samples (e.g. honey to wine or pepper to paprika etc.) were not comparable. The real problem was whether a general, always applicable, solution of classification and recognition existed or not. To answer the above question a new graphic procedure, the relative aroma-chromatogram construction method has been tried. In our work substitution of retention times depending on gas chromatographic conditions was performed by determining the relative position of the compounds related to normal C_{10}, C_{14} and C_{20} standards.

At first sight differences are dominant among the aroma-maps of the same-type samples, too (see Figs. 1, 2 and 3), but the thorough visual study of the figures brings an unexpected result. Similar patterns, polygons and straight lines occure in the diagrams. The explanation of this phenomenon is really hard because the amounts of fragrance compounds are influenced by so many factors, e.g. composition of soil, sunny

hours/year, annual rainfall, weather just prior to harvest, etc. On the other hand it is supposed, that the ratios of the main aroma and fragrance components of the plants are genetically coded and determined and are therefore a fixed characteristic of each plant. Obviously normalization suggests an overall and constant measurement efficiency for all components that is not necessarily the case, but the method does give good results in our hands. We assume, the method has found those substances the ratios of which do not change with the growing conditions. That could be a logical explanation for the occurrence of similar patterns in the aroma-maps.

Since the relationship or identity of the aroma characteristics causes the occurrence of very similar or the same "constellations" in the set of points, the identification can be performed precisely by searching for and finding similar polygons. No similarities can be observed among aroma-maps of different sample extracts nor among unrelated same-type samples, e.g. characteristic and non-characteristic acacia honeys, relative and non-relative lavenders, etc.

4. Conclusions

The present work contributes to the development of a method for the graphic recognition and identification of aroma patterns, and the following tasks were worked out: (a) the elaboration of sample preparation methods producing extracts that represent the samples' real aroma-character, (b) determination of the optimal GC separation conditions of flavour and fragrance compounds, (c) creation of a stationary phase dependent "absolute" x-axis by measuring the PTRIs in each chromatograhic run, (d) the identification of as many compounds as possible and matching the chemical structures to PTRIs by GC-MS, (e) conducting aroma extract identification experiments by the construction of *"constellation-maps"*.

The "polygonal" method has proved its abilities in the recognition and identification of honeys, wines, grape-musts and herb essential oils by providing ready visualisation of the aroma properties. The run by run PTRI determination and peak area normalization lead to nearly distortion-free measurement of the compound ratios, that are much more characteristic of the aroma patterns than the absolute amounts themselves. They also make possible sample identification by using an ordinary FID-equipped gas-chromatograph, because the degree of relationship or identity of the samples can be deduced from the presence of similar polygons. These measurements applying relative mass-spectra construction principals promise the possibility of paprika variety identification too, that is of primary importance in Hungarian red pepper production and export.

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