

## RECOGNITION EXPERIMENTS OF THE VINTAGE YEAR 1997 HOT AND RED PAPRIKA (*CAPSICUM ANNUUM*) VARIETIES GROWN IN KALOCSA

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Comparison of Hungarian ground red paprika volatiles of known origin (identical to cultivated varieties grown in Kalocsa, Hungary) and provenance has been performed following simultaneous distillation-extraction sample preparation. After polar phase capillary gas-chromatography, mass spectrometric identification of as many compounds as possible was carried out to precisely describe the aroma profile of the cultivars. For conceptualizing the results a data evaluation and interpretation method has been elaborated considering the component ratios that are much more characteristic of the cultivars than the absolute amounts themselves. Relative intensity interpretation of the peak areas (y-axis) and Programmed Temperature Retention Index (x-axis) measurement resulted in aromagrams individually characteristic of the cultivated varieties. In an identification experiment the cultivars have been recognized successfully by the graphic visualization of the results called aroma-spectra (by analogy to mass spectrometry) method.

**Keywords:** paprika volatiles, simultaneous distillation-extraction (SDE), capillary gas chromatography, mass spectrometry

“Odour impressions have always fascinated mankind. The sense of smell is either consciously or subconsciously with us every day. It controls our intake of food and our emotions” (OHLOFF, 1994). The quality of foods depends not only on their nutritional value but basically on their sensory value as well. In establishing the attractive character of foods, spices play a very important role by their aroma-quality and the composition of the fragrance compounds (KORÁNY & AMTMANN, 1997).

The main species of *Capsicum*, i.e. *Capsicum annuum* var. *grossum* S., *Capsicum annuum* var. *longum* and *C. frutescens* varieties are extensively used for their colour (BALAKRISHNAN et al., 1996), pungency (ESTRADA et al., 2001), distinct, taste and aroma (WHITFIELD & LAST, 1991). Paprikas (*C. annuum* L.) are consumed as immature (green) or mature (red) fruits, as fresh vegetables or as spices in foods bearing evident characteristic differences in their aroma and fragrance features (GOVINDARAJAN et al., 1987). In Europe, beside Spain and Turkey, Hungary is one of the most significant paprika growing and ground red paprika manufacturing countries, where *C. annuum* L.

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var. *longum* cultivars are grown for powder production. The main quality parameters of ground paprika products are the colour (CARVAJAL et al., 1998) and pungency (GOVINDARAJAN, 1986; GOVINDARAJAN et al., 1987). The primary aim of *Capsicum* drying is to conserve the perishable fruits, to reduce storage and to decrease the costs of transport (LUNING et al., 1995; RODRIGUES et al., 1999). The drying methods of the greatest significance are sun and hot-air drying (GOVINDARAJAN, 1985). The selection of the cultivars for commercial drying is traditionally based on the desired combination of intensity of colour (ABELLÁN-PALAZÓN et al., 2001), pungency and economic efficiency (GOVINDARAJAN, 1986; GOVINDARAJAN et al., 1987).

In the analysis of aroma compounds the most frequently applied sample preparation procedures are the solvent extraction, steam distillation, the combination of the two previous methods, head-space analysis and supercritical fluid extraction (SFE) as the newest one (KOBAYASHI & KAWAKAMI, 1991; ŠKERGET et al., 1998). In the last two decades headspace analysis became one of the most widely applied method for the isolation of volatile compounds of plant origin (IOFFE & VITENBERG 1984; LEAHY & REINECCIUS, 1984; ISHIHARA & HONMA, 1992; RUTH et al., 1995). Since complexity is the main distinctive feature of the essential oil constituents, and aroma profiles are highly characteristic of the spices, it is apparent to find a method focusing on the specialities of aroma structures. For this purpose gas-chromatography/mass-spectrometry is one of the best tools (KAMEOKA, 1986). In a representative work more than 125 volatile compounds have been identified (VAN STRATEN & MAARSE, 1991) in the fresh and processed paprika fruits. The aroma significance of these compounds is not yet clear in details (LUNING et al., 1994).

Recent qualifying methods that control only the visual purity and microbiological state of the paprika powders (KISKÓ et al., 1998) are not satisfactory. They give information neither on the quality nor on the quantity of the fragrance- and aroma-compounds that determine the real value of the spices. The lack of measuring methods capable of investigating the aroma-profiles of spicy and medicinal plants makes necessary the thorough research of the relating analytical fields.

Although the volatile composition is considered an important parameter of quality and identity, only few investigations have been conducted in the field of powdered red paprika aroma constituents (MATEO et al., 1997). In the present work the fragrance components of Hungarian paprika varieties cultivated in Kalocsa, both sweet and hot, were studied.

## 1. Materials and methods

### 1.1. Plant material

Red pepper samples of known origin (identical to cultivated varieties) and provenance, provided by the courtesy of Red Pepper Research-Development Ltd. (Kalocsa, Hungary) have been examined. Kalocsa and its surroundings represent one of the two

famous traditional Hungarian paprika growing and ground red pepper producing districts. The samples were as follows:

Sweet paprika cultivated varieties: Kalocsai-M-622, Szegedi-20, Kalocsai-801, Szegedi-80, Csárdás, Folklór, Remény, Rubin, Kármin, Zuhatag and Mihályteleki. Hot paprika cultivars were: Kalocsai-V-2 and Szegedi-178.

### 1.2. Chemicals, apparatuses

Chemical substances, standards and solvents used in our work were of “analytical”, “HPLC” or “GC” grade due to the requirements of the task and were purchased from Merck (Darmstadt, Germany), Carlo Erba (Milan, Italy) and Carl Roth (Karlsruhe, Germany). Although spectral clarity is not equivalent to chemical clearness, it is evident that transmittance of 90% at 200 nm wavelength ensures unusually high quality.

The glassware was of thermo resistant Pyrex quality matching the demands of an ordinary chemical laboratory. Distillation equipment and other glass tubes were Teflon-valve equipped. For the injection of the samples special precision GC syringes were used. The following chemicals and equipment were used:

1.2.1. *Solvents and chemicals.* *n*-Hexane, iso-octane, methanol, diethyl ether (HPLC grade), bidistilled water, boiling sand, sodiumsulphate.

1.2.2. *Glassware and tools.* Round bottom flasks (1 dm<sup>3</sup>), distillation equipment with condenser, Teflon-capped sample containers, GC syringes of 1, 5 and 10 µl capacity.

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

### 1.3. Sample preparation

The paprika samples (semifinal product) were kept at 5 °C in aroma-tight bags excluding light. Before taking the amount to be ground the whole sample was thoroughly mixed and homogenized. Grinding of the 100 g red pepper was performed by a laboratory mill type Lab. Mill-1 QC-114 (Labor MIM, Budapest, Hungary). Distillation was carried out immediately after grinding to prevent the loss of the most volatile compounds.

As the essential oil content of the paprika is so small that it does not form separate layer on the water surface 3 cm<sup>3</sup> of *n*-hexane was applied for the collection of the condensing volatiles.

The preparation procedure might be called the “collection of an average distillate” method. After internal standard addition (0.1 g benzyl alcohol to each 30 g paprika powder) in triplicate, ground paprika samples were poured into 3×500 cm<sup>3</sup> water and were distilled (in an apparatus described by HUNGARIAN STANDARD, 1978) into the same 3 cm<sup>3</sup> *n*-hexane to obtain a concentration high enough to capillary gas chromatography measurement. The distillation time was 1.5 h in every case. Having finished the distillation the extract was evaporated to 0.3 cm<sup>3</sup> end-volume and 0.1 cm<sup>3</sup> hydrocarbon standard (iso-octane containing C<sub>14</sub>, C<sub>16</sub>, C<sub>18</sub> and C<sub>20</sub> *n*-hydrocarbons and

benzyl alcohol, a solution chromatographed individually as the first sample of daily measurements) was added for Programmed Temperature Retention Index (PTRI) and recovery determination. This sample was gas chromatographed 9 times and the average was calculated. One microliter of the mixture was directly injected into the GC without any further treatment.

#### 1.4. GC-MS analysis

The measurements were performed under the following conditions:

Instrument	: Hewlett Packard 5890/ II GC-5971A MSD
Column	: 30 m×0.25 mm ID Supelcowax 10 (fused silica)
Film thickness	: 0.25 $\mu\text{m}$
Initial temperature	: $T_1 = 60\text{ }^{\circ}\text{C}$ ,
Temperature progr.	: $v_{\text{heat}} = 4\text{ }^{\circ}\text{C min}^{-1}$
Final temperature	: $T_2 = 280\text{ }^{\circ}\text{C}$ ,
Det.temp. (tf.line)	: $T_{\text{det}} = 280\text{ }^{\circ}\text{C}$
Carrier	: He, $v_{\text{lin}} = 30.0\text{ cm s}^{-1}$
Injector	: split/splitless, $p_{\text{in}} = 29\text{ kPa}$ , $T_{\text{inj}} = 250\text{ }^{\circ}\text{C}$
Injector mode	: splitless mode
Delay	: 0.35 min
Split ratio	: 100 : 1
Ion source	: EI, excitation energy 70 eV
Mass range	: $m/z = 35\text{--}350$
Scan speed	: 390 mass $\text{s}^{-1}$

## 2. Results and discussion

The primary results of the measurements were the total ion chromatograms (TIC) of the samples. For sparing space only 3 records of the examined 13 cultivars listed in the “Plant material” paragraph are shown in Fig. 1. Detailed study of the hot and sweet paprikas discovered characteristic differences among the samples in every region of the chromatograms. For instance, the ratios of pinene, myrcene, terpinene and dl-limonene eluting between 0 and 15 min are very different. In addition, many other differences can also be observed in all regions of the chromatograms. A general conclusion can immediately be drawn that pungent varieties are more fragrant and aromatic than the sweet ones considering both the number and the amount of the compounds. This statement can be generalized for all cases of our investigation. Breeders of Kalocsa explain this phenomenon by the loss of certain features during the breeding process that aims at forming the naturally hot original species into sweet cultivars with no capsaicin content.

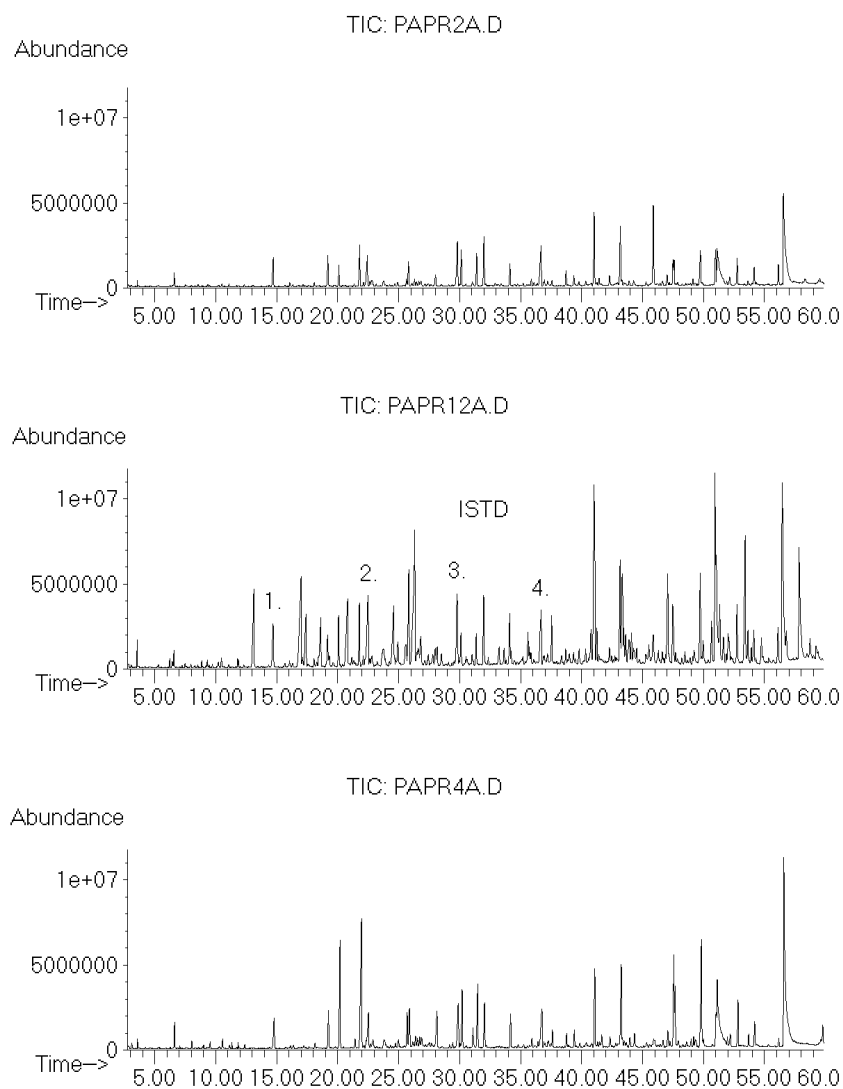


Fig. 1. Total ion chromatograms of aroma compounds extracted from sweet (upper: cv. Szegedi-20, lower: cv. Kalocsai-801) and hot (middle: cv. Kalocsai-V-2) paprika. For conditions see text

Table 1. Compounds identified in the ground paprika samples

PTRI	Chemical classes/Compound names	PTRI	Chemical classes/Compound names
<b>Terpenes and their derivatives</b>		1226	2-Hexenal, (E)-
1108	$\alpha$ -Pinene, (-)-	1286	Octanal
1180	Isoterpinolene	1385	Nonanal
1184	$\beta$ -Myrcene	1420	2-Octenal, (E)-
1198	$\alpha$ -Terpinene	1486	2,4-Heptadienal, (E,E)-
1211	dl-Limonene	1529	2-Nonenal, (E)-
1236	$\beta$ -Ocimene-X	1581	2,6-Nonadienal, (E,Z)-
1248	$\gamma$ -Terpinene	1611	3-Cyclohexene-1-acetaldehyde
1252	1,3,7-Octatriene, 3,7-dimethyl-	2008	Hexadecanal
1431	Linalool oxide(2)	2046	5-methyl-2-phenyl-2-Hexenal
1460	Longipinene	2099	16-Octadecenal
1541	Linalool	<b>Ketones</b>	
1567	$\alpha$ -Chamigrene	1311	Cyclohexanone, 2,2,6-trimethyl-
1590	(-)- $\beta$ -Elemene	1472	2-Nonen-4-one
1673	$\beta$ -Selinene	2001	2-Nonadecanone
1692	1- $\alpha$ -Terpineol	2093	2-Pentadecanone, ...-trimethyl-
1698	Aristolen	<b>Acids</b>	
1706	$\beta$ -Himachalene	2227	Decanoic acid
1711	Eremophilene	2381	Dodecanoic acid
1716	(+)-Aromadendrene	<b>Esters</b>	
1758	(-)-Aromadendrene	1771	Benzoic acid, 2-hydroxy-, methyl ester
1770	(E)- $\alpha$ -Bisabolene	1799	Dodecanoic acid, methyl ester
1794	Nerol	2028	Tetradecanoic acid, ethyl ester
1839	trans-Geraniol	2040	Pentadecanoic acid, methyl ester
2017	d-Nerolidol	2170	Hexadecanoic acid, methyl ester
2032	cis-Caryophyllene	2200	Hexadecanoic acid, ethyl ester
2048	Elemol	2252	Heptadecanoic acid, methyl ester
2066	$\gamma$ -Gurjunene	2254	Heptadecanoic acid, methyl ester
2104	Chromolaenin	2332	Octadecanoic acid, methyl ester
2122	$\delta$ -Selinene	2362	Octadecanoic acid, ethyl ester
2155	Widdrene	2372	9-Octadecenoic acid (Z)-, ethyl ester
2163	$\gamma$ -Selinene	2385	9,12-Octadecadienoic acid ... methyl ester
2263	Liguhodgsonal	2407	Ethyl linoleate
2274	cis-Farnesol	2431	Octadecatrienoic acid, (Z,Z,Z)-Me-ester
2291	(E,E)-Farnesylacetone	<b>Benzene derivatives</b>	
2336	Spathulenol	1173	Benzene, 1,4-dimethyl-
<b>Alcohols</b>		1516	Benzaldehyde
1221	2-Hexanol	1724	Benzaldehyde, 2,5-dimethyl-
1301	1-Pentanol, 4-methyl-	1930	Benzene, (1-butyl-octyl)-
1549	1-Undecanol	1949	Benzene, (1-propyl-nonyl)-
1595	3-Cyclohexen-1-ol, 4-methyl-1-(1-...)-	1978	Benzene, 1-ethyl-3,5-diisopropyl-
1958	2-Tetradecanol	1998	Benzene, 1-ethyl-3,5-dimethyl-
2113	1-Hexadecanol	<b>Naphthalene skeleton</b>	
2154	(4aR*,9aS*)-...a-Octahydro-...-5-ol	1438	Naphthalene, tetrahydro-1,1,6-trimethyl-
2422	(Z)6,(Z)9-Pentadecadien-1-ol	1686	Naphthalene, ...-tetrahydro-trimethyl-
<b>Aldehydes</b>		1720	Naphthalene, ...-octahydro-4a,8-dime..
1078	Butanal, 3-methyl-	1822	Naphthalene, 1,2-dihydro-...-trimethyl-
1139	Hexanal	2128	Naphthalene, 1,2,3,4,4a,5,6,8a-octahy
1201	Heptanal	2309	Naphthalene, 2-decyldecahydro-
		2349	1-isopropyl...-Naphthalene

PTRI	Chemical classes/Compound names	PTRI	Chemical classes/Compound names
	<b>Hydrocarbons</b>		
1096	1,3-Cyclopentadiene, 5-tert-butyl-	1741	Paprika-D
1273	Decane, 2,2-dimethyl-	1849	Paprika-E
1279	Decane, 2,2,8-trimethyl-	1911	Paprika-F
1295	Cyclohexane, 1,4-dimethyl-, trans-	1927	Paprika-G
1349	Tridecane	1970	Paprika-H
1349	Tridecane, 2-methyl-	2111	Paprika-I
1367	Cyclododecane	2288	Paprika-J
1453	Tetradecane, 2-methyl-		<b>Sulphur compounds</b>
1456	Tridecane, 3-methyl-	2174	2-Ethylthiobenzothiophene
1460	1,3,5,8-Undecatetraene	2178	1-Ethylthiobenzothiophene
1466	3-Heptene, 2,6-dimethyl-	2182	3-Ethylthiobenzothiophene
1468	Cyclopropane, 1,1-dimethyl-2-nonyl-	2235	1a,7b-dihydroazirine(.)benz...dithiophene
1500	Pentadecane		<b>Phenolic compounds</b>
1520	Cyclotetradecane	1903	Phenol, 2,6-bis(1,1-dimethylethyl)4-methyl-
1558	Pentadecane, 4-methyl-	2243	Phenol, 2,4-bis(1,1-dimethylethyl)-
1563	Pentadecane, 2-methyl-		<b>Nitrogen containing substances</b>
1570	Pentadecane, 3-methyl-	1169	1H-Pyrrole, 1-methyl-
1627	3-Hexadecene, (Z)-	1192	2-Dimethylaminopyridine
1643	1-ethynyl-2-methyl-1(E)-Cyclododecene	1461	Pyrazine, tetramethyl-
1649	1-Hexadecene	1492	1,2,4- decahydro-Methenoazulene
1661	Hexadecane, 2-methyl-	2065	5-acetyl-6-methyl-Benzimidazolone
1707	Heptadecane	2145	2,4,6-Trimethyl-1,3-benzenediamine
1749	1-Octadecene	2335	1H-Indole
1761	Heptadecane, 2-methyl-	2381	6-acetyl-7-hydroxy-2,2-dimethylbenzopyran
1878	1-Cyclohexyl-1-butyne		<b>Others</b>
1899	Nonadecane	1235	2-pentyl-Furan
2078	Heneicosane	1250	3,5-dimethyl-Phenol
2151	1-ethyl-2-methyl Cyclododecane	1407	4-Ethyl-2,6-xyleneol
2163	Docosane	1638	1H-Benzocycloheptene, ...-octahydro-...,
2195	Cyclotetradecane	1892	3-(4-Methoxy...-5-methylphenyl)propene
2207	1,13-Tetradecadiene	1895	6-Acetyl-5-hydroxy-1,8-dimethyl-1,2,3
2213	7-Hexadecene, (Z)-	2126	1,1'-Biphenyl, 3-chloro-4-methoxy-
2235	Cyclohexadecane	2189	Dihydro-6,7-dimethyl...[1,2-b]-furan
2246	Tricosane	2386	Nootkatone
2280	4-Hexadecen-6-yne, (E)-		<b>Carotenoid derivatives</b>
2293	5-Eicosene, (E)-	1329	6-methyl-5-Hepten-2-one
2295	Cyclotetradecane	1615	$\beta$ -Cyclocitral
2298	3-Tetradecen-5-yne, (E)-	1814	$\beta$ -Damascenone
2315	Cyclohexadecane	1825	Dihydro-.beta.-ionone
2323	1,4-Cyclononadiene	1841	$\alpha$ -Ionone
2374	3-Eicosene, (E)-	1923	$\beta$ -Ionone
2393	3-Octadecene, (E)-		
2402	Nonadecane		
	<b>Unknown compounds (with trivial names)</b>		
1315	Paprika-A		
1496	Paprika-B		
1600	Paprika-C		

The detailed chemical study of the sample extracts was performed by the MS identification of as many components as possible. It proved that hot varieties are richer both in the primary compounds of plant origin (terpenes, sesquiterpenes and their derivatives) and in the secondary constituents formed by enzymatic (e.g. lipoxygenase) and chemical processes like Maillard and Strecker degradation, oxidative decomposition of carotenoids, etc. The identified compounds sorted into chemical classes are listed in Table 1.

The table shows that the compounds belong to many different chemical classes. The sensory significance of that has not been clarified yet (LUNING et al., 1994; MATEO et al., 1997). In cases of low intensity peaks the search in the Wiley138.L spectral library was performed manually after proper background correction of the apex-spectra, so the reliability of the identification is suitably high. The names of the compounds in the list are the ones used by the spectral library. In certain cases (from Paprika-A to Paprika-J) when the research algorithm was unable to recognize the constituents, names were given by us (a special Pepper spectral library has been created with these trivial names) to control if these substances are common components of the paprika samples or not. Most of them proved common compounds and were identifiable under the trivial names given by us.

Our work aimed at mapping the aroma and fragrance structure of the Hungarian ground red paprika cultivars. This task could be considered more or less completed by the identification of 173 compounds with a match quality of 80% or better. The detailed chemical knowledge of the components could not solve the second important problem of the work, namely the recognition of the different cultivated varieties. In our work the identification of the possible greatest number of compounds strategy has been followed, assuming that maximal known substances allow to find "marker" constituents characterizing individually the cultivar in question. This assumption seemed to work evaluating the records in pairs, comparing the compound lists of two cultivars to each other, but was not applicable for the whole stack of data. Unfortunately, in most of the cases components that could distinguish two varieties were common with others.

General and effective evaluation procedures of so confused and fuzzy data sets are the principal component analysis or any other mathematical statistical interpretation methods (cluster analysis, pattern recognition). These merely mathematical solutions, however, do not care about the standardization of the analytical work. Consequently, their use requires the case by case education of the evaluating system and the results cannot be generalized easily and compared to that of other laboratories. In current work similarly to our previous investigations conducted in wine (KOVÁCS et al., 1999), medicinal herb and honey (KORÁNY et al., 2000) volatile examinations, the elaboration of a data interpretation method, taking the component ratios into account and based on the principals used in relative mass spectra construction, has been tried.



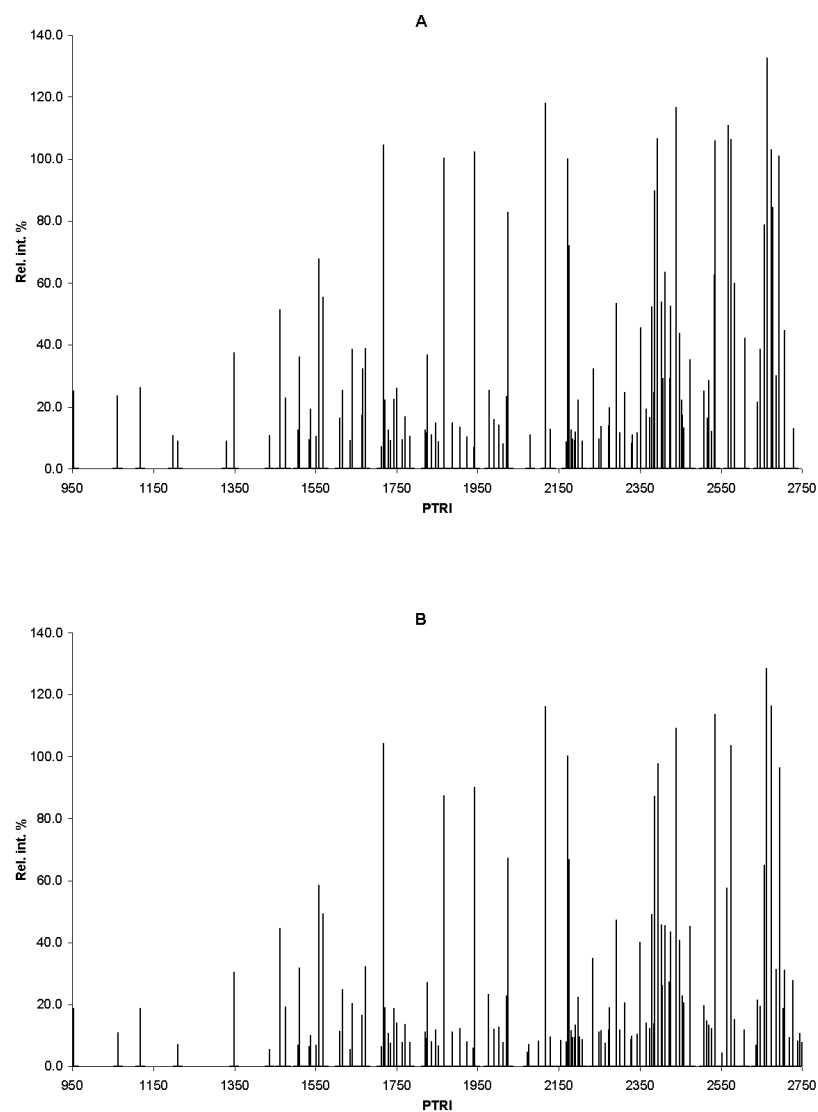


Fig. 2. The relative aromagrams of two (A and B) unknown hot paprika samples identified successfully, they proved variety Kalocsai-V-2 having broken the codes

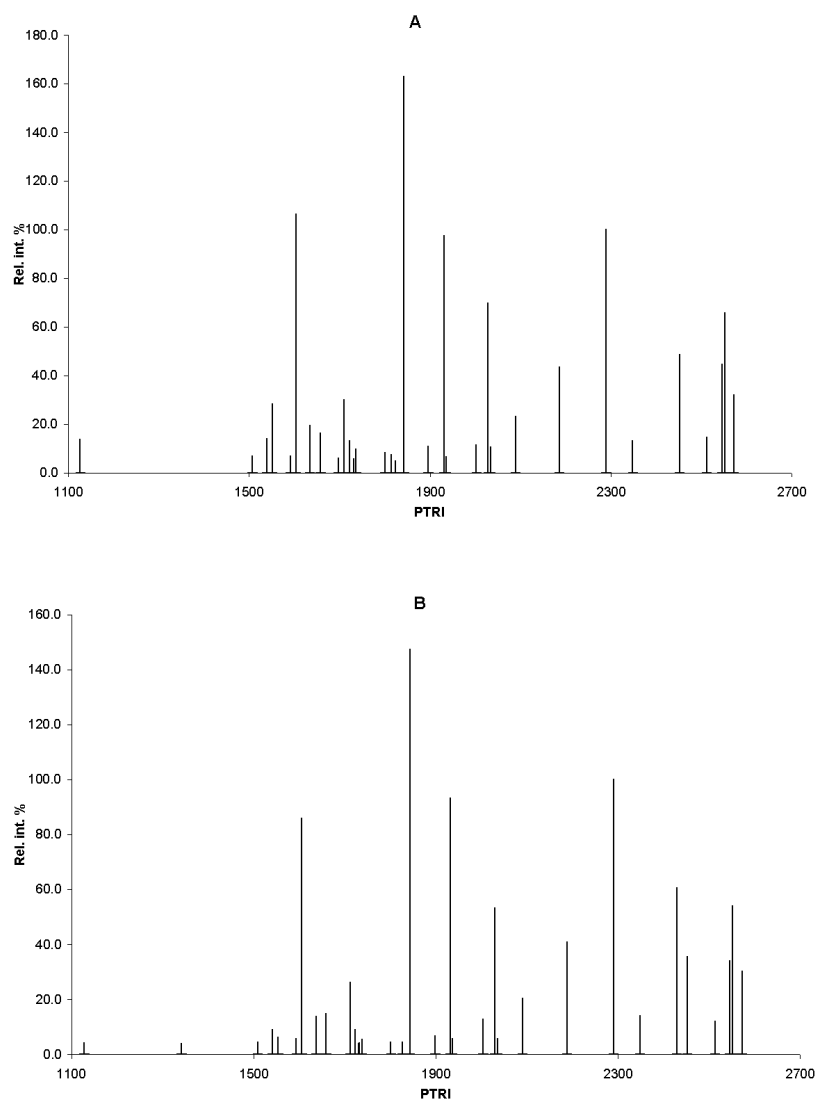


Fig. 3. The identification of two sweet paprika samples. Both (A and B) proved to be Szegedi-20 variety by their "aroma-spectra"

The results show that the reproducibility of the PTRI values is  $\pm 2$  index units with respect to the mean and they are in accordance with literature (LUNING et al., 1995). In case of minor components (under 3 rel.%), the reproducibility of relative intensities is approximately  $\pm 10\%$ , for medium components of 3–8 rel.% it is between  $\pm 5$ –8% and for major constituents (above 8 rel.%) it is lower than  $\pm 5\%$ . The values are expressed in % of the mean of the compound in question. Benzyl alcohol internal standard addition prior to sample preparation allowed measuring the recovery of the whole analytical process. It varied between 36 and 52% that seems rather low, but the great number of chromatographic peaks ensures the sensitivity of the measurements that is necessary to describe the aroma structures of ground red paprikas in detail and with the complexity that is necessary to successful recognition.

Depicting the relative intensity – PTRI data pairs in two dimensional coordinate systems led to mass spectra-like diagrams. In these pictures the relationship or identity of the samples can be recognized by the appearance of similar bands of bar-structures. The transformation of the chromatograms into “aroma spectra” are shown in Fig. 2 and Fig. 3. The similarity, what’s more the identity of the samples is obvious and can immediately be established. We found the relative aroma spectra method capable of recognizing the cultivated paprika varieties grown in 1997 in three cases of an experiment (two of them shown in Figs 2 and 3) that was performed with coded samples. One identification trial failed (not shown for space reasons) because of the presumable inhomogeneity of the paprika (called semi-final product by the producer) samples.

The conceptualization of the recognizability phenomenon is not simple and self-explanatory. Although the metabolic repertoire of biological organisms is genetically coded, it is plausible to argue that the aroma materials of plant products are genetically fixed characteristics (EVANS, 1996). A constant aroma profile, however vaguely defined, implies that the ratio of the aroma-determining components is constant within a certain range. In fact, this is what one can see on the aroma maps presented here, with the caveat that there is no warranty that the extraction method used here recovers all aroma components. On the other hand, it is natural to expect that a variety of non-genetic factors (such as seasonal, agronomic, technological conditions, etc.) will influence the aroma component ratios, sometimes even distorting them beyond the range accepted as characteristic by expert tasters. Therefore, this method (in fact any objective method) will have to be carefully calibrated against results obtained by tasting panels.

### 3. Conclusions

The comparison of hot and sweet ground red paprika samples of identical cultivated varieties and provenance shows that pungent cultivars (close to wild, natural species) are more aromatic and fragrant than bred sweet ones. This phenomenon can be observed both in the number and in the amount of the compounds, both in the case of the primary compounds of plant origin and in the case of the secondary components. The

constituents bearing the fresh (green) bell pepper note are practically missing from the aroma map. Finding “marker” compounds characteristic of the cultivars individually brought no results. Transformation of the absolute chromatograms into relative “aromagrams” by run PTRI measurement and normalization of the peak areas to methylhexadecanoate resulted in the visual identification of the cultivars of the 1997 harvest. Present graphic method has proved useful in the recognition and identification of honeys, wines, grape-musts and herb essential oils as well. Identification experiments with the same paprika cultivars of next year are under way.

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