Visualization of the Gas Chromatography/Mass Spectrometry Data of Muscat Ottonel Must and Wine Measurements

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Summary: The lack of interpretation methods useful in evaluating the aroma-profiles of wines makes it necessary to thoroughly investigate alternative evaluation procedures. By adding three appropriate normal hydrocarbon standards to all sample extracts, measuring the Programmed Temperature Retention Indexes of the components and by normalizing the peak areas to that of the l-alpha-Terpineol (in musts) or Benzeneethanol (in wines), the aroma features could be visualized. The relationship or identity of the aroma patterns could be deduced from the presence or absence of similar polygons in the "constellation-map" of the components.

Introduction

Pollinated flowers of the grapevine grow ripe autumn by autumn. Flavour, fragrance, aroma and colour compounds, all treasures of the whole year's sunshine hiding in the ripe grapes have to be transferred into the wine as perfectly as possible. Tasting a wine we enjoy the unity of its flavour, taste and sight. Fortunately no one thinks of the complex effect caused by several thousands of organic and unorganic components.

The quality control of wines and the determination of certain wine constituents are the tasks of analysts. Both classic and modern instrumental methods are used in the measurements of food products and in wine analysis. The development of instrumental analysis brought serious changes in the speed and reliability of the determinations. In most countries it is impossible to sell wines without a quality certificate obtained by up-to-date methods. The investigation of toxic metals for instance Pb, Cd, Hg is an ordinary demand. Exclusion of the use of artificial aroma and colourant substances and the chromatographic proof of originality are expected in several countries (Italy, France). Some quality parameters can be defined well and are prescribed in standards. Others may depend on many factors like provenance, vintage cultivated variety ...*etc.* and can not be standardized. Currently qualification and classification of wines are performed by educated experts. At present state of analytics it is not possible to replace their work by instrumental measurements, but all opportunities must be taken to support and prove their results by objective analytical investigations *(Rapp, A.* 1988).

Transforming absolute gas chromatograms into relative ones by normalizing the individual areas to that of the largest peak can almost ensure a distorsion free measurement of the compound ratios in the linearity range of the instrument. This can also compensate for the effect of sample preparation to an extent not worse than the internal standard method. These relative chromatograms bear the same advantages that relative mass spectra do when compared to the absolute ones and can be handled identically from the physical point of view. The main difficulty is, that retention times are highly dependent on gaschromatographic parameters and independent of the chemical quality among different type samples. That means, totally different compounds may have the same retention time on the same column under different conditions (Kameoka, H. 1986). The correct solution to this problem is to use **P**rogrammed **T**emperature **R**etention **I**ndex measurements.

Theoretical

In gas chromatography at constant speed column heating the members of homologous series (n-alkanes, olefins...*etc.*) elute equidistantly, their 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 **R**elative **R**etention **T**imes (RRT, retention times 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 run togehter with the alkanes 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 of the linear function. The procedure described above means the aroma and fragrance compounds' relative position determination related to n-alkanes and called PTRI measurement. Under fixed analytical conditions the PTRIs characterize the components almost as individually as their chemical names.

The other problem in the evaluation of chromatographic runs is, that peak areas depend both on the sample preparation efficiency and peak detection sensitivity namely on the integration parameters to too high an extent. The problem is analogous to the spectral interpretation difficulties in mass-spectrometry, where results are dependent on the number of molecules in the ion source and the electron multiplier sensitivity as well. The normalized spectrum creation method transforms the absolute peak areas proportional to the number of fragment ions into relative ones by dividing them by the area of the largest peak. This solution substitutes the absolute peak area measurement with area ratio determination, where numerators and the nominator of the fractions are equally affected by the conditions (number of molecules, sensitivity...etc) of measurement. Thus the area ratios remain constant in the linearity range of measurement making the relative spectra always recognizable.

The same method has to be adopted in gas chromatography as well, because every chromatogram contains a largest peak suitable for the described normalization. The procedure gives the possibility of increasing or reducing the detected peak numbers without affecting the existing results. It is much more flexible and precise than the area-percent method and does not depend on the peak detection sensitivity. A further advantage 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. Obviously this statement suggests an overall and constant measurement efficiency for all components that is not necessarily achieved, but the method does give good results.

Material and methods

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

The glassware used was of thermoresistant Pyrex quality. Distillation equipment and other glass tubes were equipped with teflon-valves. Special precision GC syringes were used for injection of samples. The following chemicals and equipment were used:

Solvents and chemicals:

n-hexane, iso-octane, methanol, diethylether (HPLC grade), doubledistilled.water, boiling chips, sodium sulphate.

Glassware and tools:

round bottom flasks (1 L), distillation equipment with condenser, teflon-capped sample containers, GC syringes of 1, 5 and 10 µl capacity.

Instrumentation:

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

Samples (must and wine) of known origin and provenance provided by courtesy of farmers and primary producers have been examined. They are as follows:

Muscat ottonel must and wine, from Gyöngyös region,

Preparation of the wine samples required the combination of distillation and extraction. In the first step the alcohol from 500 mL wine together with the volatiles was distilled with 80 mL of condensate (*i.e.* 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 from three 500 mL samples of the same wine have been combined and extracted three times with 80 mL of specially cleaned n-pentane. The pentane extract was evaporated to 0.5 mL in a cold N_2 stream and brought up to 1 mL with iso-octane containing the C_{10}, C_{14} and C_{20} n-hydrocarbon standards. The samples were gas chromatographed using 5 parallel injections and the average of the 5 runs was calculated.

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

Instrument : Hewlett Packard 5890/ II GC - 5971A MSD
Column : 60 m x 0,25 mm Supelcowax 10 (fused silica)
Film thickness : 0.25 µm
Initial temperature:
$T_1 = 60°C$, $t_1 = 5.00$ min
Temperature progr.:
$v_1 = 4.0°C/min$, $T_2 = 280°C$
Final temperature:
$T_2 = 280$ °C
Det.temp (tf.line):
$T_{det} = 280$ °C

Carrier:
He, 155 kPa, const. pres. mode, 29.6 cm/s

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:
3.9 D/s

Results and discussion

The great separation power of the long capillary and the excellent features of the GC-MS equipment used in our experiments resulted in chromatograms of high resolution and repeatibility as shown in *Figure 1*. The upper part of the figure depicts a run of the must, the lower that of the wine sample. The compounds elute with symmetrical peak shape and can be measured well compared to the background.

In the evaluation procedure as many components as possible listed in *Tables 1* and *2* were identified by the mass spectrometer. All substances found in the must should be called primary because they originate from the plant, but only the terpene and terpene derivatives (*e.g.* Linalool and l-alpha-Terpineol) are considered really characteristic of the *Muscat ottonel* variety. Chemical substances in "*Italic*"occure more than once in the samples because of the great similarity of the terpene spectra and the low absolute resolution (ΔM = 0.5 Dalton) of the mass spectrometer. Consequently, their identification is uncertain inspite of the high match quality and suggests that they are close relatives only. The components in "*bold*" are primary ones and can repeatedly be recognized in the same places (PTRIs) of the chromatograms in each sample, so their presence is certain. Eleven of these components (*bold* and *underlined*) are common to both wine and must. Due to the high concentrations of these compounds both the must and the wine bore excellent sensory features.

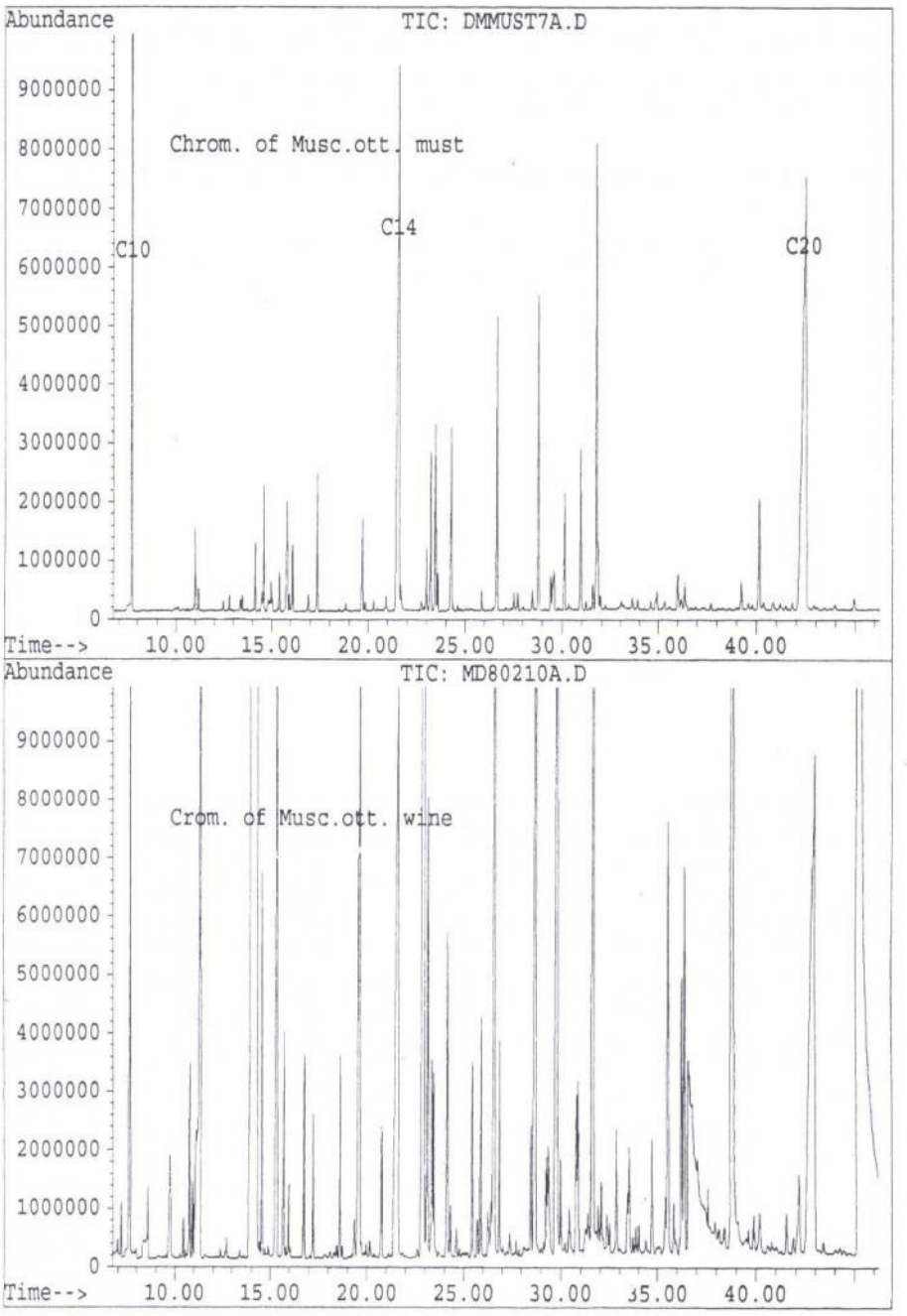

Figure 1 – Representative chromatograms of Muscat ottonel must and wine

File	: D:\RESEARCH\DATA\WINE\DEX\97AUTUMN\MD80210A.D
Operator	: Kovacs, scan, slss, AEPC155kPa
Acquired	: 11 Feb 98 9:49 am using AcqMethod AROMSLSS
Instrument	: 5971 – In
Sample Name	: Muscoaft2minbottl (3x500mlsltdext, iC8+3std) 1u
Misc Inf	: NSpwax60mx0.25IDchAEPCconspres155kPa
Vial Number	: 1

The greatest disadvantage of relative retention time *versus* relative peak area diagrams described in our previous aroma research work (*Korány, K., Amtmann, M.* 1997.) was, that the meaning of the "x"-axis changed with each sample type. The problem could be overcome by adding normal-alkane standards to all sample extracts and measuring the **P**rogrammed **T**emperature **R**etention **I**ndexes of the components in each chromatographic run as discussed in the Theoretical chapter.

Table 1 Identification list of Muscat ottonel must completed with the PTRIs

Index	Compound	Qual
934	Acetic acid, ethyl ester	87
936	Ethane, 1,1-diethoxy-	90
951	Ethanol	90
1029	Butanoic acid, ethyl ester	95
1058	Butane, 1,1-diethoxy-3-meth	78
1063	1-Propanol, 2-methyl-	83
1090	Pentane, 1-(1-ethoxyethoxy)	78
1096	Muscatmust-A (corr.)	95
1101	Muscatmust-B	98
1107	1-Butanol, 3-methyl-, acetat	91
1139	*.alpha.-Terpinolene**	93
1147	**.beta.-Myrcene**	95
1167	**.alpha.-Terpinene**	98
1189	1-Butanol, 3-methyl- (impur	83
1199	Muscatmust-D (corr)	95
1205	*p-Mentha-1,5,8-triene*	87
1211	Muscatmust-C	95
1222	Hexanoic acid, ethyl ester	97
1234	Muscatmust-D (corr)	94
1237	**.gamma.-Terpinene****	95
1241	**1,3,6-Octatriene,3,7diMe*****	97
1263	Acetic acid, hexyl ester	90
1278	**.alpha.-Terpinolene**	98
1314	*Linalool deriv. in Muscat w.*	96
1320	Muscatmust-G	94
1335	6-methyl-5-Hepten-2-on	93
1339	Ethyl-lactate	83
1344	1-Hexanol	83
1349	Muscatmust-H	94
1352	**Rose oxide**	94
1389	Octanoic acid, methyl ester	81
1433	2,5,7-TriMe-1,2,3,4-tetr.	86
1722	Octanoic acid, ethyl ester	96
1445	**Linalooloxide (2)**	90
1452	**p-Mentha-1,5,8-triene**	94
1456	Naphtalene, 1,2,3,4-tetrahy	93
1474	**trans-Linalooloxide**	90
1478	*Geraniol (deriv.)*	90
1492	**cis-Ocimene**	95
1510	*Geraniol (deriv.B)*	90
1520	**(+)-m-Mentha-1,8-diene**	95
1534	Benzaldehyde	97
1543	**Linalool**	95
1551	1-Octanol	83
1565	Benzene, 4-(2-butenyl)-1,2-d	92
1576	Benzene, 1-ethyl-3,5-dimeth	90
1604	Muscatott-A	97
1622	3-Cyclohexene-1-acetaldehy	80
1626	Muscatmust-N	93
1633	Decanoic acid, ethyl ester	97
1640	*5,7-Octadien-2-ol, 2,6-dimet*	91
1664	*5,7-Octadien-2-ol, 2,6-dimet*	91
1667	Butanedioic acid, diethyl est	94
1688	Muscatmust-O	91
1695	**l-.alpha.-Terpineol**	91
1742	Naphtalene, 1,2-dihydro-1	92
1776	**Nerol**	87
1777	Muscatmust-S	95
1803	Acetic acid, 2-phenylethyl e	90
1811	**.beta.-Damascenone**	91
1815	Benzene, 2-(1,3-butadienyl)	96
1821	**trans-Geraniol**	94
1828	Hexanoic acid	90
1852	Benzenemethanol	91
1900	Benzeneethanol	97
1914	4,5,9,10-dehydr.-Isolongifol	90
1930	6-Chloro-4,5-dimethyl-2-(d	98
1959	4,5,9,10-dehydr.-Isolongifol	95
2040	Octanoic acid	90
2252	Phenol, 2-methyl-5-(1-met	87

* Copounds in *Italic* are uncertainly identified (see: text on the bottom of page 4).
** Copounds in **bold** are common in must and wine.
*** Compounds in **bold** are identifiable certainly

Table 2 Identification list of Muscat ottonel wine completed with the PTRIs

Index	Compound	Qual
951	Ethanol	90
1055	*.alpha.-Terpinene*	80
1071	Hexanal	93
1084	*dl-Limonene*	93
1097	Muscatmust-A	98
1101	Muscatmust-B	98
1120	*1,3-Cyclohexadiene, 1,*	83
1137	*.alpha.-Terpinolene*	97
1145	**.beta.-Myrcene**	91
1149	**.gamma.-Terpinene**	87
1164	**Isocineole**	89
1165	.alpha.-Terpinene	97
1177	1-Butanol, 3-methyl-	78
1185	**dl-Limonene**	96
1203	*p-Mentha-1,5,8-triene*	81
1220	*.alpha.-Pinene*	90
1233	Muscatmust-E	99
1235	**.gamma.-Terpinene**	96
1239	*.Delta.3-Carene*	95
1261	Muscatmust-F	98
1275	**.alpha.-Terpinolene**	97
1282	**Isoterpinolene**	91
1321	Muscatmust-G	98
1334	6-Me-5-Hepten-2-one	94
1344	1-Hexanol	83
1348	Muscatmust-H	95
1361	Muscatmust-I	98
1365	Benzene, 1-methyl-3-	90
1371	*2,4,6-Octatriene, 3,4-*	93
1378	3-Hexen-1-ol, (Z)-	93
1391	2-Nonanone	80
1396	Nonanal	96
1414	*p-Mentha-1,5,8-triene*	81
1431	2,5,7-TriMeL-1,2,3,4-t.H.	80
1437	*p-Mentha-1,5,8-triene*	96
1444	*p-Mentha-1,5,8-triene*	89
1448	**Linalooloxide (2)**	90
1451	**p-Mentha-1,5,8-triene**	95
1455	Naphthalene, 1,2,3,4-	97
1461	Phenol, 4-(1,1-	86
1466	2-Furancarboxaldehyde	90
1477	*Neroloxide (deriv.)*	98
1485	Muscatmust-J	96
1490	Naphtalene, 1,2,3,4-	87
1499	Benzene, 1,2,3,4-tetramethyl	93
1523	Muscatmust-I	99
1527	3,6-DiMe-2,3,3A,4,5,7A	87
1540	**1-Oxaspiro(4,5)dec-7-ene**	94
1546	**Linalool**	95
1556	1-Octanol	90
1567	Naphtalene, 1,2,3,4-	95
1572	Muscatmust-L	99
1578	Muscatmust-M	98
1591	Naphtalene, 1,2,3,4-	91
1599	2-Cyclopentene, 1-	80
1608	Musc(nat.)ref.	97
1626	Muscatmust-N	97
1630	Muscatmust-N	94
1631	4,4-Dimethyl-6-methylene-	83
1640	Decanoic acid, ethyl ester	96
1647	**Tricyclene**	89
1671	*Tricyclene*	86
1679	*cis-.beta.-Terpineol*	90
1688	Muscatmust-O	97
1695	**l-.alpha.-Terpineol**	86
1700	*Isoterpinolene*	83
1733	Naphtalene, 6-(1,1-	90
1747	Naphtalene, 1,2-dihydro-1,1	96
1754	Muscatmust-P	96
1774	Muscatmust-R	96
1782	Muscatmust-P	96

(continued overleaf)

Table 2 Identification list of Muscat ottonel wine completed with the PTRIs

Index	Compound	Qual
(folytatás Table 2)		
1782	**Nerol**	95
1793	**(+)-6-exo-Hydroxicamphene**	80
1813	**.beta.-Damascenone**	91
1817	1-(2,4,6-Trimethylphenyl)	91
1821	**trans-Geraniol**	91
1824	Phenol, 5-methyl-2-(1-	80
1873	Nonadecane	91
1885	1-benzylidene-2,2,3,3-tetra	83
1889	1H-Inden-1-one, 2,3-	81
1928	1H-Inden-1-one, 2,3-dihydro-	94
1946	*p-Mentha-1(7),8(10)-*	93
2017	(E,E)-2,5-Diphenyl-2,4-hex	90
2032	Docosane	91
2040	Octanoic acid	83
2053	1H-Inden-1-one, 2,3-	90
2087	*.beta.-Maaliene*	90
2103	Naphtalene, 1,2-dihydro-	80
2148	Hexadecanoic acid, ethyl	95
2175	Phenol, 2,4-bis(1,1-	91
2183	Decanoic acid	95
2350	Dodecanoic acid	90
2545	Tetradecanoic acid	90
2674	Hexadecanoic acid	96

In *Figure 2* the relative aromagrams of *Muscat Ottonel* must samples are shown. The grapevine was harvested on the same farms of Gyöngyös region – a traditional wine producing county of Hungary – in 1996 and 1997. For normalization the largest peak area of l-alpha-Terpineol was used. On the vertical axis relative peak area data are depicted as percents of l-alpha-Terpineol. At first sight differences are dominant due to vintages, 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 very hard because the amounts of fragrance compounds are influenced by 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 *(Evans, W. C.* 1996.). We assume, the method has

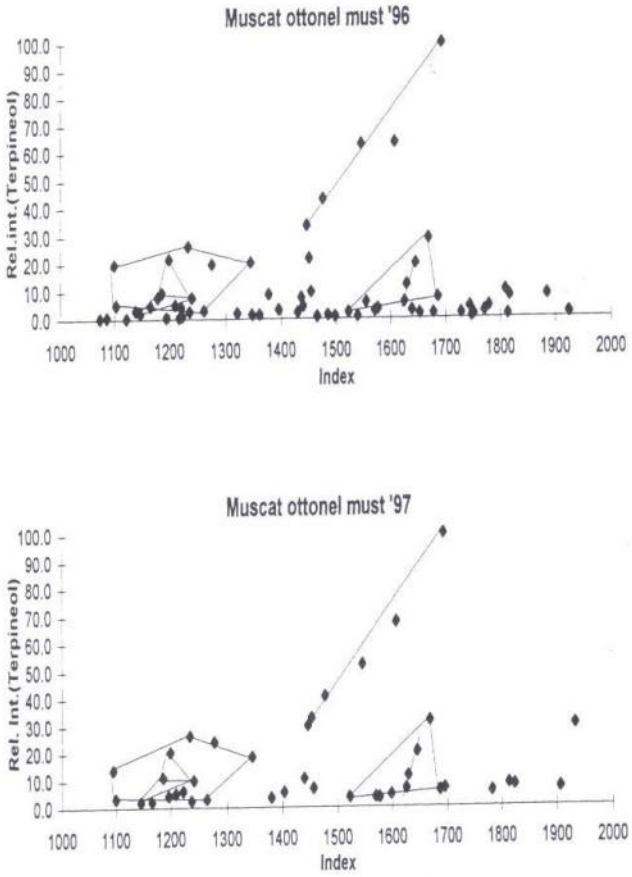

Figure 2 The constellation map of Muscat ottonel musts

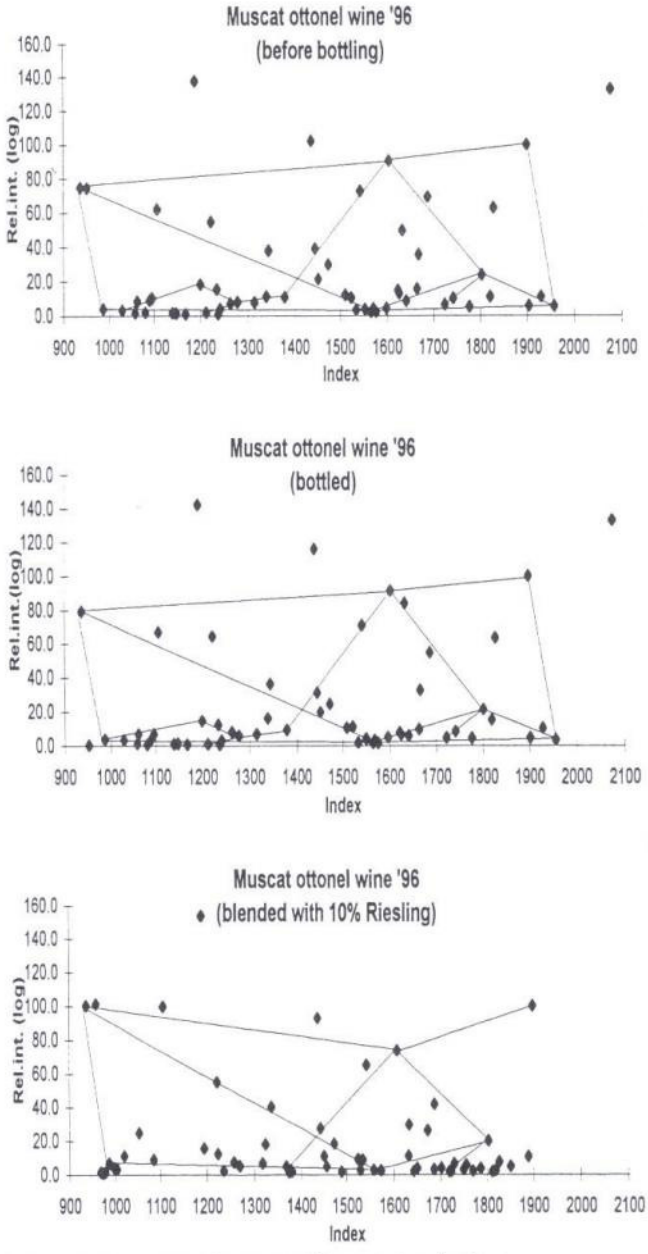

Figure 3 The constellation map of Muscat ottonel wines

found those substances the ratios of what do not change with growing conditions. That can be a logic explanation for the occurrence of similar patterns in the aroma-maps.

Compared to the must diagrams there are two main differences in the wine maps. The amount of 3-methyl-Butanol-1 is so high in the wines that it presses all other compounds into the Index-axis. Therefore another normalizing compound of medium quantity had to be chosen. It is the always present Benzeneethanol produced by yeasts. Normalization by this component causes higher values than 100 % for certain substances. To make these results depicted a logaritmic transformation was introduced for them.

In case of wines the aroma-maps are more complex, since yeasts contribute to the aroma-structure to a great extent. The results of the 1996 and 1997 vintages were uncomparable because different yeast strains were used for fermentation in the two years. Therefore the effect of bottling was studied shown in *Figure 3*. This operation contains a filtering step that may cause deep changes in the aroma-structure if unprofessionally carried out. The figure proves that the filtration operation was led properly, no changes can be observed in the compound ratios. The effect of blending with 10% Riesling causes slight differences in the maps. It must be admitted the sensitivity of the measurement should be higher and makes us to develop the method.

Conclusions

In developing an analysis-based method for the recognition and identification of must and wine aroma patterns several tasks have been accomplished: (a) development of a sample preparation method producing extracts that represent the samples' real aroma-character, (b) determination of the optimal GC separation conditions for flavour and fragrance compounds, (c) creation of a stationary phase dependent "absolute" x-axis by measuring the PTRIs in each chromatograhic run, (d) identification of as many compounds as possible and matching the chemical structures to PTRIs by GC-MS, (e) conducting recognition experiments by the construction of *"constellation-maps"*.

The run by run RTRI determination and peak area normalization leads to the nearly distortion-free compound ratio measurement, that is much more characteristic of the aroma patterns than the absolute amounts themselves. They make possible the sample identification by an ordinary FID equipped gas chromatograph as well, because the degree of relationship or identity of the samples can be deduced from the presence of similar patterns. Our results obtained by applying relative mass-spectra construction principals promise the possibility of wine recognition and identification, that is of primary importance in Hungarian wine production. The new method of visulizing aroma properties has proved its abilities in the recognition and identification of honeys (Akacia, Tilia) and herb (Lavender, Achilea) essential oils as well.

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