Characterization of volatile organic compounds in Slovak Tokaj wines

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Summary
Slovak Tokaj liquor wines are made of grapes infected by *Botrytis cinerea*, which are characterized by unique aroma. Despite their uniqueness, information about their chemical composition, the presence of organic compounds responsible for their aroma or taste, as well as suitable analytical procedures for their determination have not been reported. Thus, aim of this work was to characterize organic compounds in Slovak Tokaj liquor wines by liquid-liquid extraction and comprehensive gas chromatography coupled to high resolution mass spectrometry. The most suitable extraction and separation conditions for extraction of organic compounds from wines were determined and subsequently used for characterization of the volatile organic compounds profile of Tokaj liquor wines ("Tokajský výber 5-putňový"). In this work, selected vintage wines (from 1959 to 2004) from the most important Slovak producers were studied. In total, more than 800 volatile organic compounds were detected and 90 of them were identified by comparison of obtained retention indices with retention indices of previously analysed pure standards at the same conditions. The identified compounds belonged to esters, higher alcohols, furanoids and pyranoids, volatile acids, terpenoids, carbonyls, volatile phenols, volatile sulphur compounds and pyrrols. Obtained results demonstrated changes in chemical composition of analysed wines during maturation in wooden barrels.

Keywords
Tokaj wine; accurate mass measurement; comprehensive gas chromatography; volatile organic compounds

Tokaj liquor wines are considered as unique and famous commodities with a protected designation of origin in Slovakia. These sweet wines are produced from withered, shrivelled grapes infected by *Botrytis cinerea* (noble rot), which is essential for the establishment of their typical aroma. By the activity of the fungus, new compounds are produced, while some compounds present in grapes are decomposed [1]. Crucial conditions for the achievement of high grape quality and production of noble-rotten berries are unique geographic, geological and climatic ones. There are only five geographical-viticultural environments in the world that fulfil these requirements, and Tokaj is one of them [2]. The climate in Tokaj is relatively warm, in which water from the rivers provides the necessary moisture of both soil and air, while the north mountain massif retains atmospheric humidity. The soils are made from clay and loess on a volcanic tuff subsoil in which the wine cellars can be easily carved. Tuff cellars with *Cladosporium cellare* and *B. cinerea* on grapes are the two most important elements that form the characteristic expression of all Tokaj wines.

One of the most important characteristics influencing the wine quality and consumer preferences is its aroma. Up to date, more than 1 000 aromatic compounds have been identified in wines. Those belong to different chemical classes such as alcohols, esters, terpenes, aldehydes or ketones, covering a wide range of volatilities, solubility, concentration and polarities [3]. This great variety of volatile organic compounds (VOC) makes the flavour of wine very complex and a specific combination of VOC in each wine can be used for differentiation of one wine from another. Many of these

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compounds are chemically unstable and are prone to oxidation or chemical degradation. Moreover, the concentration range of volatile compounds in wines can vary from a few nanograms to hundreds of milligrams per litre. Therefore, the suitable extraction technique facilitating identification and quantification of wine volatile compounds is of great importance. Nowadays, there is a wide range of analytical tools available for extraction of VOC from wines. These methods include techniques based on volatility (static and dynamic headspace), sorptive capacity (solid phase extraction (SPE), solid phase microextraction (SPME), stir bar sorptive extraction) and solubility (liquid-liquid extraction, simultaneous distillation liquid extraction) [4]. Despite this great variety of analytical methods, liquid-liquid extraction (LLE) continues to be the reference technique for the extraction of volatile compounds from wine [3]. Although LLE is being replaced by solvent-free techniques, it has the advantages of having capacity to extract a wide range of compounds of different volatilities (low, medium and high volatility) in one single step, it provides high repeatability and has the possibility to carry out simultaneous extractions [5].

Many studies dealt with comparison of LLE with solvent-free techniques. For example, Hernanz et al. [6] compared SPE and ultrasound-mediated LLE for determination of volatile compounds in wine. The authors concluded that LLE led to higher recoveries for a greater number of odourants that could be implicated in the aroma of wines. Cabredo-Pinillos et al. [7] performed a similar study and their results showed that LLE presented higher values of pre-concetration factors. Castro et al. [3] compared continuous LLE and SPME, with results demonstrating that both methods provided adequate detection and quantitation limits, with linear ranges for correct analysis of volatiles in sherry "fino" wines. However, higher repeatability of LLE was reported. There is no doubt that, despite several disadvantages such as time consumption, high solvent consumption and necessity of solvent evaporation from the sample, LLE has many advantages over other techniques and shows good analytical performance for correct identification and quantification of VOC in complex matrices as wines. After the extraction and pre-concentration steps, VOC are usually identified and determined by gas chromatography - mass spectrometry or by gas chromatography - flame ionization detection [8].

The aim of this work was characterization of organic compounds present in Slovak Tokaj liquor wines using comprehensive gas chromatography (GC×GC) coupled to high resolution time of flight mass spectrometry (HRTOF-MS) with LLE as a pre-concentration method. LLE parameters, such as the choice of the suitable organic solvent and the volume of the organic phase, were optimized in order to achieve good extraction performance. Moreover, GC×GC conditions, such as type of the stationary phase and separation time in the second dimension, were optimized. Optimal extraction and separation conditions were consequently applied to analysis of 9 samples (1959–2004) obtained from the most prominent producers of Slovak Tokaj wines.

MATERIALS AND METHODS

Samples and chemicals

For analysis, 9 samples of Tokaj liquor wines (Tokajský výber 5-putňový, Tokaji Aszú 5-puttonyos) originating from Slovak Tokaj Wine Region were used. Samples were obtained from three Slovak wine-making companies, namely, Ostrožovič (Veľká Tŕňa, Slovakia), Tokaj Zlatý strapec (Viničky, Slovakia) and Tokaj & Co., (Malá Tŕňa, Slovakia). List of the samples is shown in Tab. 1. *n*-Hexane (98.0 %) and dichloromethane (99.8 %) were obtained from Sigma Aldrich (St. Louis, Missouri, USA), sodium chlo-

Tab. 1. List of analysed samples.

Abbreviation	Producer	Vintage	Alcohol [g·l^{-1}]	Residual sugar [g·l^{-1}]
1959 ZS	Tokaj Zlatý Strapec	1959	90.7	124
1972 ZS	Tokaj Zlatý Strapec	1972	90.7	123
1983 ZS	Tokaj Zlatý Strapec	1983	102.6	122
1990 TCo	Tokaj & Co.	1990	94.7	124
1993 OST	Ostrožovič	1993	98.6	124
1993 ZS	Tokaj Zlatý Strapec	1993	94.7	123
2000 ZS	Tokaj Zlatý Strapec	2000	90.7	124
2003 TCo	Tokaj & Co.	2003	98.6	125
2004 OST	Ostrožovič	2004	102.6	125

ride was obtained from Chemapol (Prague, Czech Republic).

Determintion of sugars and alcohol

Concentration of reducing sugars was determined by the Shoorl method [9]. Concentration of alcohol was determined pycnometrically (according to the standard method of International Organization of Vine and Wine OIV-MA-AS312-01A) [10].

Liquid-liquid extraction of organic compounds

Twenty millilitres of wine sample were mixed with 2.0 g of sodium chloride and the mixture was transferred to a separatory funnel. The mixture was extracted with 5 ml of *n*-hexane by hand-shaking for 5 min. After separation of phases, the organic phase was collected, and the extraction was repeated two more times. In total, three extraction steps were performed for each sample. All three fractions were combined, which resulted in 15 ml of final extract. Subsequently, the final extract was centrifuged for 10 min at 3 600 ×*g*. Finally, the extract was concentrated to 1 ml by evaporation in a flow of nitrogen and water bath temperature of 55 °C using Multivap-8 Concentrator (LabTech, Brno, Czech Republic). Extraction was performed three times and extracts were subsequently analysed by GC×GC-HRTOF-MS.

Gas chromatography-mass spectrometry analysis

GCxGC-HRTOF-MS (LECO, St. Joseph, Michigan, USA) consisting of an Agilent 7890 gas chromatograph (Agilent Technologies, Palo Alto, California, USA), a jet modulator and a high-resolution time-of-flight mass spectrometer (LECO) was used for sample analysis. For separation, DB-FFAP and HP-5 columns, 30 m × 0.25 μm × 0.25 μm (Agilent Technologies) in the first dimension and a 1.6 m × 0.25 mm × 0.25 μm Rxi-17Sil (Restek, Bellefonte, Pennsylvania, USA) in the second dimension were used. Helium with flow-rate 1 ml·min^{-1} was used as a carrier gas. The temperature program started at 40 °C for 10 min, with slow temperature gradient of 2 °C·min^{-1} to final temperature 220 °C kept for 5 min. One microlitre of the sample extract was injected into splitless injector heated at 250 °C. A jet modulator was kept at a temperature by 15 °C higher than the actual oven temperature with a modulation period of 6 s. The temperature of the transfer line was kept at 250 °C. HRTOF-MS spectra were obtained at ionization energy 70 eV, the temperature of the ion source was set to 250 °C and the detector voltage was maintained at 1 860 V during the analysis. The signal acquisition rate was 100 spectra per second in the *m*/*z* range of 29–550. Primary processing of the obtained data was performed using LECO ChromaTOF-HRT 1.90.60 Software and NIST 2014 mass spectral library (National Institute of Standards and Technology, Gaithersburg, Maryland, USA). The compounds were identified by exact mass (tolerated mass accuracy was 1 mDa), comparison of the obtained mass spectra with those stored in the library and by comparison of determined retention indices with those obtained for standards (VOC was considered as identified if the difference between experimental and reference retention index was less than 20 units). Relative peak areas were calculated from three replicates as an average value.

RESULTS AND DISCUSSION

Optimization of liquid-liquid extraction parameters

The first part of the work was focused on optimization of the LLE procedure to reach efficient extraction of organic compounds from the studied Tokaj liquor wines. The optimization of LLE working conditions was performed with sample 1993 OST (Tab. 1) and included the most crucial parameters, such as polarity of the organic solvent, volume of organic solvent and phase ratio, organic and inorganic modifiers, which influence extraction performance. The number of extracted compounds, their peak areas, as well as the quality and purity of the obtained mass spectra were used as criteria to select the most suitable LLE conditions.

The most important parameter that has significant effect on extraction performance is polarity of the used solvent, since the physico-chemical properties of extracted compounds must be in correlation with properties of the used solvent. In this work, two types of solvents were studied, i.e. non-polar *n*-hexane and the more polar dichloromethane. In previous studies, the most frequently used solvents were dichloromethane and freon [11–15]. Thus, firstly used solvent was dichloromethane. It was determined that this solvent extracted approximately 900 compounds belonging to various non-polar and medium-polar chemical classes. The highest peak areas were recorded for carboxylic acids and their esters. Often, the peaks for polar and medium-polar compounds were overloaded or provided tailed peak shape, which resulted in a significant peak broadening. As a result, it was not possible to record sufficiently pure mass spectra in vicinity of such peaks or to correctly identify organic compounds. Indeed, only 15 % of detected peaks were identified with a match score better than 900. Therefore, a less polar sol-

Tab. 2. Optimization of liquid-liquid extraction working conditions.

Sample volume [ml]	Type of organic solvent	Volume of organic solvent [ml]	Amount of added sodium chloride [g]	Emulsion formation* [%]
20	Dichloromethane	3	0	100
20	Dichloromethane	3	2	100
20	Dichloromethane	3	5	90
20	Dichloromethane	5	0	90
20	Dichloromethane	5	2	90
20	Dichloromethane	5	5	90
20	Dichloromethane	10	2	90
20	*n*-Hexane	3	0	70
20	*n*-Hexane	3	2	70
20	*n*-Hexane	3	5	60
20	*n*-Hexane	5	0	50
20	*n*-Hexane	5	2	30
20	*n*-Hexane	5	5	30
20	*n*-Hexane	10	2	30

* – the percentage of emulsion formation refers to the whole extract volume.

vent *n*-hexane was used in order to decrease the overload of medium-polar compounds. With the use of *n*-hexane, a lower number of compounds was extracted (approximately 720), but the problematic co-eluted peak clusters were separated. The lower number of detected peaks was compensated by significant improvement of quality of the obtained mass spectra, which resulted in easier and more efficient identification of organic compounds.

It is well known that extraction efficiency improves with the increasing volume of organic phase. Unfortunately, the concentration of analyte decreases, which leads to the general practice to minimize the volume of the organic phase and to reduce or even exclude the evaporation step. Therefore, the volume of the organic phase (phase ratio) was optimized in this study. As documented in Tab. 2, three volumes (e.g. 3 ml, 5 ml and 10 ml) of the organic solvent were tested with a constant volume of the wine sample (20 ml). However, the main challenge for the extraction of organic compounds from wines during experiments was the formation of emulsions. To reduce this effect, sodium chloride was added to the wine sample. Unfortunately, the use of dichloromethane promoted the formation of emulsion and the phase boundary was not formed, independently of the amount of the added sodium chloride. On the contrary, the use of *n*-hexane partially reduced this effect. In case of hexane (3 ml), the formed emulsion represented 70 % of the final extract volume. An increase in the volumes of *n*-hexane (use of 5 ml or 10 ml) led to better formation of the phase

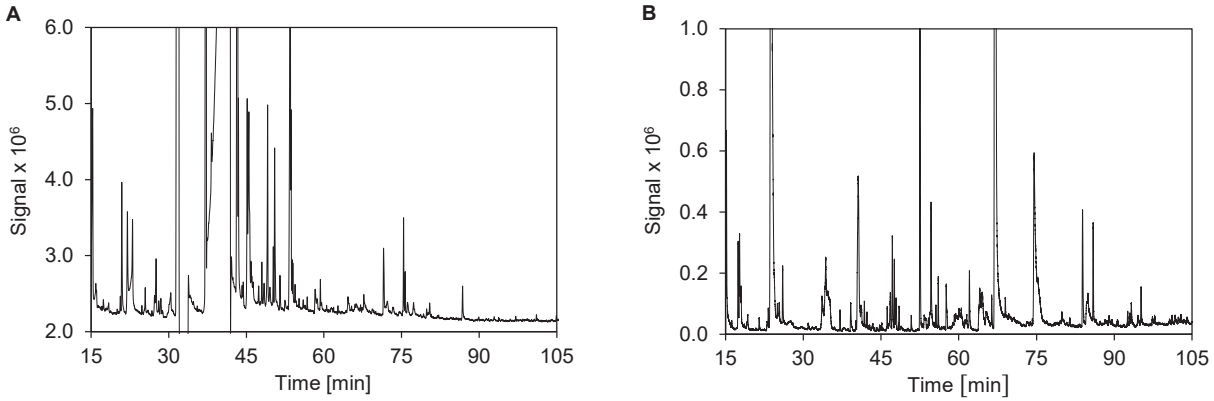

Fig. 1. Chromatograms of the Tokaj liquor wine extract.

A – HP-5 column, B – DB-FFAP column.

boundary and, with the added sodium chloride (2 g), the emulsions represented up to 30 % of the extract volume. Addition of sodium chloride at further increased amounts did not help and it was observed that, in both cases of 5 ml and 10 ml of solvent use, the emulsion formation was the same. Therefore, 5 ml of *n*-hexane and 2 g of sodium chloride were used to obtain the highest possible concentration of the analyte. Extracts were centrifuged for 10 min in order to quantitatively collect the organic phase. Final extracts were concentrated to 1 ml and analysed by GC×GC-HRTOF-MS.

Optimization of separation conditions

Optimization of the working procedure continued by selecting of suitable GC separation conditions and it began with the selection of a proper stationary phase for the separation of the extracted organic compounds. Full exploitation of the advantages of two-dimensional separations requires a fully orthogonal separation system. This means that separation in the first and second dimensions must be governed by independent separation mechanisms. This was achieved by selecting two stationary phases with different polarities. The stationary phases most frequently used for separation of VOC in wine samples are non-polar 5% phenyl – 95% dimethyl polysiloxane (HP-5) [16] or polar modified polyethylene glycol stationary phase (DB-FFAP) [16, 17]. Both stationary phases of the same column dimensions were used in the first GC dimension. A polar stationary phase Rxi-17Sil was used in the second dimension to achieve orthogonality of both separation systems. The maximum separation efficiency is achieved by enhancing separation in the first dimension, while second dimension is used to separate possible impurities from the effluent from the first dimension [18]. Thus, the extract of Tokaj liquor wine was analysed firstly by one-dimensional gas chromatography with use of non-polar HP-5 and polar DB-FFAP columns. The obtained chromatograms are shown in Fig. 1. It was clearly shown that with the use of HP-5 column, the organic compounds eluted within the time range from 15 min to 55 min, while most peaks were detected between 30 min and 45 min (Fig. 1A). In this time frame, a high number of co-elutions and formation of huge peak clusters could be observed, which was caused by presence of organic compounds with similar boiling points. This could be overcome by utilization of stationary phases with orthogonal character. Indeed, the use of DB-FFAP stationary phase promoted a better use of the separation space, as is documented in Fig. 1B. To ensure orthogonality of the two-dimensional separation space, a medium-polar stationary phase Rxi-17Sil was used in the second dimension.

In the next step, modulation period was optimized. Modulation periods of 4 s, 6 s and 8 s were tested. The obtained chromatograms are shown in Fig. 2. It was found that the best use of the two-dimensional separation space was achieved with a modulation period of 6 s. A significant wrap around effect was observed with a modulation period of 4 s, while no peaks were found at a retention time of the second dimension higher than 6 s.

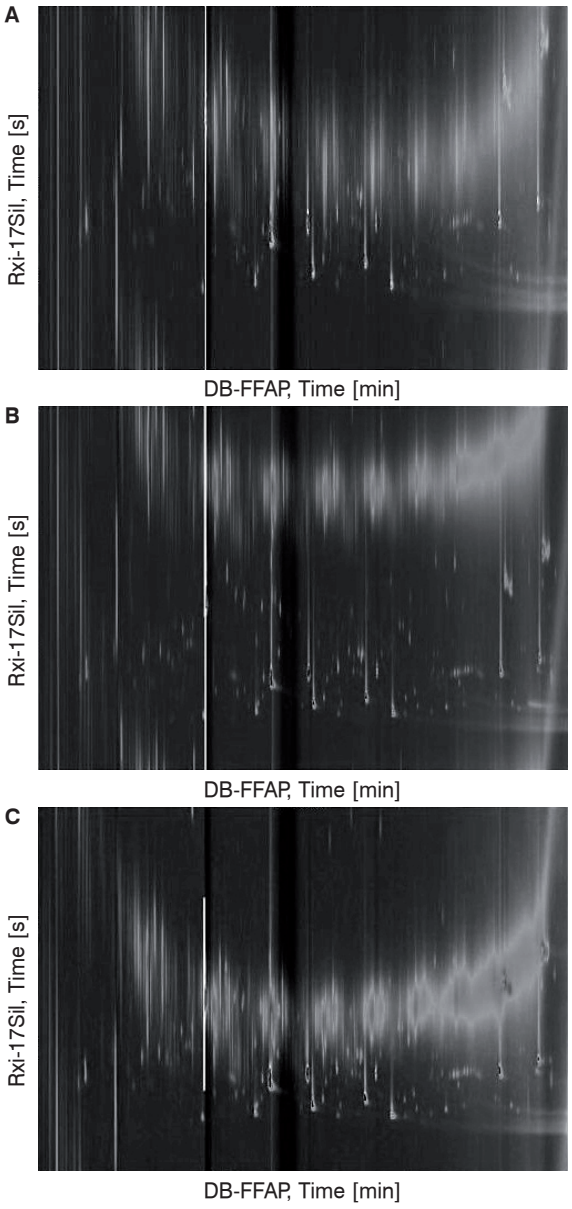

Fig. 2. Optimization of modulation period.

A – modulation period 4 s, B – modulation period 6 s, C – modulation period 8 s.

Volatile organic compounds profile

VOC from nine Slovak Tokaj liquor wines sweet wines of various vintages were extracted using LLE under optimized conditions and analysed by GC×GC-HRTOF-MS in order to comprehensively describe VOC profile of these unique products. The compound was considered as identified only if its retention characteristics in both dimensions (expressed by linear retention indices) as well as the recorded mass spectra were in agreement with those recorded for a standard. Tab. 3 shows VOC identified based on the analysis of standards. Normalized peak area was used to describe and compare quantity of each VOC found in wine samples.

In total, more than 800 organic compounds were detected in the studied samples, while only 90 were identified by standards and 31 were present independently of the wine vintage or producer. VOC profiles of wines were represented mostly by esters (25), furanoids and pyranoids (20), and by volatile acids (12). Minor occurrence was determined for terpenoids (8), higher alcohols (7), carbonyls (6), volatile phenols (5), volatile sulphur compounds (4) and pyrrols (3).

2-Phenylethanol, diethyl succinate and ethyl lactate were the most abundant VOC in all studied wine samples. Compared to other VOC, 2-phenylethanol reached the highest values of relative peak area (11.5–33.0). This higher alcohol is a common component of VOC profile of many wines. It is a natural metabolite of grapevine formed also during ethanol fermentation by yeasts [16]. Wines produced from grapes infected by bunch rot (*Botrytis* sp.) are characteristic by increased concentrations of 2-phenylethanol, which provides pleasant rose and lilac-like aroma [17]. Among other higher alcohols, only benzyl alcohol occurred in all wine samples.

1-Hexanol, as the most common C6 alcohol that originates in grape berries, was identified in 8 of 9 samples and reached relatively high relative peak areas (up to 2.7).

Diethyl succinate (relative peak area 2.4–15.6) and ethyl lactate (relative peak area 0.4–19.6) are major wine esters that are produced by yeast *Saccharomyces cerevisiae* during ethanol fermentation [18]. Concentration of ethyl lactate rapidly increases in wines maturated in contact with yeast sediments (sur lie, bâtonnage, Champagne) and during aging of wine [18, 19]. The other esters identified in all studied samples originate from yeast metabolism (diethyl malate, monoethyl succinate, ethyl phenylacetate, 2-phenethyl acetate and diethyl malonate) or from Maillard reactions in grape skins and toasted oak wood (ethyl levulinate and ethyl vanillate) [20]. *cis*-Whiskey lactone and group of furfurals were the most abundant VOC among the group of furanoids and pyranoids. Whiskey lactones are constituents of VOC profile of toasted oak wood and are repeatedly found in wines aged in oak casks [21]. Furfural, 5-methylfurfural and furfural diethyl acetal were dominant furaldehydes in most of the samples. They are usually formed by Maillard reactions and caramelization, but they could also be generated in sugar-containing foods during storage, especially in presence of acids [16]. Wine lactones can originate from grapes (mostly from noble-rotten berries) and can also be synthesized during fermentation, wine maturation or extracted from wood barrels [21]. γ-Lactones and whiskey lactones significantly contribute to overall wine aroma [21]. γ-Butyrolactone was the most frequently occurring γ-lactone in studied wines, followed by γ-nonalactone, γ-ethoxybutyrolactone and γ-octalactone. Other dominant furanoids and pyranoids identified in studied samples were δ-octenolactone, phtalolactone, coumaran, nutty furan and lavender lactone.

Seven of 12 identified volatile acids were present in all investigated wine samples, hexanoic and octanoic acids being the most abundant. All identified acids are metabolites of microorganisms (yeast and lactic acid bacteria) participating in winemaking process and are components of VOC profile of various wines [18, 22]. While aliphatic acids originate from metabolism of lipids, benzeneacetic acid (and consequently mandelic acid) can be formed from amino acid phenylalanine or from 2-phenylethanol and benzeneacetaldehyde [23].

Abundance of terpenoids in analysed samples was relatively poor, linalool oxide (furanoid) was the only terpenoid found in all samples. Among typical wine terpenoids and C13 norisoprenoids, α-terpineol, β-damascenone and 1,1,6-trimethyl-1,2-dihydronaphthalene were also identified. Generally, Slovak Tokaj liquor wines are made from cuvée consisting of Tokaj varieties Furmint (more than 80 %), Lipovina (Hárslevelű) (up to 15 %) and Muscat Lunel (up to 5 %). Muscat Lunel is the only aromatic variety used for production of Tokaj wines and due to its low content, the contribution to final terpenoids profile is negligible. Furthermore, during long term oxidative maturation of wine, terpenoids and carotenoids oxidize to form oxides and norisoprenoids.

Benzeneacetaldehyde, butyrovanillon and four derivatives of benzaldehyde were identified among the group of carbonyls, while benzeneacetaldehyde was present in all samples and its relative

Tab. 3. List of identified volatile organic compounds in Slovak Tokaj liquor wines.

Chemical class	Compound	Retention index	Relative peak area								
			1959 ZS	1972 ZS	1983 ZS	1990 TCo	1993 OST	1993 ZS	2000 ZS	2003 TCo	2004 OST
Higher alcohols	1-Hexanol	1378	0.547	1.633	0.955	nd	2.656	1.620	1.621	0.116	0.537
	3-Hexen-1-ol	1410	nd	nd	nd	nd	0.066	nd	nd	nd	nd
	1-Heptanol	1500	0.003	0.016	nd	nd	0.124	0.015	0.053	nd	nd
	2-Ethyl-1-hexanol	1543	nd	nd	nd	1.042	0.120	nd	0.060	0.022	0.068
	1-Octanol	1616	nd	nd	nd	nd	0.017	0.010	0.012	nd	nd
	Benzyl Alcohol	1948	0.098	0.140	0.063	0.107	0.101	0.087	0.076	0.016	0.056
	2-Phenylethanol	1979	19.998	15.160	19.122	28.069	11.520	19.121	18.990	11.841	33.007
Carbonyls	Benzaldehyde	1563	0.172	0.114	0.128	0.013	0.059	0.098	0.062	nd	nd
	Benzeneacetaldehyde	1692	0.083	0.135	0.081	0.135	0.120	0.099	0.071	0.024	0.062
	Benzaldehyde, 3-methyl-	1698	0.014	0.312	nd	nd	nd	0.455	nd	nd	0.400
	Benzaldehyde, 4-methyl-	1698	nd	nd	0.223	nd	0.409	nd	0.373	nd	nd
	Vanillin	2662	0.005	0.015	0.006	0.006	0.027	0.016	0.010	0.004	nd
	Butyrovanillone	2804	0.022	0.028	nd	nd	0.051	0.032	0.036	0.003	0.019
Esters	Ethyl lactate	1368	13.062	11.978	19.545	13.767	8.451	7.323	7.787	0.437	2.216
	Ethyl 2-hydroxyisovalerate	1455	0.504	0.587	0.678	2.081	0.795	1.396	0.737	nd	0.260
	Propanoic acid, 2-hydroxy-, 2-methyl-propyl ester	1500	0.127	0.085	0.089	nd	nd	0.072	0.056	nd	0.010
	Ethyl sorbate	1526	1.022	1.381	1.357	1.141	0.541	0.702	0.864	0.101	0.009
	Ethyl 3-hydroxybutanoate	1562	nd	0.008	nd	nd	0.067	0.009	nd	nd	nd
	Ethyl 2-hydroxy-4-methylpentanoate	1594	0.345	0.278	nd	0.399	0.567	0.418	0.323	nd	0.121
	Isoamyl lactate	1621	0.663	0.282	0.504	nd	0.388	0.250	0.245	nd	nd
	Diethyl malonate	1643	0.014	0.034	0.018	0.051	0.021	0.036	0.049	0.002	0.043
	Butanedioic acid, dimethyl ester	1656	nd	0.004	nd	nd	0.005	0.004	0.004	nd	0.004
	Ethyl levulinate	1667	0.220	0.361	0.254	0.235	0.143	0.260	0.292	0.023	0.076
	Butanedioic acid, ethyl methyl ester	1700	0.092	0.111	nd	nd	nd	0.028	0.061	nd	nd
	Ethyl decanoate	1703	nd	nd	nd	nd	0.061	0.016	nd	nd	nd
	Dodecanoic acid, ethyl ester	1703	nd	nd	nd	nd	nd	nd	0.024	nd	nd
	Ethyl benzoate	1715	0.017	nd	nd	nd	0.016	nd	nd	nd	0.010
	Hexanoic acid, 3-hydroxy-, ethyl ester	1738	nd	nd	nd	nd	0.013	0.004	nd	nd	nd
	Diethyl succinate	1742	10.450	13.119	15.549	14.767	14.835	11.206	12.082	2.452	10.159
	Ethyl phenylacetate	1848	0.330	0.402	0.334	0.291	0.394	0.404	0.486	0.046	0.375
	Glutaric acid, diethyl ester	1852	0.103	0.154	0.129	nd	0.114	0.114	0.133	0.007	0.050

Tab. 3. *continued*

Chemical class	Compound	Retention index	Relative peak area								
			1959 ZS	1972 ZS	1983 ZS	1990 TCo	1993 OST	1993 ZS	2000 ZS	2003 TCo	2004 OST
	Succinic acid, butyl ethyl ester	1866	nd	0.100	nd	nd	0.173	nd	0.076	nd	nd
	2-Phenethyl acetate	1876	0.141	0.096	0.114	0.066	0.205	0.126	0.172	0.042	0.228
	Diethyl dl-malate	2127	0.780	2.877	3.414	5.755	2.409	2.297	1.981	0.663	2.280
	Monoethyl succinate	2486	1.299	2.995	3.050	2.220	2.599	1.983	1.235	0.561	1.117
	Triethyl citrate	2571	0.190	0.805	0.948	0.582	0.162	0.413	0.378	0.004	0.153
	Ethyl vanillate	2733	0.026	0.039	0.029	0.010	0.056	0.034	0.038	0.018	0.023
	Ethyl homovanillate	2798	nd	nd	nd	nd	0.012	0.008	0.009	0.006	nd
Furans and lactones	Furfural diethyl acetal	1500	0.041	0.230	nd	0.100	0.200	0.141	nd	0.071	0.229
	Furfural	1510	0.970	0.458	0.334	0.485	0.745	0.507	0.032	0.256	0.845
	3-Furaldehyde	1514	nd	0.090	nd	nd	0.045	nd	nd	0.027	0.040
	2,5-Furandicarboxaldehyde	1619	nd	nd	nd	nd	0.004	nd	nd	nd	nd
	1-Propanone, 1-(2-furanyl)-	1629	0.020	0.029	nd	nd	nd	0.015	nd	nd	0.091
	5-Methylfurfural	1630	0.386	0.121	0.088	0.152	0.191	0.142	0.029	0.052	0.216
	Nutty furan	1670	0.035	0.024	nd	0.024	0.055	0.014	0.004	0.034	0.039
	γ-Butyrolactone	1672	0.083	0.027	0.041	0.054	0.045	0.042	0.010	0.045	0.037
	Ethyl 2-furoate	1681	0.273	0.193	0.156	0.276	0.306	0.298	0.016	0.194	0.161
	Lavender lactone	1711	0.005	nd	nd	0.005	0.006	0.008	nd	0.004	0.004
	γ-Ethoxybutyrolactone	1776	0.009	0.010	nd	0.008	nd	nd	nd	0.002	0.007
	cis-Whiskey lactone	2014	0.661	0.704	0.373	0.526	0.512	0.932	0.144	0.663	0.197
	2-Acetyl-2-methyltetrahydrofuran	2058	nd	0.006	nd	nd	0.006	0.006	nd	nd	0.029
	γ-Octalactone	2089	0.039	nd	nd	0.090	0.079	nd	0.008	nd	nd
	γ-Nonalactone	2089	0.201	0.121	nd	nd	nd	0.167	nd	nd	0.070
	Pyran-2-one, 6-pentyl-	2248	0.025	nd	nd	0.004	nd	0.007	nd	nd	nd
	δ-Octenolactone	2300	0.034	0.070	0.036	0.039	0.047	0.046	0.003	0.007	0.013
	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	2384	0.028	0.007	nd	0.012	0.015	0.011	nd	nd	nd
	Phtalolactone	2424	0.064	0.034	0.037	0.045	0.073	0.032	0.005	0.007	0.021
	Coumaran	2493	0.156	0.040	0.074	0.059	0.090	0.032	nd	0.028	0.016
Phenols	Guaiacol	1931	0.006	0.005	nd	nd	0.020	0.008	0.007	0.008	0.009
	4-ethylguaiacol	2106	0.013	0.122	0.115	0.325	0.076	0.194	0.138	0.014	0.028
	Eugenol	2248	0.006	nd	0.024	0.018	nd	nd	0.030	0.007	0.013
	Phenol, 4-ethyl-	2262	0.048	0.240	0.258	0.589	0.122	0.207	0.254	0.043	0.022

Tab. 3. *continued*

Chemical class	Compound	Retention index	Relative peak area								
			1959 ZS	1972 ZS	1983 ZS	1990 TCo	1993 OST	1993 ZS	2000 ZS	2003 TCo	2004 OST
	4-Vinylguaiacol	2283	nd	nd	nd	0.030	0.060	nd	0.023	0.004	0.014
Pyrrols	Ethanone, 1-(1H-pyrrol-2-yl)-	2047	0.006	nd	0.004	0.007	0.006	nd	nd	nd	nd
	1H-Pyrrole-2-carboxaldehyde	2097	0.022	0.027	0.050	nd	0.066	0.016	0.022	0.051	0.032
	1H-Pyrrole-2-carboxaldehyde, 1-methyl-	2188	0.021	0.043	0.050	0.067	0.034	0.037	0.043	0.004	0.035
Sulphur compounds	Ethyl 3-(methylthio)propanoate	1614	0.012	0.015	0.008	0.008	0.019	0.003	0.010	nd	0.006
	Methionol	1778	0.031	0.031	0.027	0.066	0.079	0.045	0.042	nd	0.045
	Benzothiazole	2012	0.022	nd	nd	nd	0.017	0.027	0.031	nd	0.011
	1-(2-Thienyl)-1,2-propanedione	2145	0.035	0.045	0.057	0.107	0.109	nd	nd	nd	nd
Terpenoids and noriso-prenoids	Linalool oxide (furanoid)	1435	0.224	0.193	0.081	0.103	0.096	0.172	0.155	0.013	0.093
	Nerol oxide	1479	nd	nd	nd	nd	nd	nd	nd	0.001	nd
	Ocimenol	1726	nd	0.021	nd	0.007	nd	0.016	0.008	nd	nd
	α-Terpineol	1757	nd	0.014	nd	nd	0.034	nd	0.014	0.018	0.025
	1,1,6-Trimethyl-1,2-dihydronaphthalene	1783	nd	0.023	nd	nd	0.043	0.022	0.022	nd	0.004
	β-Damascenone	1870	0.006	0.032	nd	nd	0.012	0.019	0.021	nd	0.018
	Myrcenol	2045	nd	nd	0.004	0.035	0.016	nd	nd	nd	nd
	Santolina triene	2325	nd	nd	nd	0.016	nd	nd	0.021	0.004	nd
Volatile acids	Isobutanoic acid	1626	0.040	0.025	0.020	0.049	0.063	0.008	0.039	0.028	0.003
	Butanoic acid	1644	0.048	0.037	nd	nd	0.081	0.028	0.032	0.002	nd
	Isovaleric acid	1674	0.008	nd	nd	0.008	nd	0.019	nd	nd	nd
	Hexanoic acid, 2-methyl-	1734	0.212	0.174	0.180	0.239	0.397	0.238	0.277	0.107	0.281
	Hexanoic acid	1918	0.934	1.274	0.977	1.455	2.039	1.439	1.427	0.416	1.326
	Mandelic acid	2003	0.034	0.052	0.102	0.074	0.025	0.040	0.035	nd	0.016
	Hexanoic acid, 2-ethyl-	2029	0.005	0.004	nd	nd	0.018	0.025	0.022	0.006	nd
	Heptanoic acid	2038	0.002	nd	nd	nd	0.017	0.009	0.005	nd	nd
	Octanoic acid	2145	0.335	0.522	0.909	1.503	1.242	0.574	0.546	0.720	1.326
	n-Decanoic acid	2371	0.005	0.018	0.005	nd	0.092	0.022	0.028	0.022	0.061
	Benzeneacetic acid	2679	0.035	0.113	0.062	0.062	0.114	0.124	0.110	0.014	0.113
	n-Hexadecanoic acid	3037	0.217	0.460	0.344	0.687	0.484	0.603	0.447	0.968	0.651

Abbreviations of samples are explained in Tab. 1. nd – not detected.

peak area varied from 0.02 to 0.14. Benzeneacetaldehyde is a product of biological or Strecker degradation of phenylalanine [23, 24], while benzaldehyde occurs in grape berries naturally. It is also produced by wine yeasts and *Botrytis cinerea* [2]. Vanillin and butyrovanillon are part of VOC profile of oak barriques used for maturation of Tokaj wines.

VOC profiles of our "Tokajský výber 5-putňový" wines contained five volatile phenols, the most abundant being 4-ethylphenol and 4-ethylguaiacol, which were identified in all studied samples. These volatile phenols are formed from *p*-coumaric acid that is naturally occurring in grape and are considered as markers of *Brettanomyces*-caused fault of wine [16]. However, until now, no *Brettanomyces* contamination or related off-flavours in Tokaj liquor wines were reported. Other volatile phenols such as guaiacol, eugenol or 4-vinylguaiacol, which are responsible for spicy character of wine aroma [25], were detected at lower concentration levels (Tab. 3).

Among the four identified volatile sulphur compounds, ethyl 3-(methylthio)propanoate, methionol and benzothiazole are metabolites of microorganisms and common constituents of wine VOC profile [26]. 1-(2-Thienyl)-1,2-propanedione is characterized by intense praline-like and woody odour and until now, it was identified only in coffee [27].

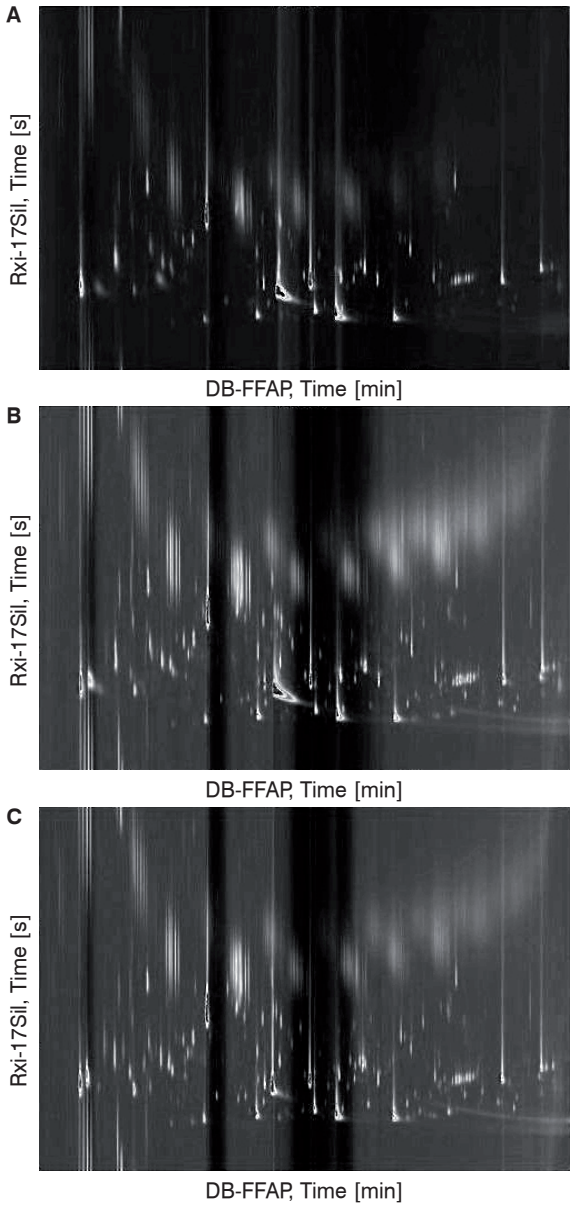

Fig. 3. Obtained chromatograms of Tokaj liquor wines.
A – 1990 Tco, B – 1993 ZS, C – 1993 OST.
Abbreviations of samples are explained in Tab. 1.

Comparison between vintages and producers

The chemical composition of wines changes during maturation in wooden barrels or glass bottles and is influenced by various parameters such as temperature, transport of oxygen or maturation time. These affect overall taste and aroma perception of the wine [28]. The unique selection of studied samples (vintage 1959, 1972, 1983, 1993 and 2000) allowed to evaluate the effect of maturation time on VOC composition. Compared samples were obtained from the same producer (Tokaj Zlatý Strapec), thus they were produced by the same technology. The number of detected compounds varied from 640 to 780, while the lowest number of compounds was observed for the youngest vintage 2000. In remaining vintages, the differences in the number of detected compounds were significantly lower and varied in the range 750–780. Changes in chemical composition of chemical classes during wine aging are known to take place [14, 18, 22] and some trends could be seen also in our study. The most remarkable trends were observed for groups of esters, furans and lactones. For example, relative peak area of ethyl lactate for older vintages (1959, 1972, 1983) was two times higher compared to younger ones (1993, 2000). A descending trend was observed for furfural, benzaldehyde and phtalolactone. Furthermore, coumaran was not found in the recent vintage but it was detected in vintage 1993 followed by an increasing trend back to 1959 where it had the highest relative peak area. Similar observation was recorded also for γ-ethoxybutyrolactone. Even more noticeable changes were observed for ethyl benzoate, which was detected only in samples from the oldest vintage of 1959. On the contrary, an opposite trend was recorded for ethylester of deca-

noic acid, 4-vinylguaiacol and santolina triene, which were present only in the recent vintage and were not detected in older samples.

As shown in GC×GC chromatograms of samples 1990 TCo, 1993 OST, 1993 ZS at Fig. 3 (different producers), VOC profiles were similar and possible differences were observed only in relative composition of some individual VOC. Thus, it can be concluded that there were no important differences between various producers. Based on the obtained results, it was possible to distinguish wines produced by Tokaj & Co. due to absence of some organic compounds in its VOC profile, e.g. positional isomers of methyl benzaldehyde or isoamyl lactate.

CONCLUSIONS

This work was focused on characterization of VOC profiles of Slovak Tokaj liquor wines using LLE followed by GC×GC-HRTOF-MS. Firstly, the LLE parameters, such as type and volume of organic solvent and addition of inorganic modifier, were optimized. Optimal conditions were achieved when 20 ml of sample, 5 ml of *n*-hexane and 2 g of sodium chloride were used. In the next step, separation conditions, i.e. type of the stationary phase in the first dimension and time of separation in the second dimension, were optimized. HP-5 or DB-FFAP columns were used for the separation of VOC in the first dimension. Results showed that the more polar column DB-FFAP was more suitable for this purpose. Modulation periods of 4 s, 6 s and 8 s were used in the second dimension, while 6 s was the optimum time, where wrap around effect was not observed and the separation space was used effectively. Under these optimal conditions, more than 800 organic compounds were detected in Slovak Tokaj liquor wines (Tokajský výber 5-putňový) wines, while only 90 were identified by standards and 31 were found in all studied samples. The most abundant group of identified VOC were esters (25), followed by furanoids and pyranoids (20), volatile acids (12), terpenoids (8), higher alcohols (7), carbonyls (6), volatile phenols (5), volatile sulphur compounds (4) and pyrroles (3). Some of the identified VOC (4-ethylphenol and 4-ethylguaiacol) are markers of *Brettanomyces* infections of wine which, until now, were not reported in Tokaj liquor wines. Moreover, 1-(2-thienyl)-1,2-propanedione characterized by its intense praline-like and woody odour and until now identified only in coffee, was found in analysed samples. Obtained results showed that the vintage has a significant effect on the quality as well as on quantity of identified VOC. No important changes were observed between various producers, which can be perceived as confirmation that all producers are using the traditional technology of winemaking in Tokaj region.

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