Compositional profiling of Slovakian wines from distinct production systems by analysis of main saccharides and glycerol

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Summary
The aim of this study was to assess the quality of Slovakian wines with protected geographic indication coming from conventional and organic production in accordance with the European wine regulations, as well as to differentiate them on the basis of compositional analysis of some wine constituents, mainly saccharides and glycerol. For this purpose, the sum of glucose and fructose, glucose/fructose and glycerol/ethanol ratio were determined. A technique of high performance liquid chromatography (HPLC) was utilized for testing 14 organic and 21 conventional dry white and red wines as well as grape berries from both cultivation systems. Independent from the grape cultivar tested, the saccharide concentration was in the range of 0.15–3.26 g·l^{-1} and glycerol 4.97–9.78 g·l^{-1} in wines. The mean saccharide concentration was 232.91 g·l^{-1} and 248.21 g·l^{-1} in fresh grape juice from organic and conventional crop, respectively. The analysis of variance revealed a significant difference between organic and conventional production in fructose ($P < 0.001$) and glycerol level ($P < 0.01$). The principal component analysis demonstrated significant differences between white and red wines. The present results are a basis for further differentiation of Slovakian wines with regard to varieties.

Keywords
wine; organic; conventional; glucose; fructose; glycerol; HPLC

Glucose, fructose and glycerol, together with other grape juice constituents, play a role in creating the wine quality. Formation of saccharides in grapes differs according to vine cultivar, and is influenced by environmental and viticultural practices [1]. Grape berries contain primarily glucose and fructose, which represent approximately 99% of the saccharides concentration at the end of grape maturation [2]. The saccharose level is seldom above 10% of total saccharides. In most of *Vitis vinifera* cultivars, the ratio of glucose to fructose is close to 1 at ripeness, higher fructose levels appear in overripe grape berries [1].

Glucose and fructose are fermentable saccharides, which are converted to ethanol and carbon dioxide by yeasts in course of fermentation. Utilization of these saccharides is dependent on the innate properties of the yeast strain used in fermentation process, and on the external conditions like ethanol and nitrogen addition, as well [3]. Glucose and fructose are consumed by yeasts with different rates. The most studied species of yeast, *Saccharomyces cerevisiae*, appears to be glucophilic, but other yeast species, such as *Candida stellata* or *Zygosaccharomyces bailii*, may prefer fructose to glucose [4]. The average glucose/fructose ratio is 0.58/1.0 in fully fermented wine, but this may change to a great extent [5]. According to the residual concentration of saccharides (commonly referred to as residual sugar), wines are classified as dry, medium dry, medium sweet and sweet [6]. Fructose may taste nearly twice as sweet as glucose in wines [7] and is a basic component of sweet dessert wines. In most wines, there is very little saccharose and is detectable only if the enzyme saccharase is inhibited during pressing of grape berries [5]. The exception happens when saccharides are purposely added either during or post fermentation process to raise the final alcohol concentration (referred to as chaptalization) [8].

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Glycerol, as a by-product of fermentation by *S. cerevisiae*, is usually found in the greatest abundance after ethanol and carbon dioxide. The significant role of glycerol in grape processing and wine production has been reviewed by SCANES et al. [9]. Glycerol positively contributes to the sensory quality of wine in terms of sweetness, fullness and smoothness [10]. Although its concentration is low in grape juice, occasionally may be present in high levels in grape musts infected by moulds *Botrytis cinerea* [11]. The concentration of glycerol formed by *S. cerevisiae* in wine varies between 1–15 $g \cdot l^{-1}$, with an average concentration of 7 $g \cdot l^{-1}$ [9]. Formation of glycerol during fermentation is influenced by many environmental factors, such as grape variety, fermentation temperature, nitrogen source, addition of sulfur dioxide, pH, aeration rate, yeasts strain and inoculation ratio [10, 12].

The addition of saccharides to grape juice is prohibited in most jurisdictions or is strictly regulated, e.g. in France. Saccharides may be added either as saccharose or "invert sugar", which is quickly converted to ethanol. The detection of wine chaptalization consists in distinguishing between grape-derived and non-grape-derived ethanol, which can be preferably achieved by measuring the carbon isotope $^{13}C/^{12}C$ ratio [12, 13]. This technique may be also used at wines adulterated with industrial-grade glycerol, differentiating the botanical origin of glycerol sources [14, 15]. The simplest evidence of illegal addition of glycerol to wines is based on the defined ratio between glycerol and ethanol. The concentration of glycerol should be in the range of 6–10% of the ethanol concentration in authentic wine. However this ratio is variable due to natural variability of relevant wine constituents. After all, industrial grade glycerol may be added to wine with a low natural glycerol concentration and will still remain within the range specific for authentic wines [16].

The aim of this work was to provide information on the levels of saccharides and glycerol in geographically protected wines produced by distinct technologies in Slovakia. Differences between wine components were statistically assessed in main interaction for organic versus conventional wine and in sub-interaction for white versus red wine. The relative ratio of glucose/fructose and glycerol/ethanol was also determined to learn more about the quality of the wines. For detection and quantification of wine constituents, a HPLC method with refractive index detection was utilized.

MATERIALS AND METHODS

Chemicals and materials

Standards of D-(–)-fructose (99.9%), D-(+)-glucose, anhydrous (99.5%) and saccharose (99.5%) were purchased from Sigma Chemical (St. Louis, Missouri, USA). Glycerol, p.a. (99.3%) was purchased from Lachema (Brno, Czech Republic). Acetonitrile Chromasolv was obtained from Sigma-Aldrich Chemie (Steinheim, Germany). Water was purified in Rodem 6 water purification device equipped with UV-lamp (Ecotest, Zemné, Slovakia). The syringe micro filters of 0.45-μm pore size with cellulose membrane from Agilent (Waldbronn, Germany) were used for filtration of grape juices.

Stock standard solutions were prepared in water at a concentration level of 10 $g \cdot l^{-1}$ for saccharides and 20 $g \cdot l^{-1}$ for glycerol. The stock solutions were diluted with water to prepare calibration standards in the range of 0.025–10 $g \cdot l^{-1}$ for saccharides and 0.1–20 $g \cdot l^{-1}$ for glycerol.

Wine and grapes samples

Fourteen samples of organic white and red wines were obtained from a producer in the vineyard region Modrý Kameň situated in Central Slovakia. The wines were made of various grape varieties (Chardonnay, Pinot Blanc, Traminer Red, Rhine Riesling, Cabernet Sauvignon, Blaufränkisch, Pinot Noir). The counterpart wines (21 white and red wines) and conventional grape samples were obtained from the neighbouring vineyard. All wines originated from the vintage 2002–2009. Majority of the wine samples were high-quality dry wines, a minor fraction of the wines represented medium dry, medium sweet and sweet wines. The grape berries were collected during the vintage 2010–2011 and included Traminer Red, Pinot Noir and Cabernet Sauvignon grape varieties.

Wine samples were analysed directly after microfiltration. Regarding the grape berries, a 50 g portion was manually crushed in a mortar with a pestle, filtered through a paper and a microfilter. The grape juice sample was diluted ten-fold with deionized water and injected to an analytical column.

HPLC analysis

A HPLC system Agilent Technologies 1100 Series equipped with a refractive index detector (RID), quaternary pump, degasser, column thermostat unit and autosampler (Agilent) was used. All instrument units were monitored and controlled by Agilent ChemStation computer pro-

gramme. The separation was performed on a Kromasil 100-5NH_2 column, 250 × 4.6 mm i.d. (EKA Chemicals, Separation Products, Bohus, Sweden) at 30 °C. The RID optical unit was permanently warmed up to 40 °C. All samples were injected in 20-μl volumes and eluted isocratically with the mobile phase acetonitile : water, 75 : 25 (v : v). The flow rate of the mobile phase was 1.0 ml·min^{-1}. The peaks were identified by retention times and quantified by external calibration.

Statistics

Each sample was analysed minimally in duplicate and results are reported as mean concentration ± standard deviation (*SD*). Excel XP Software (Microsoft, Redmond, Washington, USA) was used for construction of calibration graphs as well as for determination of differences between means by analysis of variance (ANOVA). In this analysis, the difference was taken as significant at $P < 0.05$ (95% confidence level). The program QC.Expert version 2.5 (TriloByte Statistical Software, Pardubice, Czech Republic) was used for determination of linear correlation between individual wine constituents. To distinguish the organic wine samples from the conventional ones, multivariate statistic calculation, employing methods of canonical discriminant analysis and classification, was performed by means of Unistat v. 6.0 (Unistat, London, United Kingdom) statistical software, taking into consideration all the experimental data. The principal component analysis (PCA), a widely used multivariate analytical statistical technique to reduce dimensionality of the data by linear combinations of original dependent variables to a smaller set of new uncorrelated variables, was applied for data visualization.

Method validation

The HPLC method was validated by means of calibration and estimation of the range of linearity, limit of detection (*LOD*) and limit of quantification (*LOQ*), precision (as an internal repeatability) and accuracy (as a recovery rate). The calibration measurements were performed with calibration standards. *LOD* and *LOQ* values were calculated as an average response increased by triple and decuple the standard deviation, respectively. The precision of the method (expressed as relative standard deviation, RSD_r) was determined at two concentration levels: 1.0 g·l^{-1} and 10 g·l^{-1} for glycerol and 0.5 g·l^{-1} and 10 g·l^{-1} for saccharides. The accuracy was assessed through recovery test by the addition of a known amount of the standard to a wine sample.

RESULTS AND DISCUSSION

Method for wine analysis

The HPLC method, routinely utilized for determination of saccharides in our laboratory, was modified for simultaneous separation of glycerol, fructose, glucose and saccharose in wine and grape juice. A small adjustment of flow rate of the mobile phase was carried out as well as adaptation of temperature of analytical column, in order to gain better separation capability and higher responses for the compounds of choice. As a result, the duration of analysis, assuring reliable separation of all wine components, was a maximum of 25 min. Satisfactory precision and accuracy were attained in the method validation assessment (Tab. 1). The refractive index detector responses were linear in the range of 0.40–20 g·l^{-1} for glycerol, 0.05–10 g·l^{-1} for fructose, 0.10–10 g·l^{-1} for glucose and 0.08–10 g·l^{-1} for saccharose. In the calibration measurements, the correlation coefficients obtained were higher than 0.999 for all studied compounds. Fig. 1 shows typical HPLC profiles of white wines containing different residual saccharides and glycerol, as obtained using the optimized method.

Tab. 1. Validation parameters of HPLC-RID.

Compound	Retention time [min]	*LOD* [g·l^{-1}]	*LOQ* [g·l^{-1}]	Precision			Accuracy	
				Repeatability, RSD_r [%]			Recovery rate, white / red wine [%]	
				0.5 g·l^{-1}	1 g·l^{-1}	10 g·l^{-1}	2 g·l^{-1}	3 g·l^{-1}
Glycerol	5.330 ± 0.020	0.22	0.40	–	2.75	6.30	–	82 / 73
Fructose	8.889 ± 0.230	0.04	0.05	3.64	–	2.38	86 / 82	–
Glucose	10.576 ± 0.390	0.08	0.10	11.11	–	7.11	114 / 96	–
Saccharose	14.059 ± 0.331	0.05	0.08	2.17	–	2.55	101 / 81	–

LOD – limit of detection, *LOQ* – limit of quantification, RSD_r – repeatability relative standard deviation.

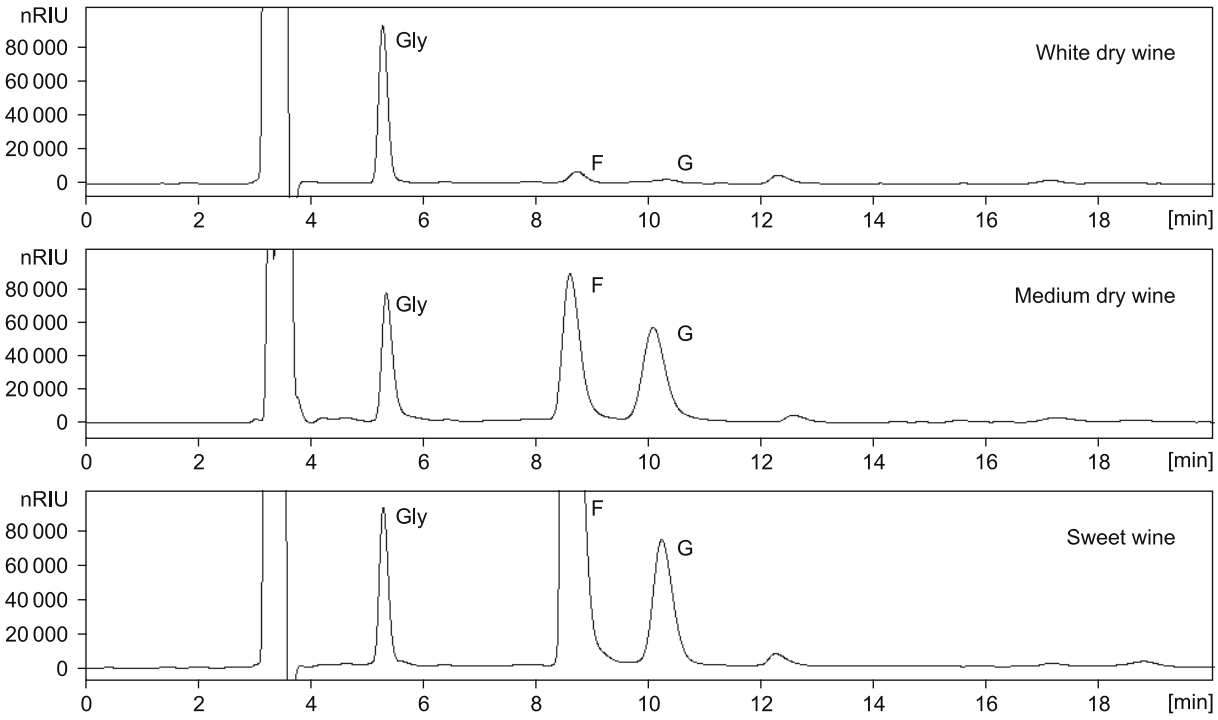

Fig. 1. Typical HPLC-RID record in white dry, medium dry and sweet wine.

Gly – glycerol, F – fructose, G – glucose.

Quality assessment of wines and grape juices by compositional analysis

The dominant group of wine samples involved 14 organic and 21 conventional dry white and red wines. Tab. 2 presents particular results for glucose, fructose, glycerol and ethanol contents, and glucose/fructose and glycerol/ethanol ratio in wines. The concentration of ethanol in grams per litre was determined indirectly by transformation of ethanol volumetric percentage declared on the bottle etiquette, via conversion tabular values. Tab. 3 lists all results acquired by analyses of fresh grape juices, in which also saccharose was detected. Grape juices had to be diluted tenfold with deionized water to adjust the concentration of saccharides to the concentration range of linear response.

Analysis of saccharides

Concentration of saccharides is one of the most significant factors in fermentation. Although the level of produced ethanol is related to the level of saccharides initially present in the juice, this relationship is not precise [17]. Residual saccharides in wine refer to the saccharides remaining after fermentation. This value is important for assessment of the fermentation completeness and for final sensory and microbial stability of wine. The concentration of residual saccharides in wines was estimated as a sum of glucose and fructose concentrations. According to the residual saccharides labelling indication for classification of wines in the European Union [6], dry wine should comprise maximal 4 $g \cdot l^{-1}$ of residual saccharides. Within the group of dry wines analysed, the concentration of residual saccharides was in the range of 0.15–3.26 $g \cdot l^{-1}$, independent from grape variety. Two wine samples exceeded the upper value (8.13 $g \cdot l^{-1}$ and 6.81 $g \cdot l^{-1}$). In all conventional wines tested, the concentration of fructose varied from 0.28 $g \cdot l^{-1}$ to 2.25 $g \cdot l^{-1}$, which was much lower than the published data of up to 9.12 $g \cdot l^{-1}$ [18], however well corresponding with fructose levels 0.2–2.0 $g \cdot l^{-1}$ found in conventional white wines [19].

Regarding the grape juice, it usually contains approximately equal levels of glucose and fructose. In our work, also saccharose was detected in the samples, but at markedly lower concentrations than glucose and fructose in the three organically and conventionally cultivated grape berries (Pinot Noir, Traminer Red, Cabernet Sauvignon) in two vintage years 2010–2011 (Tab. 3). The concentration of saccharose in fresh grape juices ranged from 0.23 $g \cdot l^{-1}$ to 1.11 $g \cdot l^{-1}$, whereas

the interval of glucose and fructose concentration was 95.34–171.30 g·l^{-1} and 78.12–119.16 g·l^{-1}, respectively. In the study on the quality characteristics of 23 clones of *Vitis vinifera* cv. Kalecik Karasi [20], glucose and fructose levels between 120.59–136.45 g·l^{-1} and 112.51–123.19 g·l^{-1}, respectively, were determined. In comparison with that study, our work included different grape cultivars from organic and conventional production, therefore the interval of saccharides concentration was much broader.

Examination of two comparably homogenous samples from the group of red grape cultivars (Pinot Noir and Cabernet Sauvignon, Tab. 3) in

Tab. 2. The sum of glucose and fructose, glycerol and ethanol concentration, glucose/fructose and glycerol/ethanol ratio of different wine varieties and wine production systems.

Grape variety	Sample identification	Glucose + Fructose* [g·l^{-1}]	G/F*	Glycerol* [g·l^{-1}]	Ethanol[a] [g·l^{-1}]	Gly/Eth × 100 [%]
Chardonnay	D1-O	2.78 ± 0.07	0.15 ± 0.01	4.97 ± 0,13	90.9	5.5
	D2-O	2.22 ± 0.07	0.93 ± 0.06	6.96 ± 0.01	102.7	6.8
	D3-O	5.40 ± 0.60	0.32 ± 0.12	7.15 ± 0.09	102.7	7.0
	D4-C	0.97 ± 0.02	1.54 ± 0.07	7.00 ± 0.10	98.9	7.1
	D5-C	0.81 ± 0.10	2.59 ± 0.16	6.21 ± 0.01	90.9	6.8
	D6-C	3.26 ± 0.52	1.01 ± 0.17	7.79 ± 0.09	114.6	6.8
Traminer Red	D7-O	0.15 ± 0.01	1.42 ± 0.12	6.18 ± 0.01	98.9	6.2
	D8-C	0.99 ± 0.03	2.29 ± 0.04	6.86 ± 0.05	86.9	7.9
	D9-C	1.68 ± 0.18	1.66 ± 0.41	6.57 ± 0.05	95.0	6.9
	D10-C	1.53 ± 0.29	1.89 ± 0.57	6.30 ± 0.09	95.0	6.6
Rhine Riesling	D11-O	4.79 ± 0.11	0.99 ± 0.04	5.87 ± 0.01	95.0	6.2
	D12-C	2.74 ± 0.02	0.64 ± 0.01	6.64 ± 0.07	98.9	6.7
Pinot Blanc	D13-O	2.82 ± 0.02	0.18 ± 0.01	7.59 ± 0.01	102.7	7.4
	D14-O	4.21 ± 0.30	0.32 ± 0.08	7.88 ± 0.46	102.7	7.7
	D15-O	8.13 ± 0.10	1.51 ± 0.01	9.01± 0.01	106.4	8.5
	D16-C	0.93 ± 0.10	1.17 ± 0.02	6.37 ± 0.05	86.9	7.3
	D17-C	1.62 ± 0.15	0.71 ± 0.05	7.53 ± 0.55	102.7	7.3
	D18-C	1.50 ± 0.25	1.00 ± 0.18	6.69 ± 0.15	102.7	6.5
	D19-C	6.81 ± 0.57	1.93 ± 0.07	6.91 ± 0.23	102.7	6.7
Pinot Noir	D20-O	0.38 ± 0.01	3.17 ± 0.08	9.65 ± 0.04	102.7	9.4
	D21-O	0.94 ± 0.09	2.26 ± 0.70	9.73 ± 0.01	102.7	9.5
	D22-O	1.37 ± 0.34	6.53 ± 1.33	9.44 ± 0.03	109.0	8.7
	D23-C	1.42 ± 0.09	2.49 ± 1.11	7.93 ± 0.03	95.0	8.3
	D24-C	1.68 ± 0.28	1.75 ± 0.01	7.64 ± 0.01	90.9	8.4
Blaufränkisch	D25-O	0.33 ± 0.02	1.51 ± 0.11	8.86 ± 0.01	101.2	8.8
	D26-C	0.71 ± 0.06	1.55 ± 0.22	8.00 ± 0.08	98.9	8.1
	D27-C	0.78 ± 0.12	2.15 ± 0.21	7.25 ± 0.04	86.9	8.3
	D28-C	1.24 ± 0.12	1.43 ± 0.10	8.80 ± 0.06	102.7	8.6
	D29-C	0.66 ± 0.05	1.31 ± 0.19	6.90 ± 0.13	86.9	7.9
Cabernet Sauvignon	D30-O	1.69 ± 0.18	1.21 ± 0.08	8.37 ± 0.03	98.9	8.5
	D31-O	1.44 ± 0.50	3.00 ± 1.17	9.78 ± 0.01	112.2	8.7
	D32-C	0.54 ± 0.04	0.26 ± 0.05	8.69 ± 0.06	102.7	8.5
	D33-C	4.77 ± 0.63	1.53 ± 0.24	9.17 ± 0.07	102.7	8.9
	D34-C	1.93 ± 0.35	2.10 ± 0.75	8.70 ± 0.01	95.0	9.2
	D35-C	1.88 ± 0.25	2.69 ± 0.01	8.60 ± 0.05	95.0	9.1

Sample identification (product type-technology): D – dry, O – organic, C – conventional.
* – results are expressed as mean ± standard deviation, a – ethanol concentration converted from percentage by volume to gram per litre (tabular values). G/F – glucose/fructose ratio, Gly/Eth – glycerol/ethanol ratio.

Tab. 3. Concentration of saccharides and glycerol in fresh grape juice from the vintage 2010 and 2011.

Grape cultivar	Product type technology	Glucose [g·l^{-1}]	Fructose [g·l^{-1}]	Saccharose [g·l^{-1}]	G/F	Glycerol [g·l^{-1}]
Pinot Noir	O^a	110.07±13.46	90.01±3.08	0.49±0.01	1.28±0.02	0.99±0.47
	O^b	187.56±1.49	119.16±0.01	1.01±0.11	1.58±0.01	0.59±0.02
	C^a	95.34±2.50	78.12±0.69	0.54±0.01	1.21±0.02	2.81±0.56
	C^b	175.17±7.49	117.60±0.45	Nd	1.49±0.06	Nd
Traminer Red	O^a	102.19±2.28	81.16±0.86	0.23±0.01	1.23±0.01	1.10±0.07
	O^b	173.95±2.50	116.29±0.69	0.97±0.52	1.50±0.01	0.49±0.03
	C^a	116.28±3.03	89.82±1.31	0.37±0.01	1.27±0.02	0.74±0.11
Cabernet Sauvignon	O^a	112.09±0.98	84.01±0.47	Nd	1.32±0.01	0.43±0.04
	O^b	137.75±3.56	90.97±0.13	1.11±0.41	1.52±0.04	Nd
	C^a	111.27±3.24	83.74±1.00	Nd	1.32±0.01	0.36±0.08
	C^b	171.30±2.02	109.27±0.58	Nd	1.57±0.01	Nd

Product type technology: O – organic, C – conventional. a – vintage 2010, b – vintage 2011.
Results are expressed as mean ± standard deviation.
G/F – glucose/fructose ratio, Nd – not detected.

the interaction variety versus production system showed that the accumulation of saccharides in grapes during the maturation period was nearly equivalent in both cultivation systems. In summary, the mean concentration of saccharides (not directly given in Tab. 3) was 232.91 g·l^{-1} and 248.21 g·l^{-1} in organic and conventional red cultivars, respectively. Following literature data, Bunea et al. [21] found much lower mean values of saccharides in white wine cultivars from organic and conventional cultivation, which represented 180 g·l^{-1} and 191 g·l^{-1}, respectively. Liu et al. [22] analysed 98 grape cultivars and determined 45.86–122.89 g·l^{-1} of glucose and 47.64–131.04 g·l^{-1} of fructose. Present results confirm a high variance of saccharides concentration in different grape cultivars, with a slight tendency to increase the concentration of saccharides in conventionally cultivated grapes. The overall data on saccharides concentration in ripe grapes range from 150 g·l^{-1} to 270 g·l^{-1} [1, 17].

Glucose/Fructose ratio (G/F)

The G/F ratio may indicate which one among the residual saccharides dominates in wine, and may also give some information on conditions of fermentation. Basically, this ratio is derived from actual glucose and fructose concentrations in grape juice or wine. In fermented wines, the G/F ratio is usually less than 1, but may be strongly dependent on variety, yeast strain and fermentation procedure [23]. According to the results presented in Tab. 2, the G/F ratios in wines differed to a large extent between/within grape varieties and cultivation systems. Pinot Noir and Cabernet Sauvignon variety wines exhibited most diverse results, in which glucose surpassed fructose concentration several times, hence both resulted in a high G/F ratio. Only in few wine samples the G/F ratio was lower than 1. The variations in the obtained ratios may be probably attributed to the large period of vintages (2002–2009) that included changing weather conditions and accordingly its influence on grapes' crop.

The G/F characteristic is also a major indicator of the grape maturity for determination of harvest time. As was already mentioned, this ratio is near to 1 in ripe berries, but may vary from 0.71 to 1.45 [24]. In our case, the G/F ratios in grape juices were not so scattered as in wines and varied from 1.22 to 1.57 in the three grape varieties during two vintages (Tab. 3). Variability in the G/F ratio among grape varieties at ripe stage has been previously described. For example, Keskin et al. [19] published the ratio from 0.90 to 0.955 in Kalecik Karasi cultivar; Sabir et al. [25] reported the mean value of 1.09 in five ecological grape cultivars produced in Mediterranean conditions. Soulis and Avgerinos [26] presented the value of 1.55 found in table grape cultivar Razaki in the final over-ripe stage, evidently with superfluity of glucose, which most corresponds to our findings.

Glycerol/Ethanol ratio (Gly/Eth)

The Gly/Eth ratio is generally regarded as highly variable that may reveal a systematic adulteration of wine with low amounts of glycerol, when the glycerol concentration exceeds the 10% level of the ethanol concentration.

The estimation of both wine-derived glycerol

and ethanol under our condition showed that glycerol did not exceed the concentration of 10 g·l^{-1} in wines; the highest glycerol level of 9.78 g·l^{-1} was determined in Cabernet Sauvignon variety wine. The declared ethanol concentration in all analysed dry wines, calculated from the ethanol percentage by volume, was within the range of 95.0–114.6 g·l^{-1}; comparably the ordinary ethanol concentration ranges between 65 g·l^{-1} and 120 g·l^{-1} in wines [1, 27]. The actual percentage of the Gly/Eth ratio ranged from 5.5 to 9.5 in all wine samples analysed, which falls within the required limit interval of 6–10% of the ethanol concentration (Tab. 2). The obtained results did not show any adulteration of wines using this simple conventional technique. Although high Gly/Eth ratios may indicate an addition of unnatural glycerol, on the contrary, it can be reduced by addition of distilled ethanol to the wine [28]. It is worth to point out that the estimation of Gly/Eth ratio depends on formation of glycerol during fermentation, hence is markedly influenced by many environmental factors as mentioned in the introduction section and, therefore, its utilization as a decisive parameter should be taken with caution. It should be also taken into account that, in recent years, there is an increasing demand for wines with high glycerol and reduced ethanol concentration. For this purpose, attempts have been made in selection and testing *Saccharomyces* species with enhanced glycerol production [29, 30]. Presumably, new and more reliable approaches for evaluation of such wines are expected to be established.

Concerning fresh grape juice, we found a natural quantity of glycerol at levels of 0.36–2.81 g·l^{-1} (Tab. 3), that corresponded with other reported values varying between 1.70 g and 3.02 g of glycerol per litre [31].

Statistical and multivariate data analysis

In order to study the differences between organic and conventional wines originating from one vineyard locality, the obtained data underwent one way factor ANOVA analysis. As a source of variations in the fructose, glucose and glycerol composition of wines, the production system (organic/conventional) as well as the type of wine (white/red) was considered. For evaluation of the significance of a grape variety and a vintage year, larger sampling and homogeneity of wine samples is commonly required. Therefore, the mentioned variables were excluded from this analysis in whole. Fig. 2 illustrates mean values of large data files on fructose, glucose and glycerol concentrations in white/red wines from organic/conventional production that were used in the statistical assessment of wines.

Testing the significance of difference between white/red wines (associated in one group from all white/red grape varieties) and organic versus conventional wine, a difference was found in fructose concentration that was higher in organic white wines ($P < 0.001$). The glycerol level was increased in red wines ($P < 0.01$), but was independent from wine production system. Similar to some published data [12, 23], much higher glycerol concentration can be commonly found in red wines than in white, what is actually in agreement with our finding.

In an examination of relative relationships between total saccharides (commonly referred to as total sugar), glycerol and ethanol concentration in wines, linear regression analysis with Pearson's correlation was applied, results are depicted graphically in Fig. 3. A moderate positive correlation was indicated between total saccharides and glycerol in organic white wines and conventional red wines ($r = 0.5238$ at $P < 0.01$ and $r = 0.5167$

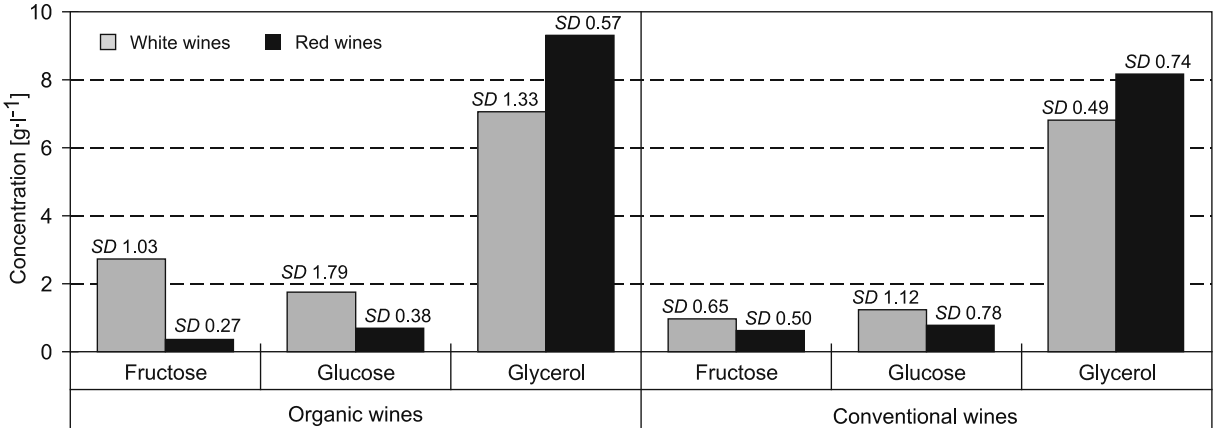

Fig. 2. Mean concentration of fructose, glucose and glycerol in tested wines.

SD – standard deviation.

at $P < 0.001$, respectively). Similarly, total saccharides were positively related to ethanol in organic white wines ($r = 0.5238$ at $P < 0.001$). High positive relation was determined between total saccharides and ethanol in conventional white wines ($r = 0.7182$) at a significance level of $P < 0.001$.

PCA was applied to the saccharide and glycerol data set consisting of 35 samples, in order to visualize the main sources of variability. Two principal components were extracted, covering 90.9% of the original data variance. The score plot of these two components is shown in Fig. 4. The recognition of wine varieties is not clear, but differences between red and white wines are substantial. From the loadings of the variables, the concentration of total saccharides and fructose were dominant variables in the first principal components, representing 64.7% of the total variance, while glycerol dominated in the second principal component. This is in accordance with results from ANOVA analysis that confirms these variables' significance. Similarly to PCA, the canonical discriminant analysis did not reveal any differences between wine varieties according to the level of examined parameters by

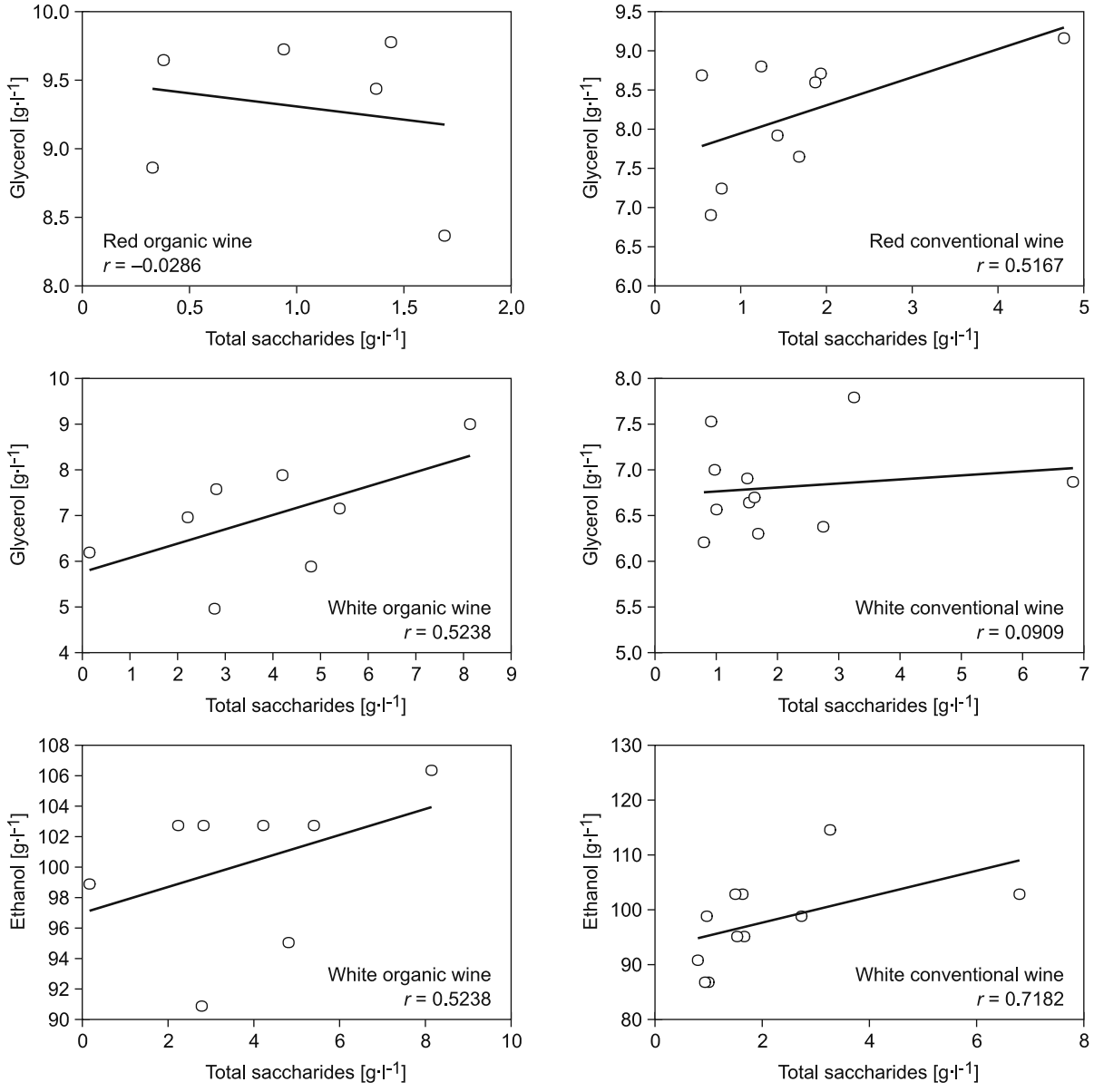

Fig. 3. The Pearson's correlations between total saccharides, glycerol and ethanol concentration.

r – correlation coefficient.

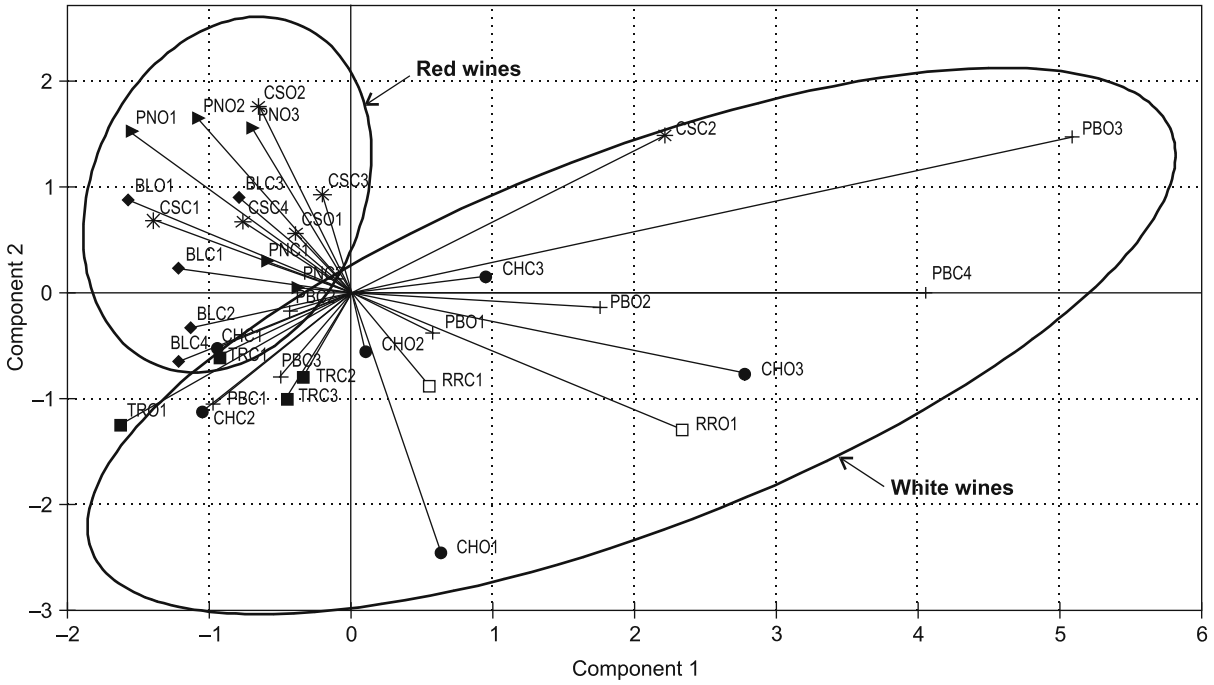

Fig. 4. Differentiation of varietal wines according to the concentration of saccharides and glycerol using principal component analysis.

CHO – Chardonnay organic, CHC – Chardonnay conventional, TRO – Traminer Red organic, TRC – Traminer Red conventional, RRO – Rhine Riesling organic, RRC – Rhine Riesling conventional, PBO – Pinot Blanc organic, PBC – Pinot Blanc conventional, PNO – Pinot Noir organic, PNC – Pinot Noir conventional, BLO – Blaufränkisch organic, BLC – Blaufränkisch conventional.

the classification procedure, which correctly rated only one variety (Traminer Red) from the other ones. By discrimination of data according to the type of grape production (conventional versus organic), 70% of wine samples were correctly identified in the recognition ability testing. The concentration of fructose was the predominant variable for discrimination and classification of wines. The later result is also in a good conformity with the ANOVA results, which found fructose significantly higher in organic white wines compared to their conventional counterparts.

CONCLUSION

Present research provides the first information on quality of wines that come from two separate production systems in Slovakia. Within this research, an attempt was achieved to identify differences between wines by partial compositional analysis using HPLC technique. Evaluation of the wines and grape juices based on analysis of saccharides and glycerol did not show relevant variations of these compounds in comparison with the European wine regulations set for dry wines. The derived glucose/fructose ratio that is strongly dependent on the concentration of individual saccharides in grape juice/wine may be rated as an informative indicator of grape maturity and fermentation process conditions. In the wines tested, this ratio showed scattered results even within the grape varieties with similar levels of total saccharides.

The analysis of variance (ANOVA) that did not include wine variety and vintage year, revealed some differences between organic and conventional wines in fructose and glycerol concentration. Applying linear correlation analysis, moderate correlative dependences were found between total saccharides and glycerol/ethanol ratio. However, the obtained results of the correlation analysis cannot definitely support the correlation causality of the variables and may be only guiding for an extended statistical evaluation. Principal component analysis found significant differences between white and red wines using the examined data. Discrimination of wines according to affiliation to organic or conventional production resulted in 70% of correctly classified wines.

This study represents primary information useful for further distinguishing of wines produced in Slovakia accordingly to their varieties. The optimized high performance liquid chromatography

method employed in this study is a useful tool for wine quality estimation because of its simplicity and applicable reliability.

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REFERENCES

1. Hartmeier, M. – Reiss, M.: Production of beer and wine. In: Esser, K. – Bennett, J. W. – Osiewacz, H. D. (Ed.): The Mycota X. Industrial Applications. Berlin, Heidelberg, New York : Springer, 2002, pp. 49–65.
2. Varandas, S. – Teixeira, M. J. – Marques, J. C. – Aguiar, A. – Alves, A. – Bastos, M. M. S. M.: Glucose and fructose levels on grape skin: interference in *Lobesia botrana* behaviour. Analytica Chimica Acta, *513*, 2004, pp. 351–355.
3. Berthels, N. J. – Cordero Otero, R. R. – Bauer, F. F. – Thevelein, J. M. – Pretorius, I. S.: Discrepancy in glucose and fructose utilisation during fermentation by *Saccharomyces cerevisiae* wine yeast strains. FEMS Yeast Research, *4*, 2004, pp. 683–689.
4. Ciani, M. – Ferraro, L. – Fatichenti, F.: Influence of glycerol production on the aerobic and anaerobic growth of the wine yeast *Candida stellata*. Enzyme and Microbial Technology, *27*, 2000, pp. 698–703.
5. Belitz, H. D. – Grosch, W. – Schieberle, P.: Food Chemistry. 3rd revised Edition. Berlin, Heidelberg : Springer 2004. 1081 pp. ISBN 3-540-40817-7.
6. Commission Regulation (EC) No 753/2002 of 29 April 2002 laying down certain rules for applying Council Regulation (EC) No 1493/1999 as regards the description, designation, presentation and protection of certain wine sector products. Official Journal of the European Communities, L118, 2002, pp. 1–54.
7. Lee, C. K.: The chemistry and biochemistry of the sweetness of sugars. Advances in Carbohydrate Chemistry and Biochemistry, *45*, 1987, pp. 199–351.
8. Bakker, J. – Clarke, R. J.: Wine: Flavour chemistry. 2nd ed. Oxford : Wiley-Blackwell, 2012. 418 pp. ISBN 978-1-4443-3042-7.
9. Scanes, K. T. – Hohmann, S. – Prior, B. A.: Glycerol production by the yeast *Saccharomyces cerevisiae* and its relevance to wine: a review. South African Journal of Enology and Viticulture, *19*, 1998, pp. 17–24.
10. Remize, F. – Sablayrolles, J. M. – Dequin, S.: Re-assessment of the influence of yeast strain and environmental factors on glycerol production in wine. Journal of Applied Microbiology, *88*, 2000, pp. 371–378.
11. Ravji, R. G. – Rodriguez, S. B. – Thornton, R. J.: Glycerol production by four common grape molds. American Journal of Enology and Viticulture, *39*, 1988, pp. 77–82.
12. Jackson, R. S.: Wine science: principles and applications. S. Diego : Academic Press, 1994. 186 pp. ISBN 978-0-12-373646-8.
13. Guyon, F. – Gaillard, L. – Salagoïty, M.-H. – Médina, B.: Intrinsic ratios of glucose, fructose, glycerol and ethanol 13C/12C isotopic ratios determined by HPLC-co-IRMS: toward determining constants for wine authentication. Analytical and Bioanalytical Chemistry, *401*, 2011, pp. 1551–1558. DOI: 10.1007/s00216-011-5012-5.
14. Cabañero, A. – Recio, J.L. – Rupérez, M.: Simultaneous stable carbon isotopic analysis of wine glycerol and ethanol by liquid chromatography coupled to isotope ratio mass spectrometry. Journal of Agricultural and Food Chemistry, *58*, 2010, pp. 722–728.
15. Calderone, G. – Naulet, N. – Guillon, C. – Reniero, F.: Characterization of european wine glycerol: stable carbon isotope approach. Journal of Agricultural and Food Chemistry, *52*, 2009, pp. 5902–5906.
16. Fauhl, C. – Wittkowski, R. – Lofthouse, J. – Hird, S. – Brereton, P. – Versini, G. – Lees, M. – Guillou, C.: Gas chromatographic/mass spectrometric determination of 3-methoxy-1,2-propanediol and cyclic diglycerols, by-products of technical glycerol, in wine: interlaboratory study. Journal of AOAC International, *87*, 2004, pp. 1179–1188.
17. Dharmadhikari, M.: Composition of grapes. Vineyard and Vintage View, *9*, 1994, pp. 3–8.
18. Moro, E. – Majocchi, R. – Ballabio, C. – Molfino, S. – Restani, P.: A rapid and simple method for simultaneous determination of glycerol, fructose and glucose in wine. American Journal of Enology and Viticulture, *58*, 2007, pp. 279–282.
19. Gorinstein, S. – Moshe, R. – Deutch, J. – Wolfe, F. H. – Tilis, K. – Stiller, A. – Flam, I. – Gat, Y. A.: Determination of basic components in white wines by HPLC, FT-IR spectroscopy, and electrophoretic techniques. Journal of Food Composition and Analysis, *5*, 1992, pp. 236–245.
20. Keskin, N. – Bingöl, Ö. – Kuru, I. S.: Evaluation of some berry quality characteristics in *Vitis vinifera* cv. Kalecik Karasi clones. Journal of Agriculture and Biology, *14*, 2012, pp. 461–464.
21. Bunea, C. I. – Ardelean, M. – Pop, N. – Bunea, A. – Babes, A. – Calugar, A.: Influence of variety and type of cultivation (organic and conventional) on quality, in five wine grape varieties grown in Cluj county, Romania. Bulletin UASVM Horticulture, *67*, 2010, pp. 179–182.
22. Liu, H. F. – Wu, B. H. – Fan, P. G. – Li, S. H. – Li, L. S.: Sugar and acid concentration in 98 grape cultivars analyzed by principal component analysis. Journal of the Science of Food and Agriculture, *86*, 2006, pp. 1526–1536.
23. Beleniuc, G. – Marin, G. – Ranca, A.: Influence of fermentation condition and yeast species on the glycerol concentration in wines. Acta Horticulturae (ISHS), *640*, 2004, pp. 375–378.

24. Amerine, M. A. – Ough, C. S.: Recent advances in enology. Critical Reviews in Food Technology, *2*, 1972, pp. 407–515.
25. Sabir, A. – Kafkas, E. – Tangola, S.: Distribution of major sugars, acids and total phenols in juice of five grapevine (*Vitis*, spp.) cultivars at different stages of berry development. Spanish Journal of Agricultural Research, *8*, 2010, pp. 425–453.
26. Soulis, T. P. – Avgerinos, A. S.: Changes in the glucose:fructose ratio during the growth period of the Razaki grape. Journal of Agriculture and Food Chemistry, *35*, 1984, pp. 527–530.
27. Mataix, E. – Luque de Castro, M. D.: Simultaneous determination of ethanol and glycerol in wines by a flow-injection pervaporation approach with in parallel photometric and fluorimetric detection. Talanta, *51*, 2000, pp. 489–496.
28. Amerine, M. A. – Ough, C. S.: Methods for analysis of musts and wines. New York : John Wiley & Sons, 1980. 341 pp. ISBN 0471050776 / 0-471-05077-6.
29. Ehsani, M. – Fernández, M. R. – Biosca, J. A. – Julien, A. – Dequin, J.: Engineering of 2,3-butanediol dehydrogenase to reduce acetoin formation by glycerol-overproducing, low-alcohol *Saccharomyces cerevisiae*. Applied and Environmental Microbiology, *75*, 2009, pp. 3196–3205.
30. Orlić, S. – Arroyo-López, F. N. – Huić-Babić, K. – Lucilla, I. – Querol, A. – Barrio, E.: A comparative study of the wine fermentation performance of *Saccharomyces paradoxus* under different nitrogen concentrations and glucose/fructose ratios. Journal of Applied Microbiology, *108,* 2010, pp. 73–80.
31. Nieuwoudt, H. H. – Prior, B. A. – Pretorius, I. S. – Bauer, F. F.: Glycerol in South African table wines: an assessment of its relationship to wine quality. South African Journal of Enology and Viticulture, *23*, 2002, pp. 22–30.

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