Selection of autochthonous *Saccharomyces cerevisiae* strains for production of typical Pinot Gris wines

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Summary
Local yeast microflora on grapevines and around the wineries may well prove to play an important, and hitherto unappreciated, role in the subtler aspects of wine terroir characteristics. The objective of the present study was to isolate and compare the indigenous strains of *Saccharomyces cerevisiae* present in a vineyard in the Nitra wine region (Slovakia) and to select the most suitable ones for winemaking process. Yeast populations were collected in 2012–2015 from the surface of berries set to maturity, must, fermenting must and young wine produced by spontaneous fermentation of Pinot Gris. Five *Saccharomyces* strains were isolated and, based on technological properties, four strains were selected for microvinification. In young wines, general parameters were monitored but also aromatic profiles were analysed by gas chromatography-mass spectrometry and by sensory evaluation. Wines prepared by strains 7613 and 7913 contained more than 40 $mg \cdot l^{-1}$ of ethyl acetate, 23 $mg \cdot l^{-1}$ of 2-methyl-1-propanol and less than 17 $mg \cdot l^{-1}$ of 2-phenyl ethanol. These wines were evaluated by tasters as having exotic fruits aroma. Based on these results, two *S. cerevisiae* strains were recommended as starter cultures for making of terroir Pinot Gris wines, so the results of the work can be used in winemaking practice.

Keywords
autochthonous yeast; *Saccharomyces cerevisiae;* selection; technological properties; volatile organic compounds; sensory evaluation; terroir wine

Wine flavour is the product of a number of factors such as grape variety, geographical and cultural conditions of grape growing and of the grape microbiome. As the importance of *Saccharomyces cerevisiae* role in winemaking has long been established, the use of commercial yeast cultures in fermentation is an ordinary practice to ensure a reproducible product and to reduce the risk of wine spoilage. However, this can cause a progressive substitution of local microflora and a consequent reduction of microbial biodiversity, which can lead to a loss of typicality and complexity of the wine. Indeed, knowledge of the autochthonous yeast strains will help to preserve and employ the most representative strains [1].

"Terroir" refers to "an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied viticultural and oenological practices develops, giving distinctive characteristics for the products originating from this area" [2]. This assumption is at the base of the Appellation of Origin systems around the globe, with a strong impact on the wine market since they drive the consumers' choice. The selection and the employment of autochthonous microorganisms could be a powerful instrument to improve the organoleptic and sensory characteristics of wine produced from indigenous grape cultivars [3]. In fact, autochthonous yeasts are the microorganisms better adapted to a specific must, which detain characteristics determined by the variety of the grapes and the terroir [4]. Pure cultures of noble wine yeasts are obtained by isolation and selection

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from grapes or spontaneously fermented must, and their properties are tested thoroughly before their introducing into production practice, which is very time consuming. Many research groups explored the opportunity to isolate, select and use indigenous *S. cerevisiae* strains with good technological properties and quality traits, as a strategic asset for winemakers to unequivocally link a wine with its environment of production [5–7]. Compared to spontaneous fermentation, selected pure *S. cerevisiae* cultures have several advantages. They guarantee a smooth and undisturbed start and continuation of fermentation, with subsequent bacterial degradation of acids. They assure complete fermentation of last sugar residues and allow a cleaner fermentation with minimal undesirable byproducts such as volatile acids or sulphur compounds [8].

Pinot Gris is a white grapevine variety of the species *Vitis vinifera*. Currently, it is grown on approximately 100 ha in Slovakia, representing 0.7 % of the total area of the vineyards. It is a valuable variety of traditional Slovak assortment. Berries are small (11–12 mm), round, light red with abundant wax coating on berry skin that produces a greyish red colour. Taste is full and very sweet. Also the sugar content in must is high, on average 19 $kg.hl^{-1}$. The wines produced from this grape are alcoholic, very harmonic, extractive and full-bodied, with a distinctive varietal aroma [9].

The aim of the work was to isolate autochthonous *S. cerevisiae* strains to produce authentic Pinot Gris varietal wines from Nitra wine region in Slovakia. The results of isolation and identification of wine yeasts from Pinot Gris grapes of Nitra wine region, together with data on their technological properties and possible application in practice previously verified by microvinification, have not been published yet. By applying the selected strains in winemaking process, terroir wines could be produced under controlled fermentation conditions within the examined region of Slovakia in the future.

MATERIALS AND METHODS

Mixed yeast populations were collected from the surface of grape set to maturity, must, fermenting must and young wine of Pinot Gris cultivar in 2012–2015 from Nitra wine region in Slovakia. Ten berries with 50 ml of sterile saline solution (0.9%) were shaken on an orbital shaker PSU-10i (Biosan, Riga, Latvia) at 3 Hz at laboratory temperature for 3 h. Must was prepared from 1 kg of grapes. A volume of 100 ml of must was spontaneously fermented for 14 days to young wine.

A volume of 100 μl of the homogenized saline solution, must, fermenting must (third–fifth day of fermentation) and spontaneously fermented wine were plated on Wallerstein Laboratories Nutrient (WLN) medium (Sigma-Aldrich, St. Louis, Missouri, USA) [10], individual yeast species were distinguished by different colony morphologies and colours, subsequently separated by serial dilutions and isolated to pure cultures. All isolated yeast strains were kept on slants of yeast extract-peptone-dextrose (YPD) agar medium (yeast extract 5 g, D-glucose 10 g, peptone 5 g, agar 20 g, distilled water 1 000 ml; final pH 6.5 ± 0.2 at 25 °C; autoclaved at 121 °C for 20 min; autoclave PS20A, Chirana, Stará Turá, Slovakia).

Yeasts were identified phenotypically, based on assimilation of carbon and nitrogen sources using biochemical tests (API test ID 32C; BioMérieux, Marcy l'Etoile, France) according to manufacturer's instructions.

Testing of technological properties

Tolerance to ethanol, osmotolerance and thermotolerance was tested in yeast extract-dextrose (YD) broth (yeast extract 10 g, D-glucose 20 g, distilled water 1 000 ml; final pH 6.0–6.5 at 25 °C; autoclaved at 121 °C for 20 min). For ethanol-tolerance testing, ethanol was added to the broth in concentrations from 0 % to 20 % v/v; for osmotolerance testing, glucose was added in levels from 0 % to 60 % w/w. Broth portions of the volume of 5 ml were inoculated by 100 μl fresh yeast suspension (1-day old culture was resuspended in 1 ml of distilled water, diluted to 3 McFarland units (McF; DensiCHEK plus, BioMérieux) and biomass formation was observed after 6 days' cultivation at 26 °C. For thermotolerance testing, YD broth portions of the volume of 5 ml were inoculated by 100 μl fresh yeast suspension and incubated at temperatures 4 °C, 26 °C, 37 °C and 54 °C (incubator FOC 225L, Velp Scientifica, Usmate, Italy). Biomass formation was observed after 6 days' cultivation.

Microvinification

Inoculum was prepared as follows: 5 × 10 ml of YD broths with *S. cerevisiae* strains (7613, 7913, 8412, 12314, 3215) were incubated at 26 °C (incubator FOC 225L) and shaken on an orbital shaker at 3 Hz for 24 h. After that, they were centrifuged (centrifuge 5804R, Eppendorf, Hamburg, Germany) at 5 200 ×*g* and the sedimented biomass was resuspended in 1 ml of distilled water and diluted to 0.5 McF (DensiCHEK plus).

A volume of 500 ml of must Pinot Gris from

Nitra wine region sterilized by heating in a microwave oven (10 min at 800 W; Perfecto MW311, Délonghi, Treviso, Italy) was inoculated by the prepared inoculum. No sulphur dioxide was used in the microvinification process. The must was fermented in 0.75 l bottles at 16 °C for 14 days. Five wine samples were prepared in duplicate.

Determination of general parameters

Three parameters (alcohol concentration, total acidity, volatile acidity) were determined according to the official methods of International Organisation of Vine and Wine (OIV) [11]. pH was measured with a pH meter OP-208/1 (Radelkis, Budapest, Hungary) by differential method [12]. Sugar concentration was determined by refractometry (refractometer MR210ATC, Milwaukee Electronics, Milwaukee, Wisconsin, USA) according to Commission Regulation No. 2676/90 [13] and expressed as sums of glucose and fructose in grams per litre. General parameters were analysed in duplicate and the results expressed as the mean of four determinations ± standard deviation.

Identification of volatile organic compounds

The head space solid-phase microextraction method previously described by SÁDECKÁ et al. [14] was used for sample extraction in a modified version. For each analysis, 10 ml of wine sample, 2 g of NaCl and 10 μl of 4-methyl-2-pentanol (10%) as internal standard was poured into a 20 ml vial. For heating (at 50 °C) and stirring (7 Hz) of the samples, a heated magnetic stirrer (RH digital, IKA, Staufen, Germany) with PTFE stirring bar was used. The compounds were thermo-desorbed by placing the fibre for 1 min into the gas chromatograph injector at 230 °C. A 6890N gas chromatograph (Agilent Technologies, Santa Clara, California, USA) coupled to a 5973 inert mass selective detector (Agilent Technologies) was used, the column was DB-WAXetr, 30 m × 0.25 mm with 0.50 μm film thickness (Agilent Technologies). The analyses were performed in split mode 20 : 1. The oven temperature was held at 35 °C for 1 min, then raised to 220 °C at a rate of 5 °C·min^{-1}. The total analysis time was 47 min. Mass spectra were acquired in selected ion monitoring (SIM) mode.

The concentrations of volatile organic compounds (with internal standard) were estimated from five-point calibration curves (0.1–50.0 mg·l^{-1}) with correlation coefficients (r^2) of 0.9976–0.9998. Each sample with internal standard was measured in duplicate and the results were expressed as the mean of four determinations ± standard deviation.

Sensory evaluation

In total, 5 staff members (1 man, 4 women) coming from the same workplace as authors participated in the sensory descriptive analysis. Tasters were asked to describe the odour of tested wines by choosing odour characteristic from the aroma list. The following twelve odour characteristics were used (exotic fruits, red fruits, banana, fruity, distillate, spice, roses, acidic, sweet, cantaloupe, nectarine, unpleasant), because they are considered as the most appropriate to describe wine samples [15].

RESULTS AND DISCUSSION

Suitable indigenous *S. cerevisiae* strains were selected based on technological properties, general parameters, aromatic profile of volatile compounds and sensory analysis, with the aim to offer, for the winemaking practice, the best starter cultures of yeasts originating from the given region. This is taken as an important issue in making of terroir wines.

WLN agar is the medium for macroscopic evaluation of yeasts. The evaluation is based on colour characterization and colony morphology specific to each fungal species. The colour coding feature is the pH indicator – bromocresol green. WLN medium is suitable for resolving colonies of non-*Saccharomyces* yeasts from *Saccharomyces* spp., the latter being dark green [16]. The yeast strains grown on WLN medium were of different colours (various shades of white, beige, green or brown). There was also a difference in their shape, most of the isolated yeasts were smoothly curved and some of them had a reduced margin or convex apex. Yeasts were round with solid margins. All of the yeast strains that were able to grow on YPD agar medium had white colour and all of them had circular shape with entire margin and convex elevation. In total, 36 yeast strains originating from Pinot Gris grapes, must, fermenting must or spontaneously fermented young wine from Nitra wine region of Slovakia were identified, therefrom 5 strains were identified as *Saccharomyces cerevisiae* (Tab. 1).

Yeast genera *Kloeckera*, *Candida*, *Saccharomyces* and *Saprochaete*, which are predominant at the time of the grape harvest [17–19], were isolated from samples. Genus variability of apiculate microorganisms was low during the 2012 observation season. Differences in total counts of isolated yeasts could be caused by climate conditions in combination with a protection system of grapevine. Strains identified as *Saccharomyces cerevisiae*

Tab. 1. Identified yeast strains.

Sample	2012		2013		2014		2015	
	Strain	Yeast species	Strain	Yeast species	Strain	Yeast species	Strain	Yeast species
Berry	7812	*Candida magnoliae*	3413	*Kloeckera apis*	10914	*Kloeckera apis*	2615	*Kloeckera apis*
			3513	*Candida pulcherrima*	11414	*Candida pulcherrima*	2515	*Candida pulcherrima*
			3613	*Saprochaete capitata*	11014	*Candida holmii*		
			3713	*Kloeckera apis*	11214	*Candida rugosa*		
			3813	*Candida pulcherrima*				
Must	8012	*Candida magnoliae*	7113	*Kloeckera apis/apiculata*	11514	*Kloeckera apis*	2815	*Kloeckera apis*
			7213	*Candida pulcherrima*	11614	*Candida pulcherrima*	2715	*Candida pulcherrima*
					11714	*Candida rugosa*		
Fermenting must	8212	*Candida incospicua*	7413	*Kloeckera apis/apiculata*	12114	*Candida rugosa*	2915	*Candida pulcherrima*
	8312	*Candida krusei*	7513	*Candida incospicua*	11914	*Kloeckera apis*	3015	*Kloeckera apis*
					12014	*Candida holmii*		
Young wine	8412	*Saccharomyces cerevisiae*	7613	*Saccharomyces cerevisiae*	12314	*Saccharomyces cerevisiae*	3215	*Saccharomyces cerevisiae*
	8512	*Candida krusei*	7913	*Saccharomyces cerevisiae*			3315	*Kloeckera apis*

Tab. 2. Ethanoltolerance, thermotolerance and osmotolerance of indigenous *S. cerevisiae* yeasts.

Strain	7613	7913	8412	12314	3215
Ethanoltolerance					
Ethanol 0 % v/v	+++	+++	+++	++	+++
Ethanol 5 % v/v	+++	+++	+++	++	+++
Ethanol 10 % v/v	++	+++	+++	++	–
Ethanol 12.5 % v/v	+	+++	+	++	–
Ethanol 15 % v/v	+/–	+/–	–	+/–	–
Ethanol 20 % v/v	–	–	–	–	–
Thermotolerance					
4 °C	+	+/–	+/–	+	+
26 °C	+	+	+	+	+
37 °C	+	+	+	+	+
54 °C	–	–	–	–	–
Osmotolerance					
Glucose 0 % w/w	+++	+++	+++	+++	++
Glucose 20 % w/w	+++	+++	+++	+++	–
Glucose 40 % w/w	+++	+++	+++	+++	–
Glucose 50 % w/w	+	+	+/–	+/–	–
Glucose 60 % w/w	–	–	–	+/–	–

(–) – no biomass formation; (+/–) – very weak biomass formation; (+) – weak biomass formation; (++) – moderate biomass formation; (+++) – strong biomass formation.

(7613, 7913, 8412, 12314, 3215), which occur mainly in spontaneously fermented musts [20, 21], were isolated from various phases of fermentation and also from young wines.

Ethanoltolerance, thermotolerance and osmotolerance of these isolated indigenous *S. cerevisiae* strains were evaluated (Tab. 2). These technological parameters of yeasts are very important from the point of view of proper fermentation process in winemaking. Four isolated strains (7613, 7913, 8412 and 12314) were tolerant to more than 12.5 % v/v of ethanol in medium and were osmotolerant enough for vinification (≥ 60 % w/w D-glucose), thus confirming their possible applicability in wine industry during the early fermentation stages [22]. The strain 3215 was weak in biomass formation and in ethanoltolerance as well as osmotolerance tests.

Based on tests results on technological properties, *S. cerevisiae* strains 7613, 7913, 8412 and 12314 were recommended for the microvinification test. Tab. 3 shows average values for the general parameters of the varietal wines Pinot Gris.

pH of wine is important to be determined since it affects the quality of the product in terms of its taste, colour, oxidation, chemical stability and other factors. Wine samples had pH in the range of 3.23–3.28, which was lower than an optimal value of 3.4 [23]. This caused retention of their fullness and freshness. Concentration of ethanol in wine samples was determined in a range of 13.4–15.1 % v/v, so they may be marked as wines with protected designation of origin according to the Slovak legislation, as they contained ≥ 9.5 % v/v ethanol [24].

The volatile organic compounds in four varietal wines prepared in duplicate were determined by gas chromatography-mass spectrometry (GC-MS). In total, 10 higher alcohols, 16 esters, 4 acids, 4 terpenoids and acetaldehyde were determined in wine samples. Concentrations of the most important volatile organic compounds in wines are shown in Tab. 4. Quantitatively, the most important higher alcohols are linear alcohols 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol (active pentanol), 3-methyl-1-butanol (isopentanol) and pentyl alcohol (as a mixture) [25]. Wine samples contained the highest levels of isopentanol (98.2–157.5 $mg \cdot l^{-1}$), 2-methyl-1-propanol (23.0–33.2 $mg \cdot l^{-1}$) and 1-propanol (15.8–29.3 $mg \cdot l^{-1}$). The determined concentrations were in the ranges commonly found in wines [26]. 1-Hexanol may greatly contribute to wine aroma, at concentrations above its aroma perception threshold (4 $mg \cdot l^{-1}$) it makes a faint impression and its flavour is described as “grassy” [27]. Additionally, at concentrations exceeding 8 $mg \cdot l^{-1}$, it gives a vegetable or herbal scent [28]. Therefore, strains producing higher concentrations of hexanol are not desirable in practice. 1-Hexanol was detected by GC-MS in wines at concentrations lower than its aroma perception threshold. In the must sample, the concentration was 1.59 $mg \cdot l^{-1}$.

Phenyl ethanol plays an important role in the aromatic profile of white wines, where its concentration is usually below the aroma perception threshold of 10 $mg \cdot l^{-1}$ [29] and is characterized by honey and spicy aroma, also being able to evoke the smell of roses or lilies [30]. Wines fermented by strains 7613, 7913 and 8412 had similar concentrations of this compund, 16.8 $mg \cdot l^{-1}$, 16.1 $mg \cdot l^{-1}$ and 17.6 $mg \cdot l^{-1}$, respectively. On the other hand, wines fermented by strain 12314 contained 40 $mg \cdot l^{-1}$ of 2-phenyl ethanol.

Esters are desirable aromatic constituents of a pleasant wine, which contribute to its fruity and flowery nuances [31]. Varietal wines fer-

Tab. 3. General parameters of Pinot Gris wines and grape juice.

Parameter	Samples				
	Grape juice	Wine 1	Wine 2	Wine 3	Wine 4
Alcohol (v/v) [%]	–	15.10 ± 0.50	13.60 ± 0.30	13.40 ± 0.60	13.50 ± 0.10
Total acidity [$g \cdot l^{-1}$]	7.60 ± 0.10	7.00 ± 0.20	7.30 ± 0.10	7.30 ± 0.10	7.20 ± 0.20
Volatile acidity [$g \cdot l^{-1}$]	–	0.45 ± 0.02	0.32 ± 0.05	0.26 ± 0.00	0.26 ± 0.06
Sugar concentration [$g \cdot l^{-1}$]	221.00 ± 2.20	49.00 ± 0.40	17.00 ± 0.30	24.00 ± 0.20	17.00 ± 0.30
pH	3.23 ± 0.01	3.23 ± 0.02	3.28 ± 0.02	3.23 ± 0.01	3.25 ± 0.01

Values are expressed as mean of four determinations ± standard deviation. Total acidity is expressed as grams of tartaric acid per litre. Volatile acidity is expressed as grams of acetic acid per litre. The sugar concentration is expressed as sums of glucose and fructose in grams per litre.
Wine 1 – wines fermented by *S. cerevisiae* strain 7613, Wine 2 – wines fermented by *S. cerevisiae* strain 7913, Wine 3 – wines fermented by *S. cerevisiae* strain 8412, Wine 4 – wines fermented by *S. cerevisiae* strain 12314.

Tab. 4. Concentration of selected volatile organic compounds in Pinot Gris wines and grape juice.

Compounds	Concentration [mg·l^{-1}]				
	Grape juice	Wine 1	Wine 2	Wine 3	Wine 4
Acetaldehyde	1.13 ± 0.32	12.27 ± 0.50	10.79 ± 4.36	4.56 ± 1.76	2.71 ± 1.65
Ethyl formate	0.01 ± 0.00	ND	0.44 ± 0.09	0.27 ± 0.09	0.34 ± 0.08
Methyl acetate	0.03 ± 0.01	ND	0.09 ± 0.03	0.08 ± 0.02	0.07 ± 0.02
Ethyl acetate	0.81 ± 0.09	60.88 ± 5.20	40.76 ± 5.22	37.49 ± 5.59	38.93 ± 6.48
Methanol	0.30 ± 0.02	0.59 ± 0.45	2.83 ± 0.01	0.33 ± 0.13	0.98 ± 0.15
n-Propyl acetate	0.0007 ± 0.0001	0.15 ± 0.01	0.10 ± 0.01	0.09 ± 0.02	0.08 ± 0.01
2-Methyl-2-butanol	0.70 ± 0.30	4.32 ± 0.28	3.31 ± 0.20	3.09 ± 1.06	3.38 ± 0.72
Isobutyl acetate	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*
Ethyl butanoate	< *LOD*	0.23 ± 0.06	0.51 ± 0.05	0.41 ± 0.14	0.11 ± 0.05
n-Propanol	< *LOD*	29.38 ± 1.83	16.52 ± 3.50	20.73 ± 1.88	15.89 ± 2.75
Ethyl isovalerate	0.0017 ± 0.0013	< *LOD*	0.002 ± 0.0001	0.0009 ± 0.0010	0.0004 ± 0.0003
Butyl acetate	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*
1-Propanol-2-methyl	1.33 ± 0.06	23.29 ± 3.33	23.00 ± 4.06	33.16 ± 5.85	26.47 ± 0.52
Isopentyl acetate	< *LOD*	3.27 ± 0.01	4.00 ± 0.29	2.62 ± 0.88	2.39 ± 0.81
1-Butanol	1.07 ± 0.01	2.08 ± 1.47	1.73 ± 0.20	2.20 ± 0.11	1.04 ± 0.00
Pentyl acetate	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*
Isopentanol	0.64 ± 0.07	98.20 ± 21.05	150.61 ± 5.33	157.50 ± 28.97	127.89 ± 4.65
Ethyl hexanoate	< *LOD*	< *LOD*	0.62 ± 0.09	0.36 ± 0.31	0.10 ± 0.14
1-Pentanol	0.28 ± 0.02	0.26 ± 0.02	0.27 ± 0.01	0.28 ± 0.00	0.27 ± 0.00
Hexyl acetate	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*
1-Hexanol	1.59 ± 0.10	< *LOD*	< *LOD*	< *LOD*	< *LOD*
Ethyl octanoate	< *LOD*	< *LOD*	0.94 ± 0.39	0.63 ± 0.38	0.40 ± 0.31
cis-Linalooloxid	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*
1-Heptanol	< *LOD*	< *LOD*	< *LOD*	< *LOD*	ND
Acetic acid	3.87 ± 0.15	29.34 ± 6.57	9.88 ± 13.88	11.04 ± 0.72	25.54 ± 0.94
Trans-linalooloxid	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*
3-Hydroxy ethyl butanoate	0.10 ± 0.00	0.10 ± 0.01	0.39 ± 0.21	0.37 ± 0.06	0.18 ± 0.05
Linalool	0.00005 ± 0.00007	0.00069 ± 0.00098	0.00007 ± 0.00007	0.00005 ± 0.00002	0.00004 ± 0.00004
Ethyl decanoate	< *LOD*	< *LOD*	< *LOD*	0.37 ± 0.05	0.08 ± 0.03
3-Methyl butanoate	0.01 ± 0.00	0.04 ± 0.05	0.14 ± 0.02	0.11 ± 0.04	0.00 ± 0.00
Diethyl succinate	0.00 ± 0.00	0.04 ± 0.04	0.08 ± 0.01	0.07 ± 0.01	0.03 ± 0.04
Nerol	0.00006 ± 0.00002	0.00322 ± 0.00444	0.00011 ± 0.00004	0.00023 ± 0.00003	0.00317 ± 0.00046
Geraniol	0.00000 ± 0.00000	0.00118 ± 0.00070	0.00358 ± 0.00178	0.00613 ± 0.00094	0.00173 ± 0.00093
2-Phenyl ethanol	0.59 ± 0.08	16.77 ± 3.82	16.14 ± 2.10	17.63 ± 3.42	39.91 ± 1.15
Octanoic acid	< *LOD*	< *LOD*	1.60 ± 1.81	4.23 ± 0.23	< *LOD*
Decanoic acid	0.19 ± 0.16	3.35 ± 2.25	9.68 ± 2.51	15.05 ± 0.50	< *LOD*

Values are expressed as mean of four determinations ± standard deviation. *LOD* – limit of detection; ND – not detected (value under detection limit of 0.0005–0.03 mg·l^{-1}).
Wine 1 – wines fermented by *S. cerevisiae* strain 7613, Wine 2 – wines fermented by *S. cerevisiae* strain 7913, Wine 3 – wines fermented by *S. cerevisiae* strain 8412, Wine 4 – wines fermented by *S. cerevisiae* strain 12314.

mented by strain 7613 contained the highest levels of ethyl acetate (60 mg·l^{-1}), while samples fermented by strains 7913, 8412 or 12314 contained lower and similar concentrations of this compund (40.8 mg·l^{-1}, 37.5 mg·l^{-1} or 39 mg·l^{-1}). Aroma perception threshold of ethyl acetate is 7.5 mg·l^{-1} [26], when it can resemble the nail polish. Concurrently, its concentrations determined in wine samples were within the range of the reference interval indicated for wines (22.5–63.5 mg·l^{-1}) [32]. Isobutyl acetate, butyl acetate, pentyl acetate and hexyl acetate were below the limit of detection.

Wine samples were also sensorically evaluated by a tasters' commission (Tab. 5), which evaluated wines fermented by strains 7613, 7913 and 8412 as fruity and sweet, rather than rose like. Wines fermented by strains 12314 and 8412 were evaluated as acidic and unpleasant.

Tab. 5. Sensory description of Pinot Gris wines fermented by autochthonous *S. cerevisiae* strains using odour characteristics provided by 5 degustators.

Odour	Sample			
	Wine 1	Wine 2	Wine 3	Wine 4
Exotic fruits	5	3	0	0
Red fruits	0	0	0	0
Fruity	0	5	0	5
Distillate	0	0	0	0
Spice	1	5	0	0
Roses	0	0	0	1
Acidic	0	0	5	5
Banana	1	0	5	0
Sweet	5	0	0	0
Cantaloupe	2	0	0	0
Nectarine	5	0	0	1
Unpleasant	0	0	5	5

Presented data express number of degustators who described the listed odour characteristics in the wine samples.
Wine 1, Wine 2, Wine 3, Wine 4 – wines fermented by *S. cerevisiae* strain 7613, 7913, 8412 and 12314, respectively.

CONCLUSION

Yeasts isolated directly from the vineyard are more competitive than commercial active dry wine yeasts because they are adapted to the given environment as well as to local vinicultural conditions. In this study, indigenous *S. cerevisiae* yeasts were isolated, pure cultures were prepared and their technological properties were studied. Pure yeast cultures were obtained from grapes, must, fermenting must and young wines by cultivation on WLN medium. Genera as *Kloeckera*, *Candida*, *Saprochaete* and *Saccharomyces* were isolated. It was confirmed that the four strains of indigenous yeasts identified as *S. cerevisiae* had tolerance to 12.5 % v/v ethanol or higher, and osmotolerance to 50 % w/w glucose in medium, thus confirming their possible use in wine industry. Terroir Pinot Gris young wines were prepared and studied. Wines prepared by strains 7613 and 7913 contained more than 40 $mg \cdot l^{-1}$ of ethyl acetate, 23 $mg \cdot l^{-1}$ of 2-methyl-1-propanol and less than 17 $mg \cdot l^{-1}$ of 2-phenyl ethanol. These wines were evaluated by tasters as having exotic fruits aroma. Our data of aromatic profiles and sensory evaluation indicate that autochthonous *S. cerevisiae* strains 7613 and 7913 could be recommended to manufacturers of starter cultures for making of terroir wine from Nitra wine region of Slovakia.

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