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Influence of indigenous strains of *Saccharomyces* on the biochemical composition of wine from Voskehat (Kharji) autochthonous grape variety of the Vayots Dzor region, Armenia

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ABSTRACT

Background: Armenia’s winemaking traditions go back centuries, as it is one of the oldest viticultural regions in the world. In winemaking, spontaneous fermentation using indigenous yeasts from autochthonous grape varieties is practiced obtaining unique types of wine, which is also typical of traditional Armenian wines. However, today the wine industry strives for technological standardization, while still emphasizing the value of traditional winemaking styles to preserve the unique bouquets of Armenian wines.

Objective: The aim of this study was to isolate and characterize indigenous *Saccharomyces* yeast from Voskehat grape must and analyze its effect on the formation of bioactive compounds that determine the functional health potential of white wine from Voskehat grape.

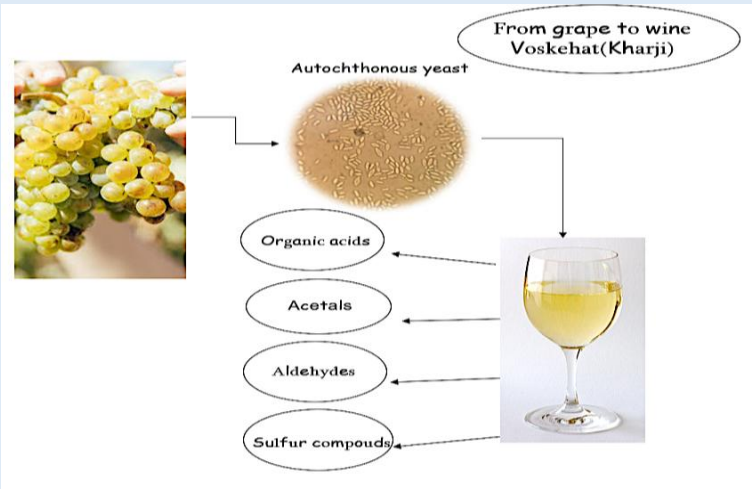
Methods: The grapes were hand-picked in the vineyards of Aghavnadzor village, Vayots Dzor region of Armenia, at the end of September, when they reached full technical maturity. Physicochemical analyses of grapes and wine were carried out by OIV (International Organization of Vine) methods. Organic acids were separated and identified by

liquid chromatography. Metabolic characteristics (glucose consumption, ethanol and organic acid production) were determined by laboratory fermentations of grape must. Residual sugar, density, ethanol concentration, titratable and volatile acidity, pH and volatile compounds were determined from 0.9-liter fermentation batches.

Results: Twenty-eight isolates were obtained from Voskehat grape must. Eighteen representative strains selected from all colony morphotypes were subjected to species identification by sequencing. The autochthonous yeast strains *Saccharomyces cerevisiae* MDC-9852 and *S. bayanus* MDC-9862 were selected based on their physiological and biochemical characteristics. The parameters used to evaluate the strains were related to growth (growth kinetics) and metabolism (glucose, sucrose, ethanol and glycerol consumption). Technological characterization of the yeast was carried out in the same Voskehat grape must from which the yeast was isolated. Physicochemical parameters of the autochthonous strains and the commercial reference strain *S. cerevisiae* VR-44 in terms of alcohol content, residual sugar, volatile acids, free and total sulfur dioxide are almost identical and are within the permissible limits. Samples fermented with *S. bayanus* MDC-9862 and *S. cerevisiae* MDC-9852 strains are characterized by a higher content of tartaric acid 1.89 and 1.99 g/l and lactic acid 3.13 and 3.51 g/l, respectively, which have a positive effect on the quality and taste of wine.

Conclusions: The studied yeast strains are characterized as effective in the process of making white wines. The aldehyde and acetal content indicators are noteworthy. The highest aldehyde values of 85.18 and 81.40 mg/l were recorded in the yeast strains *S. bayanus* MDC- 9862 and *S. cerevisiae* MDC- 9852, respectively, so it can be assumed that they can be effective in the production of sherry wines. Wine from the Voskehat grape variety fermented with the yeast strains *S. bayanus* MDC-9862 and *S. cerevisiae* MDC-9852 has a high functional potential due to the good level of biologically active organic acids.

Keywords: Voskehat grape, autochthonous yeast, *Saccharomyces* strains, fermentation, organic acids, wine quality.



Graphical Abstract: Influence of indigenous strains of *Saccharomyces* on the biochemical composition of wine

INTRODUCTION

Armenia, due to its geographical location, is characterized by a great diversity of natural zones, the climatic differences of which are favorable for the cultivation of various grape varieties for wine production [1]. Many autochthonous grape varieties are widespread in Armenia: including Areni, Voskehat (Kharji), Tigrani, Karmrahyut, Khandani, Kangan, Masala [2, 3, 4]. A unique region is Vayots Dzor, where grapes have been traditionally grown for many centuries. However, the use of a small number of local grape varieties in winemaking for wine production and, consequently, the intensive cultivation of these varieties can lead to the loss of genetic diversity of autochthonous grape varieties of Armenia [5]. The study of the yeast microbiota of autochthonous grape varieties will contribute to the preservation of genetic diversity and the isolation of technologically valuable strains of local yeast and will also be of great practical interest to produce original wines [6]. Spontaneous fermentation has been practiced in winemaking since ancient times, yielding original wines with the the region's signature terroir. This practice is widely used in winemaking. In particular, the process of producing sherry wines includes the use of only indigenous strains of yeast microflora of local grape varieties [7,8]. The qualitative characteristics of wine depend on the natural microbiota of the grapes of each wine-making region [9-11]. The species composition of autochthonous yeasts depends on many factors, including the soil and climatic conditions of the geographical region and the viticulture methods used [12]. According to the principles of precision enology, which is a new concept in winemaking, the production of premium wines demands a perfect match of yeast strains and grape varieties originating from the same locality. In this regard, there is a growing interest among microbiologists and winemakers in the use of autochthonous yeast strains that are better adapted to local grape varieties and winemaking conditions [13]. With the help of indigenous yeasts, it is possible to obtain individual wines with a unique regional character (favorable chemical composition and sensory profile), which highlights the significance of these microbes in increasing the economic value of wine [14]. Fermenting yeasts play a crucial role in wine production both through alcoholic fermentation and through the release of desirable secondary metabolites, organic acids with antioxidant, anti-inflammatory and antimicrobial properties. The taste, aroma and acidity of wine are determined by a complex mixture of organic acids, which are important for human health [15,16]. Modern winemaking uses technological advances and standardized approaches to meet growing consumer preferences for consistent sensory properties [17]. However, the inherent dynamic nature of indigenous yeast mixtures presents difficulties for maintaining consistent performance, especially when compared to their commercial counterparts. To ensure the stability and sensory qualities of traditional spontaneously fermented wines, preserving wine bouquets through the establishment of stable yeast consortia is critical. Spontaneous fermentation occurs under aseptic conditions, which emphasizes the need for stable microbial compositions. However, the stability of the consortia composition of indigenous yeast microflora strains of autochthonous grape varieties may be affected by climate change [18]. Climate change has an increasingly profound impact on vine phenology and grape composition, in turn impacting wine microbiology and chemistry, as well as sensory aspects. Among the most important effects associated with climate change is the increase in the concentration of grape sugar, which leads to an increase in the level of wine alcohol, a decrease in acidity and a change in aromatic compounds. With the increase in the concentration of grape sugar, the degree of osmotolerance of the used wine yeast

strains (i.e., their ability to grow in environments with increased osmotic pressure) becomes a critical factor, since must with a high sugar concentration causes a stress reaction in yeast, which leads to an increase in the production of fermentation by-products [19].

Higher pH can lead to significant changes in the microbial ecology of wort and wine and increase the risk of spoilage and sensory degradation [20]. This risk may be notably widespread during the early stages of fermentation before higher alcohol concentrations lead to increased microbial stability. Changes in grape quality associated with climate change will pose significant challenges to vinification and final wine quality in the future, particularly regarding the expression of varietal grape aromas, microbiological and chemical stability and sensory balance [18].

The research aims to identify and preserve the indigenous yeast strains of the microbiota of autochthonous grape varieties with technologically valuable properties and enzymatic activities, which are of great practical importance and will expand the range of production of Armenian wines.

MATERIALS AND METHODS

Sampling of Grape: The grapes were sourced from local farmers from the Areni wine region (Vayots Dzor province, Armenia). Voskehat grapes were harvested from the vineyards (0.75 ha) of Aghavnadzor village located at an elevation of 1600 m at the end of September 2024. The grapes were picked at the optimal ripening time. Intact grape samples were randomly selected from several vines within the vineyard subzones, placed in sterile 500 ml flasks and stored in a refrigerator (Samsung, Malaysia) at 3 °C. Prior to analysis, the grapes were destemmed, after which the hand-selected intact grapes were pressed to obtain the grape must.

Isolation, Selection and Identification of Autochthonous Yeast Strains: Yeast colonies were isolated by distributing serially diluted grape must samples onto glucose-peptone agar medium at 25°C (GPA – in g/100 ml: 2.0 glucose, 0.5 yeast extract, 1.0 peptone, 2.0 agar, 100 ml water, pH 7.0) (Portugalish yeast culture collection, Gulbenkian Institute of Science, Portugal). The characteristics of this must were sugar 21.50±0.54 % Brix, 5.72 ±0.14 g/l titratable acidity expressed by tartaric acid, pH 3.45±0.1. Yeast populations were quantified as colony-forming units per milliliter (CFU/mL). Yeast species were initially grouped based on growth and colony morphological characteristics. Microbial isolation was performed from single colonies. The selection of strains was carried out according to the gas-forming ability of yeast in Dunbar tubes which was determined by the formation of gas in the closed bend of the tube during the fermentation of grape juice. To study the ability of strains to assimilate carbon sources, a Sigma-Aldrich Chemie GmbH kit (Germany) was used. The ability of yeast to assimilate carbohydrates was determined in a nitrogen medium with the addition of 2% of each of the following carbohydrates: glucose, galactose, maltose, sucrose, ethanol, glycerol and raffinose (in the latter case at a concentration of 4%) and cultivation at 25^0 C [22]. The kinetic characteristics of the growth rate of the strains were quantified by measuring the optical density of the turbidity of the medium after 48 hours using a STAT FAX 1904+R biochemical analyzer (using a filter with a wavelength of 600 nm). The genus affiliation of the isolated strains was carried out based on the cultural-morphological and physiological-biochemical properties of yeast on determinants [21-22].

Molecular Genetic Analysis of Yeast: The yeast genomic DNA was isolated and purified from the investigated strains for 18S rRNA PCR amplification. For the 18S rRNA

gene amplification, the following primers FD1 (5'-ACCTGGTTGATCCTGCCAG-3') and RD1 (5'-TACAAAGGGCAGGGACAGG-3') were used. PCR amplification of the 18S rRNA gene was conducted under the following conditions: Initial denaturation: 95°C for 2 min; Cycling: 30 cycles of denaturation at 95°C for 30 sec, annealing at 59°C for 30 sec, extension at 72°C for 2 min; Final extension: 72°C for 5 min. DNA electrophoresis was conducted using a 0.8% agarose gel (Agarose I™, VWR® tablets) in 40 mM Tris-Acetate-EDTA buffer, pH 8.0, with the gel run at 100 volts for 35 minutes. DNA bands were visualized using "Millipore" GelRed® nucleic acid stain. NEB's TriDye™ 1 kb Plus DNA ladder was employed as a reference for agarose gel sizing [23]. Sequencing was carried out at Geneious Prime company (Germany). For comparative analysis of nucleotide sequences, the BLAST program was used.

Chemical Analysis: The sugar content of grapes was determined using a Carl Zeiss refractometer (Jena, Germany). The pH was measured with a PHS-25CW Benchtop pH meter (BIOBASE, China). The common oenology parameters (sugar concentration in the must, titratable acidity, volatile acidity, pH, alcohol etc.) was measured with the Official Regulation Methods established by the OIV [24]. The alcohol content was determined using the OIV-MA-AS312-01A method. Total and volatile acidity were measured in g/L using the OIV-MA-AS313-01 and OIV-MA-AS13-02 methods, respectively. The presence of free and total sulfur dioxide was measured using the OIV-MA-F1-07 method. Wine aldehydes were determined by binding them with bisulfates, then the excess bisulfate was oxidized with an iodine solution, after which the aldehyde-sulfite bond was broken in a basic medium and the isolated sulfites were subjected to iodometric titration. Organic acids were determined by liquid chromatography [25]. The HPLC system configuration and method conditions were as follows: mobile phase/eluent, H_2O with 0.5% ethanol/0.0139% concentration. Thermostatic column separation (46^0C). Variable wavelength detector (210 nm). Flow rate: 0.6 mL/min.

Statistical Analysis: The general enological parameters were assessed in five replicates. The obtained data was statistically analyzed using the mean square deviation method. Statistical significance level was considered at p-value <0.05.

RESULTS AND DISCUSSION

Isolation, selection and identification of autochthonous yeast strains: The Eight grape samples were collected within the different subzones of an individual vineyard to isolate and profile the yeast population of the Voskehat grape variety. Ripe grapes were crushed, pressed and the resulting grape juice was diluted to 10^5 power, after which 0.5 ml of the last dilution was inoculated on agarized nutrient medium GPA at 25^0 C. Yeast counting was performed after 72 hours. The populations of yeast in grape juice samples varied within the range of 1.4×10^7 to 2.1×10^7 CFU/ml. Colonies were described by morphological features and microscopic examination was performed. Microbial isolation of pure yeast cultures was performed from single colonies cultivated on GPA medium in Petri dishes. Eighteen representative strains selected from across all colony morphotypes were subjected to species across all identification by 18S rRNA sequencing analysis (Figure 1).

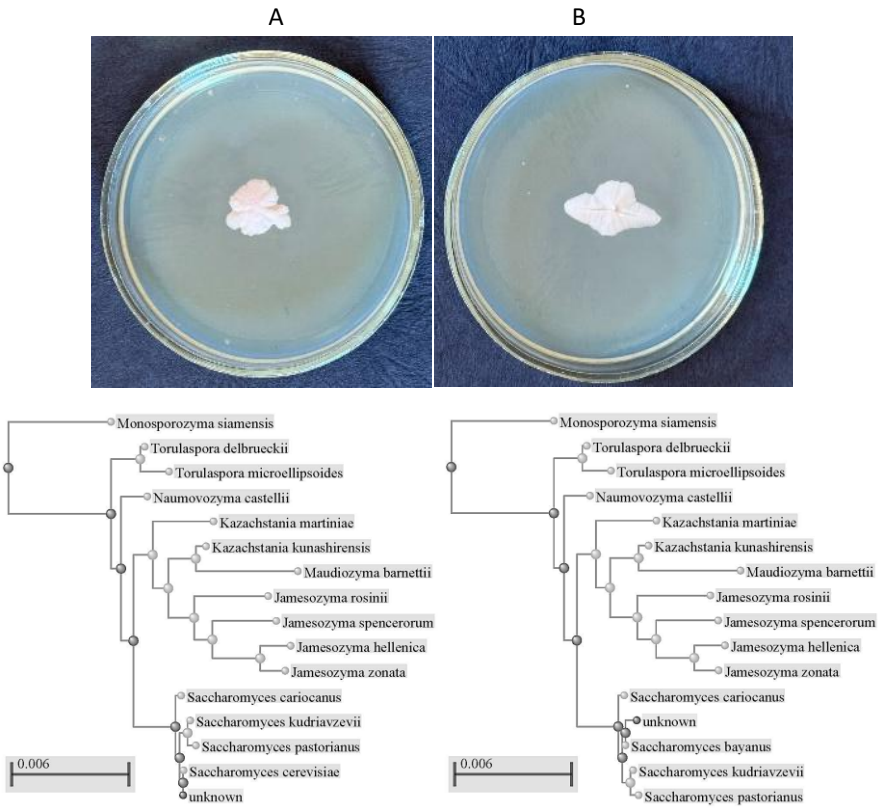


Figure 1. A - Colony morphotypes of yeast species on GPA agar medium. B - Phylogenetic tree of strains *S. cerevisiae* MDC-9852 (left) and *S. bayanus* MDC-9862.

The study of the genetic profile of yeast strains was carried out at Geneious Prime Company (Germany). As expected, the results of comparative analysis of the 18S rRNA sequence confirmed the phylogenetic relationship of the isolated strains with cultures *Metschnikowia pulcherrima* (one strain: MDC-9842), *Hanseniaspora uvarum* (three strains: MDC-9825, MDC-9831, MDC-9832), *Saccharomyces cerevisiae* (9 strains; MDC-9823, MDC-9824, MDC-9827, MDC-9830, MDC-9833, MDC-9840, MDC-9844, MDC-9852, MDC-9863) and *S. bayanus* (5 strains: MDC-9845, MDC-9862, MDC-9866, MDC-9871, MDC-9872) that correlates with literature data on the presence of these yeast species during spontaneous fermentation [26-27].

Biological characteristics of strains: Strains were evaluated based on growth (growth kinetics) and metabolism (consumption of carbohydrate sources). For determining microbial growth and the ability of strains to assimilate carbon sources, a Sigma-Aldrich Chemie GmbH kit (Germany) was used. The same medium containing nitrogen derivatives, vitamins and growth factors, but without the addition of carbohydrate sources, was used as a control. Microbial growth and the ability of strains to assimilate carbon sources were quantified by measuring the optical density of the turbidity of the medium with cultured yeast at 25^0C after 48 h (Table 1).

As presented in Table 1, the yeast microbiota strains of the Voskehat grape variety differ in growth and metabolic parameters. The results obtained for the assimilation of carbon sources by *M. pulcherrima, H. uvarum, S. cereviseae* and *S. bayanus* cultures are identical to the data on the phylogenetic identification of 18 yeast strains at the species level, which emphasizes the reliability of the traditional method of preliminary physiological differentiation of wine yeasts.

Table 1. An analysis of the carbon compounds assimilation by autochthonous yeast strains.

Strains of yeast	Glucose	Galactose	Sucroze	Raffinose	Lactose	Maltose	Inulin	D-Xylose	Arabinose	Ethanol	Glycerin
***S. cervisiae* MDC 9823**	1.423	1.222	1.289	1.121	0.069	1.305	0.219	0.266	0.241	0.227	0.158
***S. cerevisiae* MDC 9824**	1.473	1.164	1.235	1.046	0.055	1.285	0.311	0.322	0.203	0.169	0.234
H. uvarum MDC 9825	1.335	0.169	0.310	0.118	0.071	0.259	0.153	0.135	0.099	0.172	0.134
***S. cerevisiae* MDC 9827**	1.573	1.190	1.956	1.076	0.063	1.256	0.285	0.358	0.306	0.310	0.282
***S. cerevisiae* MDC 9830**	1.536	2.164	2.150	1.055	0.062	1.189	0.314	0.417	0.258	0.182	0.144
***H. uvarum* MDC 9831**	1.423	0.125	0.216	0.139	0.074	0.256	0.081	0.122	0.089	0.195	0.162
***H. uvarum* MDC 9832**	1.345	0.219	0.198	0.115	0.052	0.198	0.118	0.105	0.069	0.211	0.144
***S. cerevisiae* MDC 9833**	2.199	1.112	2.027	1.007	0.346	1.435	0.194	0.394	0.289	1.863	0.479
***S. cerevisiae* MDC 9840**	2.672	2.052	1.902	1.195	0.292	1.401	0.233	0.338	0.325	2.741	0.216
***M. pulcherrima* MDC 9842**	1.591	0.129	0.151	0.083	0.057	0.318	0.047	0.042	0.077	0.246	0.269
***S. cerevisiae* MDC 9844**	1.926	1.084	2.103	1.022	0.061	1.024	0.446	0.391	0.379	1.061	0.343
***S. cerevisiae* MDC 9852**	2.699	1.862	2.041	1.599	0.192	1.599	0.595	0.538	0.432	1.863	0.689
***S. bayanus* MDC 9862**	2.196	1.835	1.477	1.599	0.209	1.671	0.579	0.551	0.432	2.741	0.452
***S. cerevisiae* MDC 9863**	1.957	1.514	1.395	1.372	0.219	1.568	0.331	0.326	0.339	0.406	0.281
***S. bayanus* MDC 9865**	1.945	1.499	1.996	1.197	0.119	1.299	0.295	0.429	0.418	0.388	0.284
***S. bayanus* MDC 9866**	1.959	2.041	1.147	1.698	0.112	1.237	0.504	0.515	0.072	0.514	0.262
***S. bayanus* MDC 9871**	2.044	1.754	1.862	1.307	0.078	1.197	0.397	0.538	0.179	0.443	0.309
***S. bayanus* MDC 9872**	1.859	1.807	1.704	1.253	0.097	1.575	0.374	0.412	0.288	0.711	0.268

The fermentation activity of autochthonous yeast strains was assessed in Dunbar tubes. Yeast cultures (previously growing for 48 hours) were inoculated into sterilized grape juice. Incubation was carried out at 25 °C. During fermentation of the studied strains, it was noted that yeast cultures are divided into weak and strong gas formers according to their fermentation activity. Weak ones - with the release of carbon dioxide up to 3-5 ml (strains *M. pulcherrima* and *H. uvarum*) and strong ones - with the release of 8 ml or more CO_2 in 24 hours (basically strains *S. cereviseae* and *S. bayanus*). The yeast cultures *S. cereviseae* MDC-9852 and *S. bayanus* MDC-9862 were selected for further microvinification studies based on their technological characteristic of alcoholic fermentation (with release 11and 9 ml CO_2 in 24 h, respectively).

Physicochemical parameters of the grape and wine: Although the microbiota of freshly squeezed grape must contain a greater number of different yeast species belonging to the genera *Saccharomyces, Hanseniaspora, Metschnikowia, Pichia* and *Candida,* however a few hours after the start of fermentation, yeasts of the genus *Saccharomyces* become dominant [28-30]. Apart from this *H. uvarum* known as a high producer of volatile acidity, which is considered to have negative effect on the quality of wine [31]. At the same time the low fermentative power of *M. pulcherrima* makes necessary the sequential or mixed use with *S. cerevisiae* to completely ferment grape must [32-33]. In addition to ethanol, *S. cerevisiae* yeasts can produce various compounds that affect the sensory profile of wine, increasing its complexity, influencing its aromatic composition and significantly contributing to its organoleptic richness [34-35]. Moreover, the persistence of *Saccharomyces* cultures in wine is longer than that of yeasts of other genera [36]. During alcoholic fermentation with increasing *S. cerevisiae* population yeast species diversity decreases, that may be due to the relatively lower tolerance of many yeasts to ethanol compared to *S. cerevisiae* [37]. Also, non-*Saccharomyces* yeast cultures are characterized by poor sulfite tolerance, which also reduces yeast species diversity when SO_2 is added during winemaking [38-39]. On the other hand, undesirable exposure to some hazardous compounds presents at various stages of the winemaking process, in particular sulphur dioxide, may pose a risk to consumer health [40]. In this regard, biological alternatives to sulphur dioxide are of great importance for human health. [41] The use of active yeast cultures of *S. cerevisiae* ensures controlled and complete alcoholic and malo-lactic fermentation, limiting the amount of residual nutrients for undesirable microflora in wine [42] At the same time, compared to commercial yeast strains, indigenous yeasts are capable of producing higher concentrations of ethyl esters and alcohol, demonstrating the potential to improve wine quality [43]. In this regard, further studies on microvinification of Voskehat grape must were carried out with strains of *S. cereviseae* and *S. bayanus.* The enological parameters under scrutiny were the percentage of alcohol, sugar content, total and volatile organic acids, and pH of the wines.

Microvinification: The sugar content of the juice of Voskehat grape variety was 215 g/l. Titratable acidity was in the range of 5.72 g/L, while pH values were 3.45. Data were measured before fermentation and presented as mean values parameters of grape harvest of different subzones vineyard of the village of Aghavnadzor. The liquid inoculums of *S. cerevisiae* MDC 9852 and *S. bayanus* MDC 9862 autochthonous yeast strains, previously activated during 48-h, were inoculated in the prepared sterile tap glass bottles (0.9 L) for the evaluation of their enological parameters. According to literature data, the number of yeasts in fresh grape must at spontaneous fermentation varies in a wide range from

10^3 to 10^7 CFU/ mL [44,45]. In this regard yeasts were inoculated into of Voskehat must at a final concentration of $1x10^6$ CFU /ml. The inoculated musts were incubated at 23^0 C for 9 days. Fermentations were carried out in five replicates. The results depicted in Table 2 indicate that the commercial yeast VR-44 and the autochthonous yeasts MDC-9852 and MDC-9862 at the 9th day of the alcoholic fermentation demonstrated similar amounts of residual sugars which were less 2 g/l (typical to the production of dry-wines), while the percentage of alcohol was in the range of 12.25–12.41%. However, the dynamics of the alcoholic fermentation has shown that application of autochthonous yeast strain *S. cerevisiae* MDC-9852 indicated a faster and sharper decrease in total sugar content in the must from the Voskehat grape variety, while application of commercial yeast VR-44 led to a slower decrease of total residual sugars in the grape must. Although the autochthonous MDC 9852 and commercial VR 44 yeast strains belong to the same species of *Saccharomyces cerevisiae*, the dynamics of alcoholic fermentation confirm that autochthonous yeast strains in distinct matrixes often show higher functional and technological performance than allochthonous strains due to their inherent better adaptability to the original raw materials, which justifies the microbial prospection toward their potential use in food processing systems [46]. The grape acidity by tartaric acid was 5.72 g/l. Acidity values in the fermented must samples ranged from 4.05 to 4.35 g/l. The study of yeast strains has showed that the wine volatile acidity level was in the range of 0.35 and 0.39 g/l, while the maximum acceptable level of volatile acidity in wine is 1.2 g/l by OIV standards. The aldehyde and acetal content values are noteworthy. The highest aldehyde values (85.18 mg/l) were recorded at strain MDC 9862 which is typical of *S. bayanus* species, thus it can be effective in the production of sherry wines. The content of free and total sulfur dioxide is almost the same in all samples and is within the permissible limits.

Table 2. Physicochemical parameters of wines fermented by autochthonous and commercial yeast strains.

Parameters	Grape	Wine		
		MDC-9852	MDC-9862	VR-44 (commercial)
Sugar Brix, %	21.5±0.54	-	-	
Total acidity, g/l	5.72±0.14	4.35±0.1	4.27±0.1	4,05±0.1
pH	3.45±0.1	4.09±0.1	4.07±0.1	4.02±0.1
Alcoholic strength, %	-	12.41±0.31	12.32±0.44	12.25±0.44
Reducing sugar, g/l	-	0.54±0.14	0.54±0.17	0.54±0.14
Volatile acidity, g/l	-	0.35±0.1	0.39±0.2	0.37±0.1
Aldehydes, mg/l	-	81.40±0.1	85.18±0.1	66.11±0.1
Acetals, mg/l	-	27.14±0.17	35.41±0.14	24.07±0.17
Total extract, g/l		20.3±0.44	21.1±0.31	20.5±0.31
Dry extract, g/l	-	18.8±0.44	19.1±0.63	19.6±0.44
Free SO_2 mg/l	-	4.20±0.44	3.93±0.54	4.13±0.44
Total SO_2, mg/l	-	42.34±0.63	39.83±0.70	44.15±0.70
Reductions SO_2, mg/l	-	6.29±0.44	5.65±0.31	6.27±0.44

Values are expressed as mean ± standard deviation (n=5; $p< 0.05$).

Regarding the use of *S. cerevisiae* yeast in the production of fermented products, it is necessary to note their wide functional potential for health. Thus, *S. cerevisiae* yeast has a beneficial effect on the intestinal microflora, which has a positive effect on various symptoms of gastrointestinal discomfort [47]. The probiotic activity of this yeast culture for the treatment of various types of diarrheas is determined by their antimicrobial, antitoxin, and immunomodulatory effects [48]. In addition, β-glucan of the cell wall of *S. cerevisiae* yeast has a potential prebiotic function [49]. Mannoproteins contained in the cell wall of *S. cerevisiae* yeast also have high biological activity and antimicrobial properties. They are actively used in winemaking as a colloid stabilizer and an inhibitor of potassium bitartrate crystallization [50]. Overall, *S. cerevisiae* yeast is considered an important model organism for modulating population aging and validating bioactive compounds for health promotion in the functional food industry [51].

Organic acids: In recent decades, there has been a growing interest in organic acids with antioxidant, antimicrobial and anti-inflammatory properties. Wine is one of the sources of organic acids, as they are responsible for its organoleptic characteristics. [52]. The importance of determining the content of organic acids in wine is also due to the function it has on the health of consumers. These compounds bind free radicals in the human body if they are contained in the diet. For instance, most organic acids promote the absorption of iron in the human body [53]. Moderate wine consumption has been shown to have a potential therapeutic effect, counteracting the harmful effects of a high-fat diet on blood clotting, endothelial function and lipid oxidation, which contribute to the development of cardiovascular diseases. [54].

The qualitative and quantitative composition of organic acids, as fermentation products, not only affect the color, microbiological stability and sensory characteristics of wine, but also have an important impact on consumer health [55]. The results of the analysis of organic acids in grape and wine samples are shown in Table 3.

Studies have shown that wine samples had different concentrations of tartaric acid depending on the yeast strains inoculated, with the highest value recorded in the *S. cerevisiae* MDC-9852 yeast sample at 1.99 g/L. Tartaric acid is the most abundant organic acid in wine, and it gives wine its characteristic tart flavor. The quantitative indicator of tartaric acid in wine is important for health, as it has antioxidant properties [56]. The importance of tartaric acid for health has also been confirmed by studies on its effect on colon function. Tartaric acid has been shown to potentially reduce total bile acid concentrations compared to baseline values, which is an indicator of reduced risk of colon cancer [57]. Samples with strains MDC-9852 and MDC-9862 showed a lower content of malic acid, which, according to literature data, has a positive effect on wine quality [58]. A higher content of malic acid (2.12 g/L), which contributes to the sour taste of the wine, was recorded in the sample with the commercial strain VR-44. The importance of malic acid is that it has antimicrobial properties and has a positive effect on digestion by regulating the pH level in the body [59].

Table 3. The influence of autochthonous and commercial yeast strains on the synthesis of organic acids in wine.

Organic acids	Wine		
	MDC-9852	MDC-9862	VR-44 (commercially)
Tartaric acid, g/l	1.99±0.17	1.89±0.17	1.79±0.14
Formic acid, g/l	0.10±0.1	0.38±0.14	0.39±0.14
Malic acid, g/l	1.22±0.22	1.31±0.22	2.12±0.31
Shicimic acid, mg/l	7.26±0.1	12.59±0.1	10.11±0.1
Lactic acid, g/l	3.51±0.14	3.13±0.14	2.41±0.17
Acetic acid, g/l	0.39±0.17	0.33±0.2	0.32±0.22
Citric acid, g/l	0.19±0.14	0.16±0.14	0,25±0.14
Succinic acid, g/l	1.13±0.1	0.97±0.2	1.06±0.1
Fumaric acid, mg/l	9.20±0.1	6.81±0.1	4.43±0.1

Values are expressed as mean ± standard deviation (n=5; p< 0.05).

The highest content of lactic acid was recorded in wine samples fermented using autochthonous yeast strains MDC-9852 and MDC-9862 (3.51 and 3.13 g/L, respectively), which positively affected the taste assessments. Lactic acid is known to have probiotic properties, favorably affecting the beneficial microflora of lactic acid bacteria of the intestine. [60]. Since the formation of lactic acid in wines occurs mainly during malolactic fermentation, there may be a correlation between the low content of malic acid and the increase in lactic acid concentration in wine samples using autochthonous yeast strains [61]. Lactic acid, produced during malolactic fermentation in wine, can influence human health through various mechanisms, including improved lactose digestion, beneficial anti-cancer effects, and maintenance of cholesterol levels [62].

All grape must samples fermented with indigenous yeast strains contained small amounts of formic acid (0.10 to 0.39 g/L). Although formic acid is generally considered safe for human consumption in low concentrations, it can be toxic in high concentrations and may cause skin irritation or respiratory problems [63].

The levels of citric and acetic acids in the experimental wine samples are almost identical. The antioxidant and antimicrobial properties of citric acid make it important to monitor its levels. The content of citric acid in wine samples varies from 0.16 g/L (strain MDC-9862) to 0.25 g/L (strain VR-44), and acetic acid – from 0.32 g/L (strain VR-44) to 0.39 g/L (strain MDC 9852), which is consistent with the literature. Low concentrations of citric acid are due to the conversion of citric acid in the tricarboxylic acid cycle to malic acid during berry ripening [64]. Acetic acid also has an antioxidant effect on oxidative stress when the imbalance between the rate of formation and removal of free radicals is disrupted [65].

A study of the shikimic acid content in wine samples revealed differences in the concentration of the formed acid depending on the inoculated yeast strains. When using MDC-9852 yeast, the concentration was 7.26 mg/L. In wines fermented with VR-44 and MDC-9862 yeast, it was 10.11 and 12.59 mg/L, respectively. The role of shikimic acid in wine and its health benefits remains poorly understood. Shikimic acid is primarily indicative of soil and climatic conditions; in particular, in our studies, the quantitative indicators of this acid in all the samples studied were insignificant [66]. Our analysis revealed that the content of succinic and fumaric acids in wine samples

showed that the obtained values correspond to the concentrations of these acids for white wines [67]. The results show that in the case of fermentation with the MDC-9862 strain, 0.97 g/l of succinic acid was formed, and in the case of VR-44 yeast - 1.06 g/l. A slightly higher content of succinic acid was recorded in the wine sample of the MDC-9852 strain - 1.13 g/l. Succinic acid influences the wine's sensory profile, which is due to its participation in the fermentation process as part of yeast metabolism in the formation of esters. Succinic acid is a natural byproduct of alcoholic fermentation in wine. While it's a key component of wine's overall acidity and can contribute to its sensory properties according to OENO One, its direct impact on human health is not extensively studied. However, research suggests that moderate wine consumption, which includes succinic acid, may offer some health benefits, potentially including protection against neurodegenerative diseases [68]. The amount of fumaric acid in the studied samples was relatively low and varied within the following limits: minimum in the case of the commercial strain VR-44 - 4.43 mg/l; 6.81 and 9.20 mg/l in the variants with the autochthonous strains MDC-9862 and MDC-9852, respectively [69]. The content of succinic and fumaric acids in wine is significant because these dicarboxylic acids also have antioxidant properties [70].

CONCLUSIONS

According to the principles of precision enology, which is a new concept in winemaking, the production of premium wines demands a perfect match of yeast strains and grape varieties originating from the same locality. In this context, our study was aimed at the isolation, identification and genotyping of indigenous yeast strains from the extract of the autochthonous grape variety Voskehat. Representative genotypes of *S. cerevisiae* and *S. bayanus* were assessed for their vinification characteristics. The studied yeast strains are characterized as effective in the process of making white wines. In addition, they provided a good profile of organic acids during the microvinification of grape must, which has functional health benefits. The aldehyde and acetal content indicators are noteworthy. The highest aldehyde values were recorded in the yeast strains *S. bayanus* MDC- 9862 and S. cerevisiae MDC- 9852, so it can be assumed that they can be effective in the production of sherry wines. The strains *S. cerevisiae* MDC-9852 and *S. bayanus* MDC-9862 have potential for industrial use as starter cultures and are of interest in the direction of research into their vinification profiles at winery-scale.

Abbreviations: OIV: International Organization of Vine, MDC: Microbial Depository Center of the NAS RA.

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