ISOLATION OD INDIGENOUS YEASTS FROM DIFFERENT TYPES OF RED GRAPES AND DETERMINATION OF OENOLOGICAL CHARACTERISTICS OF WINE

Marija Todorovska Ivkovikj
Bioengineering LLC, Research center for applied microbiology and biotechnology ICPMB d-r Kungulovski, Skopje, N. Macedonia, m_todorovska91@yahoo.com

Abstract: The isolation, characterization and application of novel yeast strains is vital for the advancement of wine industries due to the fact that yeasts are the fundamental contributors to the microbial ecology of wine production. Wild yeasts from untreated grape must from different grapes varieties were surveyed to obtain strains suitable for fermenting a unique geolocal wine. The goal of this research is to isolate, identify, and characterize wild yeast strains from the grapes, leaves, shoots and must from the eastern part of North Macedonia and to make them available to commercial and craft wineries. In the last decade, wineries have been oriented towards the production of high quality bottled wine and simplifying the technology of making unique and competitive wines. The approach to purchasing dry wine yeasts from existing commercially available product is a limiting factor for wineries in the production of authentic local wine. The concept of making natural and authentic wines imposes the need for research and identification of different indigenous yeasts.The primary goal of this study is based on the need to obtain unique wine varieties with specific organoleptic and physico-chemical properties acquired by the action of novel wine yeasts. The production of such a wine allows for new and exciting possibilities both in classical and organic wine production such as obtaining appropriate natural wines (vin naturel).Native indigenous yeasts were isolated to be used for wine preparation and determination of sensory, oenological and chemical characteristics of wine. Different yeasts that naturally exist on the Merlot, Vranec and Burgundy grape varieties were isolated from vineyards in the Kavadarci-Negotino region of the Republic of Macedonia. A total of 77 yeast isolates were obtained. The strains were characterized on a morphological, microscopic, physiological and biochemical level using appropriate tests. Furthermore, characteristics of the isolates were determined by determining their tolerance to glucose, alcohol, temperature and determining the rate of growth by optimizing the conditions. Fermentation tests have shown that 17 of the isolates are capable of fermentation. Isolates Vinom signed (V) 01, 07, 102, 113, 130 showed the best results and a small amount of wine was made from them in laboratory conditions. Isolate Vinom 130 stood out as the best. These isolates may successfully contribute towards the improvement of wine quality and the production of novel Macedonian wines, with unique organoleptic characteristics for mass market appeal. This study shows that wild yeasts have high potential in the production of authentic, region-specific wines as well as organic, natural wines and wines with a lower alcohol concentration. These isolates could contribute for the improvement of the wider market wine quality and could also be utilized to create a unique, readily identifiable wine variety tied to region of origin. Isolates can participate in the production of new Macedonian wine with personalized characteristics and unique organic organoleptic characteristics that will make it stand out.
Keywords: Isolates, Autochthonous yeasts,,Fermentation,Red Wine,

1. INTRODUCTION

Yeasts are the most important group of microorganisms for wine producers, because without the group Saccharomyces it would be impossible to make quality wine. In addition to the genus Saccharomyces, there are many other genera and strains of yeasts that play a significant role in the vinification process, some positive and some negative. The consortium of yeasts in natural wines mainly shows inhibitory but also largely positive effects (Bagheri et al. 2017).

Historically, fermentation has played an important role in the production of several products such as bread and alcoholic drinks. Today, fermentation is also used to produce specific aromatic compounds impregnated in the final products of many industries (Alba-Lois and Segal-Kischinevzky, 2010). During the fermentation of the primary metabolites, compounds such as ethanol are formed. Fermentation, as a type of processing of cell food, works on the principle of conversion of carbohydrates to alcohol and / or organic acids; as an adaptation of microorganisms, yeasts or bacteria, to anaerobic conditions. The process is specific according to the presence or absence of specific microorganisms, i.e the profile of the fermentation population. Secondary metabolism depends on fermentable carbon, nitrogen generation, and fermentation medium. A better understanding of how these variables affect the physiology of yeast species in the production of aromatic compounds can greatly improve many industrial products. With this knowledge, the production quality could be enabled and determined or a personalized wine could be produced (Lesschaeve and Noble, 2010). During the last decade, the number of smaller wineries oriented towards

the production of high quality bottled wine with sophisticated equipment and technology that is rapidly increasing, and thus the need to develop unique, highly recognizable and competitive wines. The approach of purchasing dry canned wine fermentation yeasts from existing commercially available markets limits the production capacity to produce authentic local wine due to their genetic identity. Improving spontaneous alcoholic fermentation is a way to gain variety and character of wine (Vilanova et al., 2005). The wine-making process itself is already divided into historical or classic wines and new, modern brands (Álvares de Faria Pereira, 2016). The concept of production of natural and authentic wines imposes the need for a comprehensive analysis of the diversity of indigenous yeasts living on bunches of vineyards, selection of species and strains with acceptable and / or improved oenological characteristics, their preservation and reproduction in controlled conditions. This would allow production and use of indigenous yeasts in the context of obtaining unique wines with specific organoleptic and physico-chemical properties and reducing the need to import wine yeasts. Additionally, this way of wine production can be in great combination with organic production, with the primary goal of obtaining completely natural wine (Azabagaoglu et al. 2007).

The study of the natural diversity of wine yeasts in vineyards of regional character is essential in terms of knowing the hidden oenological potential of the untouched natural wealth of the biodiversity of wine yeasts in the Republic of Macedonia. The application of indigenous yeasts in the national winery is an extremely important strategy for maintaining the quality and ensuring the reproducibility of the wine bouquet. Wine is a complex matrix that includes compounds of different chemical nature, and volatile compounds are actually the ones responsible for the quality and aroma of the wine (Mathew et al., 2017). The microbial ecosystem of grapes and wine, including yeasts of the Saccharomyces and non-Saccharomyces species, as well as lactic acid bacteria, are considered to be a crucial factor for winemakers and oenologists, influencing wine aroma and consumer preferences. This review focuses on current knowledge of the impact of microorganisms on the aroma and taste of wine, and the biochemical reactions and pathways in which they participate, contributing to the quality and acceptability of wine. The potential use of wine yeasts and lactic acid bacteria as biological tools to improve wine quality and the advent of promising new tips allowed by pioneering technologies.

2. MATERIALS AND METHODS

2.1. Isolation of microorganisms

The samples were collected in sterile homogenization bags, properly labeled and packaged, and transported at a temperature of 4°C. Each of the samples was homogenized and inoculated with Yeast Extrat Peptone-YPA (5 g / L yeast extract, 10 g/L peptone, 25 g/L glucose and 20 g/L agar) agar and in parallel with Rose Bengal agar (Enzymatic digestion of soybean meal g/L, glucose 10 g/L, monopotassium phosphate 1 g/L, magnesium sulphate 0.5 g/L, rose bengal 0.05 g/L, chloramphenicol 0.1 g/L) with L stick technique (Yeon-Ju et al., 2011). After incubation at a temperature of 30 °C for a period of 72 hours, the cultures that were grown were supersaturated on YPA by the method of obtaining a pure culture (Figure 1). Isolation from must was carried out by maceration of the bunches and after receiving the must, it was expected to start the fermentation process under the influence of the native yeasts present on the bunches and enabled to be in contact with the sugars from the must thanks to maceration (Ok and Hashinaga, 1997). The analysis of the colonies was done according to the macroscopic morphological characteristics. The resulting colonies were differentiated according to the appearance of the colony, gloss, shape, color, opacity, yeast odor, colony lining, size, pigmentation and other characteristics. Various tests were performed to obtain detailed data for each individual isolate. The osmophility test refers to the hyperosmotic tolerance test where yeast isolates were cultured in YPB broth with different glucose concentrations of 30%, 40% and 50% and incubated at 30 ° C for 48 h (Karki et al., 2017). The test for viability and growth kinetics was performed by inoculating 100 µL suspension after McFarland into 5 mL yeast broth in five different test tubes. Each test tube was placed at different temperatures, 4 ° C, 10 ° C, 20 ° C, 25 ° C and 35 ° C (Karki et al., 2017). Alcohol tolerance was carried out with broths with different concentrations of ethanol, 10%, 12%, 15%, 18% and 20%. After inoculation, the tubes were incubated at 30 °C for a period of 72 hours, and after the incubation period, the test tubes were sown on an agar plate with YPA medium to confirm the presence or absence of growth (Todorovska Ivkovikj and Kungulovski, 2020). The test for the production of hydrogen sulfide. The test for production of hydrogen sulfide was made with commercial named BIGGY agar (Yeast extract 1 g/L, glycine 10 g/L, glucose 10 g/L, sodium sulfphite 3 g/L, bismuth ammonium citrate 5 g/L, agar 13 g/L). The degree of coloring of the substrate referred to the degree of production of the compound.

The test for sulfuric acid tolerance test was performed by sowing 100 µL of yeast suspension and inoculated into tubes with varying concentrations of sulfuric acid of 50 mg / L, 80 mg / L, 100 mg / L and 150 mg / L (Ribérau-Gayon et al 2003). Flocculation was determined by sowing 100 µL yeast suspension in 10 mL YPB in 30 mL tubes. After inoculation, the tubes were incubated at 30 °C for 72 hours. After the elapsed time with the help of agitation of

the test tube in order to visualize the flocs for 30 seconds, 15 minutes and 1 hour (Guimarães et al., 2006). Cycloheximide is a natural fungicide produced by the bacterium Streptomyces griseus (Whiffen, 1948). Different types of yeast, saccharomycete by type, tolerate different concentrations of cycloheximide (cx) and chloramphenicol (ch) (Benito et al., 2012). The test for saccharomycetes and non-saccharomycetes was performed by preparing agar plates were labeled as YPAcx and YPAch, respectively, the first with added actidion and the others with antibiotic. Isolates were sown on each of the substrates in anticipation of growth inhibition for YPAcx sucrose.

The determination of the biochemical characteristic reactions in which the individual species of yeasts participate and are specific to them and are also one of the methods of the classical determination of the new species was performed with an apparatus for that purpose, VITEK (Goessens et al., 2000). The obtained data refer to different biochemical characteristics. Ин addition to the commercially available strains of Saccharomyces cerevisiae, there are native species that are of oenological importance. 24 hour cultures were prepared from yeasts that are the best according to microbiological tests. Yeast cells were lysed with enzymes by non-optimized method. The procedure is set with an incubation process of 15 minutes at room temperature and 10 minutes at 95 ° C followed by centrifugation for 5 minutes at 15500 g. The spernant is transferred to the eppendorf and DNA extraction is performed. DNA isolation was automated in an extractor. The resulting DNA was extracted, further purified and sequenced. After all preparations the sequencing was done with MiniION and the results were seen in a database with comparison.

The kinetics of yeast were monitored for a period of 24 hours, every 2 hours. Yeast suspension, 10% of the total volume, was added to the previously prepared Erlenmeyer plants. The first measurement was made at point (Guimarães et al., 2006) and each subsequent one every 2 hours. The monitored parameters are turbidity, biomass, alcohol, glucose and cfu / mL was determined by sowing on a solidified YPA substrate and after incubation the colonies were counted (Stehlik-Tomas, 1983). Following the following protocols, wine was made in the laboratory. It was subjected to chemical analysis and also oenological. The chemical analyzes referred to the detection of volatile acids, as a total number, but also the presence of alcohol and SO_2. The aroma and taste that defined the bouque of wine were defined by an oenologist.

3. RESULTS AND DISCUSSIONS

The isolates were obtained from several vineyards at locations near Stip, Kocani, Sveti Nikole and Kavadarci. 200 were isolated, of which 77 isolates of red grape yeast, of which with further selection, the best are shown in table 1.

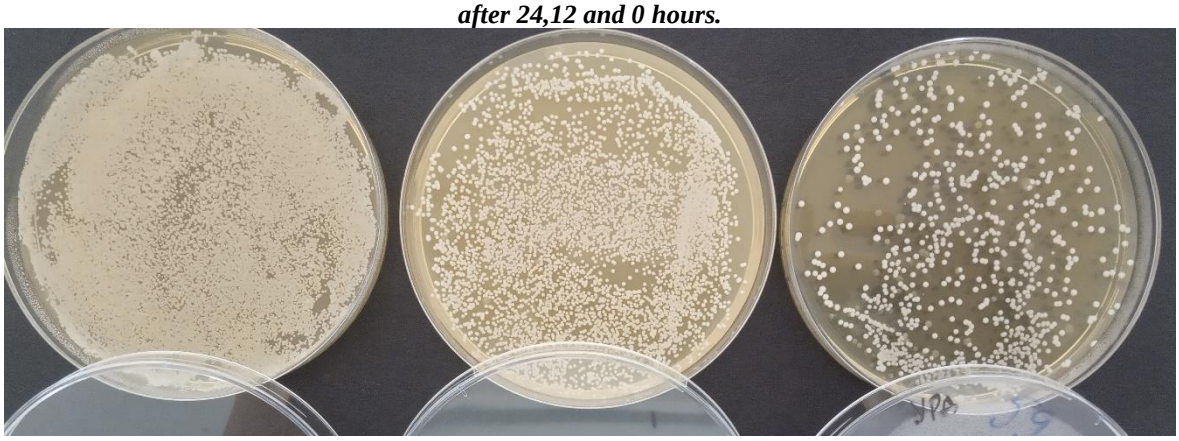
Table No. 1 The best yeast isolates selected according to the prepared tests.

	YPA ch /YPAcx		ferm entat ion	Floculatio n/precipit ation		H_2S produ ction	Alcohol tolerance	H_2SO_3 tolerance	Optimal temperature	Glucose tolerance	odor
Vinom 01	+	-	5	-	+	4	10%	150mg/L	25(4-25) °C	50%	bakery
Vinom 07	+	+	3	-	-+	3	10%	150mg/L	25(4-35) °C	50%	fruity
Vinom 101	+	+	4	-	+	3	15%	150mg/L	25(4-35) °C	50%	пекарски
Vinom 102	+	+	5	-	+	5	15%	150mg/L	35(4-35) °C	50%	bakery
Vinom 113	+	+	5	-	+	3	12%	150mg/L	35(4-35) °C	50%	bakery
Vinom 115	+	+	5	-	-	3	15%	150mg/L	25(4-35) °C	50%	nice and strong smell
Vinom 120	+	-	5	-	-	3	12%	150mg/L	25(4-35) °C	50%	bakery
Vinom 121	+	-	5	-	-	4	15%	150mg/L	20(4-35) °C	50%	nice and strong smell
Vinom 130	+	+	5	-	-	3	12%	150mg/L	25(4-35) °C	50%	nice and strong smell
Vinom 158	+	+	3	-	+	0	12%	150mg/L	25(4-35) °C	50%	bakery
Vinom 160	+	+	4	-	+	3	12%	150mg/L	25(4-35) °C	50%	fruity
Vinom 162	+	+	4	-	+	3	12%	150mg/L	35(4-35) °C	50%	fruity
Vinom 163	+	+	4	-	+	3	12%	150mg/L	35(4-35) °C	50%	fruity
Vinom 169	+	-	4	-	+	4	15%	150mg/L	35(4-35) °C	50%	bakery
Vinom 170	+	-	4	-	+	4	12%	150mg/L	35(10-35)°C	50%	nice and strong smell
Vinom 171	+	-	4	-	+	4	15%	150mg/L	35(4-35) °C	50%	fruity
Vinom 172	+	-	4	-	+	4	12%	150mg/L	35(10-35)°C	50%	nice and strong smell

A large number of isolates during fermentation produce ethanol, carbon dioxide, which is of primary importance and secondary metabolites that are responsible for the taste and smell of wine and thus the specificity of the secondary metabolites makes them selective and suitable for further work with them (Lilly et al., 2000) Improved methods of the fermentation process can enable the production of highly alcoholic wines from grape juice concentrate and thus provide an easier process for the production of dessert wines (Buescher et al., 2001). Of the 17 analyzed yeasts mentioned above, isolates Vinom 01, 07, 113 and 130 showed potential and ability for the production of red wine.

All isolates showed tolerance to exogenous glucose added to the medium. The sugar load indicates that the yeasts can carry out the fermentation process and no substrate inhibition of up to 50% glucose occurs in the environment. Seventeen yeast wine isolates showed growth and tolerance to 25% glucose load (Osho, 2005) Isolates taken from native dessert wines from Mexico showed tolerance to 35% glucose (Perrusquía-Luévano et al., 2019). 100% tested isolates are tolerant of 150 mg/L H_2SO_3. Among the isolated yeast species Aureobasidium namibiae, Sporobolomyces johnsonii, Candida apicola, Hanseniaspora uvarum, Candida thaimueangensis, Hanseniaspora opuntiae all showed tolerance to 100 ppm SO_2 (Perrusquía-Luévano et al., 2019).From the isolates in table no.1, 6 showed tolerance up to 15% alcohol, 9 of them are tolerant to 12% ethanol, and the remaining 2 to 10%. Some isolates have a tolerance of up to 17% ethanol (Casey and Ingledew, 1986) Isolates isolated during the fermentation of apple juice labeled S.cerevisiae 0271, S.cerevisiae 0269, S.cerevisiae 0260 and S.uvarum 0275 are ethanol tolerant of 10 to 12% v / v (Osho, 2005). Only isolates vinom 170 and 172 do not tolerate temperatures below 4 ° C, and for the remaining yeasts the optimal temperature growth is 35 ° C (8 isolates), for part of them 25 ° C (8 isolates) and only for Vinom 121 is 20 ° C. All are tolerant of the maximum test temperature of 35 ° C, except vinom 01 which is tolerant of temperatures up to 25 ° C. 53.8% of the isolated white grape isolates showed optimal growth at 35 ° C, some (46.2%) at 25 ° C, but all examined yeasts showed viable growth from 4 to 35 °C (Ok and Hashinaga, 1997). The production of hydrogen sulfide as a negative characteristic of yeasts is crucial in the selection of yeast isolates used by wine producers. The color intensity of the yeast colonies was denoted by 1-white colonies having the lowest production, 2-cream, 3-light brown, 4-brown, 5-dark brown and 6-black, from smallest to largest production respectively. Only one of all isolates had dark brown colonies, the rest being characterized by low to low H2S production. The addition of nitrogen to wines can help regulate H2S and reduce yeast production (Ugliano et al., 2010) Yeasts that produce large amounts of H2S are unsuitable due to the interference in the wine bouque, due to the smell that compromises the taste of bread and wine (Stratford, 1992). The test for differentiation of saccharomycetes and non-saccharomycetes yeasts was conducted in order to isolate the isolates that have fermentative ability. All isolates have excellent fermentation ability and cen be used to start the fermentation process energetically and quickly. 100% of yeasts belong to the group of sucrose yeasts and have the ability to fermentation processes. Isolates do not possess flocculation capacity and only 4 out of 17 do not precipitate well. There are isolates that have a high flocculation capacity and out of 30 isolates tested, only one does not show flocculation ability, thus simplifying the wine-making process due to the association of flocculation and the aggregation of rapidly precipitating cells (Verstrepen et al., 2003). The growth kinetics of vinom 130 isolate as the best of all isolates was studied by measurements every 2 hours.

Figure No. 1(from left to right) Monitored colony forming units from test kinetics of yeast V 130 for a period after 24,12 and 0 hours.



Determining the parameters showed an increase in yeast growth over a period of 48 hours and depletion of minimum glucose reserves from the medium to ethanol production. The criteria for selecting indigenous yeast strains for use in fermented beverages include their ability to dominate the media and enhance the desired sensory characteristics in Table 3. One of the key features when choosing a yeast is its implantation, overcoming various stresses and fermentation performance, which requires setting up and monitoring the process, including a significant amount of resources, and methods for assessing the tolerance of yeast strains are usually based on the qualitative measure of the growth of the microorganism in a medium containing a limiting compound after a certain incubation time (Dalia et al., 2017) After determining the ideal isolates for work and the correct initial inoculum, wine was made in order to determine the sensory characteristics, fermentative abilities and realization of the entire process for obtaining natural, natural, organic Macedonian personalized wine with autochthonous yeast strains. Organic acids

are important components in the production of wine because they contribute to the formation of the wine bouquet and according to the presence in the concentration share, the intensity of the taste and smell of the wine is different. Organic acids in wine include tartaric, malic, citric, lactic, acetic, amber and others and are counted and determined as total acids present in the finished wine. The concentration of organic acids varies depending on various factors such as temperature, pH, oxygen concentration and carbon dioxide (Bayraktar, 2012). High and very high correlations have been found between volatile acids and acetic acids in white grape wines, and in red wines the relationship between lactic and acetic acid is proportional (Bayraktar, 2012). The Sc24 isolate in experiments produced the wine with the highest value for volatile acids and with an alcohol content similar to the isolate with the least volatile acids produced (Blanco et al., 2014).

4. CONCLUSIONS

This study was conducted in order to isolate and characterize indigenous Macedonian yeasts from different varieties of red grapes and to determine the microbiological, physiological and cultural characteristics of the isolates. The purpose of the tolerance test by performing individual tests was to maintain and select the best isolates that could contribute to the preparation of Macedonian, organic, personalized wine with natural yeasts. The study showed that the indigenous yeasts that are part of the natural biome of the grape varieties Burgudnec, Vranec and Merlot have great potential in the production of authentic and regional specific wine with a specific aroma and bouquet as well as a characteristic chemical composition.

These isolates can contribute to the making of wine specific and unique to the local Macedonian regions and are an untapped treasure from an oenological point of view with the help of which unique well-known wines can be obtained.

ACKNOWLEDGEMENT

I express my gratitude to the Research Center for Applied Microbiology and Biotechnology ICPMB Dr. Kungulovski at Bio Engineering for the opportunity to carry out this study, the staff and most of all Professor Dr. Dzoko Kungulovski for mentoring this study.

REFERENCES

Azabagaoglu M. O., Akyil A., &Ozay A. (2007). The demand for organic wines and organic wine makerting. Journal of Environmental Protection and Ecology 8(1) 171–178.

Bahareh Bagheri, Florian F. Bauer & Mathabatha E. Setati (2017). The Impact of Saccharomyces cerevisiae on a Wine Yeast Consortium in Natural and Inoculated Fermentations. Front Microbiol 8:1988.

Bayraktar V. N. (2012). Organic acids concentration in wine stocks after Saccharomyces cerevisiae fermentation. Odesa Mechnikov National University, Ukraine.

Bobai Mathew, Mohammed Sani Sambo Datsugwai, Emmanuel Silas David & Ugboko Harriet (2017). Production of Wine from Fermentation of Grape (Vitis vinifera) and Sweet Orange (Citrus seninsis) Juice using Saccharomyces cerevisiae isolated from Palm Wine. International Journal of Current Microbiology and Applied Sciences ISSN: 6 (1) 2319-7706 868-881.

Breno Álvares de Faria Pereira (2016). The new wave of wine brands. IO Web of Conferences. 7, 39th World Congress of Vine and Wine,Article Number 03010.

Buescher W. A., Siler C. EMorris., J. R., Threlfall R. T., Main G. L., et al.(2000). Evaluation of the Vitek 2 System for Susceptibility Testing of Streptococcus pneumoniae Isolates European Journal of Clinical Microbiology and Infectious Diseases 19: 618–622.

Buescher W. A., Siler C. E., Morris J. R., Threlfall R. T., Mainet G. L et al.. (2001). High Alcohol Wine Production from Grape Juice Concentrates. American journal of enology and viniculture, 52: 345-351.

Casey G. P. & Ingledew W. M. (1986) Ethanol tolerance in yeasts. Crit Rev Microbiol. 13(3):219-80

Dalia E. Miranda Castilleja, Jesús A. Aldrete Tapia, Sofía M. Arvizu, Medrano, Montserrat Hernández Iturriaga, Lourdes Soto Muñoz et al. (2017). Growth Kinetics for the Selection of Yeast Strains for Fermented Beverages.

Lesschaeve I. & Noble A.C. (2010). 7 - Sensory analysis of wine. Woodhead Publishing Series in Food Science, Woodhead Publishing Series in Food Science, Technology and Nutrition, Pages 189-217.

Lilly M, M.G.Lamberrchtis & Pretorius I.S. (2000). Effect of increased yeast alcohol acetyltransferase activity on flavor profiles of wine and distillates. Appl Environ Microbiol. 66(2):744-53.

Luisa Alba-Lois & Claudia Segal-Kischinevzky,(2010). Yeast Fermentation and the Making of Beer and Wine. Nature Education 3(9):17.

Mar Vilanova, Isabelle Masneuf-Pomarède & Denis Dubourdieu, (2005). Influence of Saccharomyces cerevisiae Strains on General Composition and Sensorial Properties of White Wines Made from Vitis vinifera. Albariño, Food Technol. Biotechnol. 43 (1) 79–83

Marija Todorovska Ivkovikj & Dzoko Kungulovski (2020). Isolation of native yeasts from different varieties of white grape and determination of their morphology, physiology and enological characteristics, SKUN 2020 http://www.tmf.ukim.edu.mk/Data/aktuelnosti/%d0%a1%d0%9a%d0%a3%d0%9d%d0%97%d0%91%d0%9e%d0%a0%d0%9d%d0%98%d0%9a-2021.pdf (in Macedonian)

Maurizio Ugliano, Radka Kolouchova, & Paul A Henschke (2010). Occurrence of hydrogen sulfide in wine and in fermentation: influence of yeast strain and supplementation of yeast available nitrogen. J Ind Microbiol Biotechnol. 38(3):423-9.

Osho A. (2005). Ethanol and sugar tolerance of wine yeasts isolated from fermenting cashew apple juice. African Journal of Biotechnology 4(7):660-662.

Pilar Blanco, José Manuel Mirás-Avalos, E. Pereira, Daniel Fornos, & Ignacio Orriols, (2014). Modulation of chemical and sensory characteristics of red wine from Mencía by using indigenous Saccharomyces cerevisiae yeast strains. Journal international des sciences de la vigne et du vin 48 (1).

Ribérau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D.(2003) Tratado de Enologia: Quimicadelvino, estabilización y tratamentos. Buenos Aires: Hemisfério Sur. 2003 pg. 537.

Santiago Benito, Felipe Palomero, Antonio Morata, Fernando Calderón, Daniel Palmero et al. (2012). Identifying yeasts belonging to the Brettanomyces/Dekkera genera through the use of selective-differential media. African Journal of Microbiology Research Vol. 6(34):6348-6357.

Stratford M. (1992). Lectin-mediated aggregation of yeasts - Yeast flocculation. Biotechnology & Genetic Engineering Reviews 10(1) 283–341.

Susana Perrusquía-Luévano, Marlene Sarahí Cano-Herrera, César Guigón-López, María del Carmen Avitia-Talamantes, Carlos Torres-Torres et al (2019). Microbiology of high-sugar must fermentation by novel yeasts from the chihuahuan desert. FEMS Yeast Research, Volume 19 (1).

Taing Ok & Fumio Hashinaga, (1997). Identification of sugar-tolerant yeasts isolated high-sugar fermented vegetable extracts. J. Gen. Applied Microbiol, 43, 39-47.

Thais M. Guimarães , Danilo G. Moriel , Iara P. Machado , Cyntia M. T, Fadel Picheth et al. (2006) Bonfim Isolation and characterization of Saccharomyces cerevisiae strains of winery interest. Brazilian Journal of Pharmaceutical Sciencesvol. 42(1).

Thais M. Guimarães; Danilo G. Moriel; Iara P. Machado; Cyntia M. T. Fadel Picheth et al. (2006). Isolation and characterization of Saccharomyces cerevisiae strains of winery interest, Rev. Bras. Cienc. Farm. 42 (1).

Tika B. Karki, Parash Mani Timilsina, Archana Yadav, Gyanu Raj Pandey, Yogesh Joshi, et al.(2017). Selection and Characterization of Potential Baker's Yeast from Indigenous. Biotechnology Research International Volume 2017.

Whiffen A. J. (1948). The production, assay, and antibiotic activity of actidione, an antibiotic from Streptomyces griseus. J Bacteriol . 56(3):283-91.

Verstrepen K. J., Derdelinckx G., Verachtert H. et al., (2003). Yeast flocculation: what brewers should know. Applied Microbiology and Biotechnology, 61(3) 197–205.

Vesna Stehlik –Tomas (1983). Biosinteza jednostanicnih proetina s pomocu kvasaca na sirutku, Magisterski rad, Prehrambeno Biotehnoloski fakultet, Zagreb.

Yeon-Ju Lee, Yu-Ri Choi, So-Young Lee, Jong-Tae Park, Jae-Hoon Shim, et al. (2011) Screening wild yeast strains for alcohol fermentation from various fruits. Mycobiology National library of medicine PubMed 39 (1):33-9.