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COMPARATIVE ASSESSMENT OF THE LABORATORY SELECTED AND ACTIVE DRIED *SACCHAROMYCES CEREVISIAE* YEAST CULTURE IN BIOTECHNOLOGY OF THE BRANDY PRODUCTION

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Samples from different industrial grape cultivars were collected during the vintage season from the vineyard of the winery (the «Shabo» winery Company, located in the Odesa region, Ukraine). The following industrial cultivars of grapes were selected for the research: Chardonnay, Cabernet Sauvignon, Merlot, Sauvignon, Riesling Rhenish, Aligote, Rkatsiteli, Bastardo, Traminer, Telti Kuruk, Grinosh.

The grape cultivars were cultivated on the sandy soils in the district located between the Black Sea and the Dnestrovsky estuary. Grape must derived from different grape cultivars was placed into sterile glass flasks to half of the 450ml flask volume. Each flask was carefully closed with a rubber stopper with an injection needle in it. During the fermentation process, it was necessary to remove carbon dioxide, which was present as a result of active anaerobic fermentation processes in the grape must. At the end of grape must fermentation, pure yeast cultures were isolated using traditional microbiological methods by consistent inoculation of a sample into a Petri dish with a few modifications of nutrient selective agar for yeast isolation and cultivation. Primary yeast isolation was carried out using Inhibitory Mold Agar medium (Becton Dickinson Company, USA).

The yeast culture morphological properties were analyzed after the primary yeast culture isolation. Yeasts were identified by polymerase chain reaction (PCR) using universal yeast primers. After yeast culture identification, the next step in yeast cultivation was carried out on Wort Agar medium (Becton Dickinson Company, USA). Each isolated, and identified yeast culture was deposited in the Genebank of Japan, MAFF culture Collection, Tsukuba, Ibaraki, Japan and (NCYC) - Yeast Culture Collection (National Collection of Yeast Cultures, Institute of Food Research, Norwich, United Kingdom). Each yeast culture was tested for technological characteristics such as growth resistance to high temperature (+42°C) and low temperature (+6°C), growth at low pH 2.6–3.0 (acid resistance), growth in the presence of 5, 10, and 15% ethanol (ethanol resistance), and growth in the presence of high concentration potassium bisulfite (bisulfite resistance). Hydrosulfide synthesis (H_2S gassing production) was studied in addition.

Parameters of cellular metabolism in yeast suspension, such as concentration of nitrogen, protein, triglicerides, enzymatic activity and total sugar (which include glucose, fructose, and galactose) were determined. Macro- and micro-element concentrations in fermented grape must, which contained pure yeast culture was determined and included: potassium, sodium, calcium, phosphorus, magnesium, iron, chlorides. In addition to identifying parameters of macro- and micro- element concentration in grape must during and following fermentation based on a principle of photometric analysis, carried out using a biochemical analyser Respons-920 (DiaSys Diagnostic Systems GmbH, Germany).

Laboratory selected *Saccharomyces cerevisiae* wine yeast showed high enzymatic activity with short lag phase. Since of fermentation started on third day concentration of Triglicerides, Protein (total), Potassium and Sodium increased and then level of Protein (total) on the 5th day of fermentation twice decreased. Trigliceride concentration on the 5th day of fermentation continued to increase. Concentration of Iron on the 5th day of fermentation increase in geometrical progression, concentration increase in 4-5 times. Contrary Chloride concentration on the 5th day of fermentation decreased in 3-4 times. Enzymatic activity on 3rd day of fermentation maximal for Lactate Dehydrogenase, Alanine aminotransferase, Aspartate aminotransferase, Phosphatase. Since of 5th day of fermentation Enzymatic activity for Lactate Dehydrogenase, Alanine aminotransferase, Aspartate aminotransferase 3-4 times. Especially level of Phosphatase activity very decreased in 6-7 times. Comparative assessment between our Laboratory selected *Saccharomyces cerevisiae* yeast culture and Dry active commercial *Saccharomyces cerevisiae* yeast culture did not showed any difference in enzymatic activity. Both groups showed high enzymatic activity on the third day from the start of fermentation and decreasing on the fifth day since of fermentation started.

Key words: wine yeast, enzymatic activity, cellular metabolism, Saccharomyces cerevisiae.

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ПОРІВНЯЛЬНА ОЦІНКА ЛАБОРАТОРНИХ ТА СУХИХ ШВИДКОРОЗЧИННИХ ДРІЖДЖІВ *SACCHAROMYCES CEREVISIAE* ДЛЯ БІОТЕХНОЛОГІЇ КОНЬЯЧНОГО ВИРОБНИЦТВА

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Порівняльна оцінка ферментативної активності лабораторних та сухих активних комерційних дріжджів показала високу ферментативну активність дріжджових культур в обох порівнюваних груп по клітинним ферментам, макро- і мікроелементного складу, показниками клітинного метаболізму дріжджів.

Ключові слова: винні дріжджі, ферментативна активність, клітинний метаболізм, Saccharomyces cerevisiae.

В.Н. Байрактар

СРАВНИТЕЛЬНАЯ ОЦЕНКА ЛАБОРАТОРНЫХ И СУХИХ БЫСТРОРАСТВОРИМЫХ ДРОЖЖЕЙ *SACCHAROMYCES CEREVISIAE* ДЛЯ БИОТЕХНОЛОГИИ КОНЬЯЧНОГО ПРОИЗВОДСТВА

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Сравнительная оценка ферментативной активности лабораторных и сухих активных коммерческих дрожжей показала высокую ферментативную активность дрожжевых культур у обеих сравниваемых групп по клеточным ферментам, макро- и микроэлементному составу, показателям клеточного метаболизма дрожжей.

Ключевые слова: винные дрожжи, ферментативная активность, клеточный метаболизм, *Saccharomyces cerevisiae.*

INTRODUCTION

Yeast is a Laboratory and Commercial preparation consisting of dried cells of one or more strains of the yeast species *Saccharomyces cerevisiae*. A secondary contribution of yeast to bread is flavouring and aroma.

The name brandy comes from the Dutch word brandewijn, meaning «burnt wine».

Grape juices have distinctly varied properties conferred by their composition; pigmentation through the presence of anthocyanins, their glucosides and condensation products; tastes arising from the balances of organic acids, sugars and phenolic compounds; and aroma from a diverse mixture of secondary metabolites (Arora, 2003; Bayraktar, 2013b; Reynolds, 2010). This is typical for fruits and berries. From the total amounts and balance of sugars and organic acids, sweetness and sourness in fruit juices can be estimated. Average grape contains per 100g. by weight: 6.2g glucose, 6.7g fructose, 1.8g sucrose, 1.9g maltose and 1.6g of other mono and oligosaccharides. Grape juices are typically between pH 3 and 4. Brandy is distilled wine and it a completely different product to make (Arroyo-López et al., 2009; Bayraktar, 2014). Considered to be a mixer or hard alcohol. Winemaking for brandy is very simple. Like champagne, a fairly neutral base wine is ideal because the flavor comes from aging. The wine should be clean, with a low SO_2, and low Volatile Acids and must contain at about 10% alcohol. It takes approximatly about 7 gallons (31.5 L) of wine to produce 1 gallon (4.5 L) of brandy so inexpensive grapes are usually used. Wine for brandy must have no unpleasant aromas because they would be concentrated along with the alcohol. To get a more efficient concentration will be if it start with low alcohol wine. Alcohol is an organic compound which contains a hydroxyl group (ethanol $CH_3–CH_2–OH$ and small amount of methanol $CH_3–OH$, it is between 0.4% – 1% of methanol in wines and brandies) (Walker, 1998).

Fermentation is a process through which organic substances (usually carbohydrates) are transformed into alcohols by means of single-celled organisms. Brandy is obtained through the process of distillation, in which the fermented liquid mixture is heated, alcohol is extracted in the form of vapour and then it is again transformed into liquid condition by cooling. Alcohol is extracted from the mixture as a compound with a lower boiling point. Alcohol contents in a pre-distilled liquid can be increased by repeated distillations, with the reduction of the total volume of liquid (Rai, 2009; Satyanarayana et al., 2009).

The aim of this work is to establish comparative enzymatic activity between Laboratory isolated and Industrial (commercial) yeast cultures of the resulting wine fermented. To achieve this goal, the following tasks were undertaken. To determine the cellular enzymatic activity it was needed to determine among them Lactate Dehydrogenase, Phosphatase, Amylase, Alanine aminotransferase, Aspartate aminotransferase. Based on these determinations it would be easier to specify which

of yeast strain with a best powerful force of fermentation (Bayraktar, 2013a; Mukhtar et al., 2010).

Interaction of macro- and micro-element and alcohol, play an important role in forming of organoleptic properties of wine stocks, which is very important in wine biotechnology.

Buffering also plays an important role in formation of taste that results mostly from potassium ions in malic acid and, to a lesser extent, from small ions of Ca 2+ , Mg 2+, and Na +. In sparkling wines, acidic harmony is controlled by concentrations of sugar and carbon dioxide, but in dry wines it is controlled by concentrations of organic acids. Selected wine yeasts in addition to their fermentative properties have echnological and oenological properties offering winemakers better control for their alcoholic fermentation objectives: they can reveal the varietal aromas, extract compounds, help to preserve color, tannins, roundness or mouthfeel, restart fermentation.

MATERIAL AND METHODS

Samples from different industrial grape cultivars were collected during the vintage season from the vineyard of the winery (the «Shabo» winery Company, located in the Odesa region, Ukraine). The following industrial cultivars of grapes were selected for the research: Chardonnay, Cabernet Sauvignon, Merlot, Sauvignon, Riesling Rhenish, Aligote, Rkatsiteli, Bastardo, Traminer, Telti Kuruk, Grinosh.

The grape cultivars were cultivated on the sandy soils in the district located between the Black Sea and the Dnestrovsky estuary.

All tests were conducted using reagents, and specific test kits for each tested parameter, incuding: calcium, phosphorus, magnesium, iron, and chloride. The kits were produced by the BioSystems Company (S.A. Costa Brava, Spain). Test kits for investigation of Chloride were produced by the Pliva Company, (Lachema Diagnostika, Brno, Czech Republic). Investigation of electrolyte, such as sodium and potassium, were carried out using ionometric determination by a biochemical analyser ILyte Na/K with ionselective block (produced by Instrumentation Laboratory Inc., Bedford, MA, USA).

Statistical deviation and significance were evaluated by Student's t-test with P-value: $P < 0.1$; $P < 0.05$; $P < 0.01$. The Spearman rank correlation coefficient was also calculated for the tested parameters of macro- and micro-element content in the grape must. All biochemical parameters were tested in the fermenting grape must on the 3rd, and 5th days, since fermentation started. Each test was repeated three times to confirm the exact result. For both tested groups of fermentation, dispersion analysis (ANOVA) was also performed. The dispersion analysis (ANOVA), based on Fisher's test (unifactorial model), was applied. The F-criterion determined whether relevant samples belonged to one from a general aggregate, and then whether or not it was possible to pool them.

Yeast strains:

I. Laboratory yeast cultures isolated from grape must of the vineyards of «Shabo» Winery Company:

– Y-3652 *Saccharomyces cerevisiae* isolated from grape must of the cultivar Telti Kuruk, plot No.31;

– Y-3662 *Saccharomyces cerevisiae* isolated from grape must of the cultivar Sauvignon Blan, plot No.58;

– Y-3665 *Saccharomyces cerevisiae* isolated from grape must of the cultivar Chardonnay, plot No.346;

– Y-3670 *Saccharomyces cerevisiae* isolated from grape must of the cultivar Grenache, plot No.32;

II. Commercial yeast cultures for brandy production:

Yeast used in Brandy Fermentations should allow complex fruit flavors. The yeasts below are recommended for fermentation of grape must for further Brandy production.

Lalvin DV-10. Selected in the Champagne region by Station Oenotechnique de Champagne, France. Specie: *Saccharomyces cerevisiae* var. bayanus. Competitive Factor: Active (Phenotype - killer). DV-10 has strong fermentation kinetics over a wide temperature range and relatively low nitrogen demands. DV-10 is famous for its ability to ferment under stressful conditions of low pH, high total SO_2 and low temperature. Low foaming and low Volatile Acids production characterize it. DV-10 is considered a clean fermenter that respects varietal character and avoids the harsh sensory contributions of other one-dimensional "workhorse" yeasts, such as Prise de Mousse.

Type of wine: Red wine, White wine, Sparkling wine, High Brix Juice, Restart Stuck. Alcohol tolerance: 17%. MLF Compatibility: Recommended. Sensory impact: Neutral. Fermentation Speed: Fast. Lag Phase: Very short. Nitrogen Needs: Low. Volatile Acidity: Moderate. SO_2 Production: High. H_2S production: Low. Acetaldehyde Production: Low.

DV-10 recommended for such grape cultivars as: Cabernet Sauvignon (Ageable tannins); Chardonnay (Citrus); Pinot Gris (Floral, Melon/Pear); Riesling (Floral, Apple); Sauvignon blanc Grassy/Asparagus).

Lalvin ICV D-47. Selected by the ICV in the Côtes du Rhône, France. Isolate from Suze-la-Rousse for the production of full bodied barrel fermented Chardonnay and other white varietals. When left on lees, ripe spicy aromas with tropical and citrus notes are developed. Specie: *Saccharomyces cerevisiae* Competitive Factor: Active (Phenotype - killer). DV-10 recommended for such grape cultivars as: Chardonnay (Stone fruit, Nuts, Volume, Citrus); Pinot Gris (Tropical fruit, Rich mouthfeel); Riesling (Apple, Rose, Peach); Sauvignon blanc (Citrus, Rich mouthfeel). Type of wine: White and Rose.

ICV D-47 is a high polysaccharide producer known for its accentuated fruit and great volume. On most white grape varieties, this yeast elaborates wines with ripe stable fruits or jam-like aromas. Thanks to these aromas, the cuves fermented with the ICV D-47 are a good source of complexity in the blends. Moreover, ICV D-47 contributes to the wine's silkiness and persistence. Excellent results are obtained for the production of top-of-the-range Chardonnay fermented in barrels. Fermentation Speed: moderate. Glycerol Production: high. Lag Phase: very short. Nitrogen Needs: low. Alcohol Tolerance: 14 %. Volatile Acidity: moderate. SO_2 Production: moderate. H_2S production: low. Acetaldehyde production: low.

Lalvin ICV D-254. Selected by ICV in the Côtes du Rhône, France. Specie: *Saccharomyces cerevisiae*. Competitive Factor (Phenotype) – Neutral. General Sensory Contribution: Enhance varietal aromas. Lalvin ICV D-254 was selected by the ICV in 1998 from Syrah fermentations in Gallician, south of the Rhône Valley. In red wines, ICV D-254 promises high fore-mouth volume, big mid-palate mouthfeel, intense fruit concentration, smooth tannins and a mildly spicy finish. In unripe reds, ferment 25% to 50% of the lot with ICV D-254 and the balance with Lalvin ICV- GRE to help mask vegetative character. As a complement to Lalvin CY-3079, winemakers use ICV D-254 for fermenting Chardonnay with nutty aromas and creamy mouthfeel. Very malolactic bacteria compatible. Type of wine: White and Red wines. Cabernet Franc (Plum, Color stability); Cabernet Sauvignon (Berry, Jam, Color stability, Round mouthfeel); Chardonnay (Stone fruit, Nuts, Volume); Merlot (Plum, Color stability); Sauvignon blanc (Rich mouthfeel). Fermentation Speed: moderate. Glycerol Production: moderate. Lag Phase: very short. MLF Compatibility: strongly recommended. Nitrogen needs: moderate. Alcohol Tolerance: 16 %. Volatile acidity: moderate. SO_2 production: low. H_2S production: low. Suitability for co-inoculation: very recommended.

Lalvin ICV QA-23. *Saccharomyces cerevisiae* var. *bayanus* selected for enhancing the flavors and aromas of grapes utilized in the production of premium white wines. Recommended for styles such as, Chardonnay, Sauvignon blanc, the QA23 produces fresh, fruity and clean wines. Particularly suitable grape varieties are Muscat and Savignon Blanc. Lalvin QA-23 shows an advantageous fermentation curve with high final degree of fermentation. Wild yeasts and undesirable bacteria are suppressed. It does not generate undesirable fermentation by products such as SO_2, H_2S, acetaldehyde, pyruvate, ketoglutaric acid, volatile acid or ester. Lalvin QA23 can produce up to 14% alcohol by volume. The practical alcohol yield is approximately 47% of the sugar to be fermented. Recommended Styles: White - Strongly recommended, Red - Not really recommended, Rosé - May be used Late harvest - Very recommended. Description: Production of volatile acidity – low; Production of SO_2 – moderate; MLF Compatibility - Really recommended; Lag phase – moderate. Foam production - Low foam production; Sensory effect - Enhances Varietal Character; Restart stuck - Not really recommended; Fermentation speed – fast;

Temperature range - 14-28 °C; Competitive factor – active; Alcohol tolerance – 16%; Relative nitrogen needs – low; H_2S production – low; H_2S production - low; Dosage - 25-40g/hL; Glycerol production – high.

RESULTS AND DISCUSSION

All strains of *Saccharomyces cerevisiae* can grow aerobically using glucose, maltose, trehalose and fail to grow on lactose and cellobiose. However, growth on other sugars is variable. Galactose and fructose are shown to be two of the best fermenting sugars. The ability of yeasts to use different sugars can differ depending on whether they are grown aerobically or anaerobically. Some strains cannot grow anaerobically on sucrose and trehalose. All strains can use ammonia and urea as the sole nitrogen source, but cannot use nitrate, since they lack the ability to reduce them to ammonium ions. They can also use most amino acids, small peptides, and nitrogen bases as a nitrogen source. Histidine, glycine, cystine, and lysine are. However, not readily used. *Saccharomyces cerevisiae* does not excrete proteases, so extracellular protein cannot be metabolized. Yeasts also have a requirement for phosphorus, which is assimilated as a dihydrogen phosphate ion, and sulfur, which can be assimilated as a sulfate ion or as organic sulfur compounds such as the amino acids methionine and cysteine. Some metals, like magnesium, iron, calcium, and zinc, are also required for good growth of the yeast. Concerning organic requirements, most strains of *Saccharomyces cerevisiae* require vitamin B_7 – biotin. Most strains also require pantothenate for full growth. In general, *Saccharomyces cerevisiae* is prototrophic to vitamins.

Nitrogen and phosphorus are the main nutritional requirements for the yeast growth and maximum ethanol production efficiency. As a nitrogen source we used such as: Ammonium Chloride and Ammonium phosphate dibasic $(NH_4)_2HPO_4$. Phosphorus has the major role in the glycolysis cycle in the yeast cell.

Although the process of distilled beverages production is known from the ancient times, new technologies make it possible to produce brandies of increasingly better quality. The technological process of brandy production consists of four basic stages, which are: grinding, fine cutting or crushing of fruits fermentation or alcohol boiling – transformation of carbohydrates into alcohol and carbon dioxide (CO_2), by means of microorganisms and sometimes with the addition of yeast distillation – the technological operation in which alcohol is extracted from the fermented mixture aging – brandy is poured into oak barrels where it is stored from two to twelve years.

During that period, the taste, aroma and colour of a particular sort of brandy are developed. Chemists discover scientific principles and explore the ways to make the process of brandy aging faster without changes in the taste. Modern filters make it possible to remove the unwanted remains and to achieve the mild characteristics of the final product.

In Table 1 and 4 we showed parameters of cellular metabolism in the Laboratory selected and Dry active commercial *Saccharomyces cerevisiae* yeast at grape must «Sauvignon» fermentation. In Table 2 and 5 we presented concentration of macro- and micro-elements in the Laboratory selected and Dry Active commercial *Saccharomyces cerevisiae* yeast at grape must «Sauvignon» fermentation. In Table 3 and 6 the Cellular Enzyme activity for the Laboratory selected and Dry Active commercial *Saccharomyces cerevisiae* yeast at grape must «Sauvignon» fermentation was preented.

Distillation. Wine at → Brandy at → Aging → Bottle at 10% alcohol 70% alcohol + water 40% alcohol.

After ageing the brandy is diluted (cut) with water to 80º proof (40% alcohol). Brandy is aged at a higher proof then diluted with water before bottling. Product a simple, clean base wine is first made and then the unique flavors come from the processing after the primary fermentation.

Other components of the dosage can include brandy, tannins, water, or still wine. Whatever combination that will tweak the flavors to make a better tasting. The apparatus used to distill brandy from fermented liquid usually consists of a cauldron which is connected to an upright vapour tube to which alcohol vapours leave during the process of heating.The vapour tube leads to the condenser where the complete condensation of alcohol vapours into liquid condition takes place, by means of a cold water current. The apparatus that is used for brandy production is usually made of copper.

Finishing

Most of the flavor in brandy comes from barrel ageing but often small amounts of «rectifying agents» (sugar, caramel coloring, fortified wines and other flavorings) added before bottling.

In California rectifying agents must be less than 2.5% of the blend. In the U.S. Brandy must be aged in barrels for two years or be labeled «Substandard Brandy». Grappa (or marc brandy) is steam-distilled brandy made from fermented grape skins (pumice) Usually not oak aged, it's an acquired taste.

Other Brandies

Metaxa (Greece) – from sun-dried grapes; Pisco (South America) – from Muscat grapes; Applejack (US); Calvados (France) – apples; Slivovitz (Serbia, Bosnia) – from plums; Eau de Vie (France, elsewhere) unaged fruit brandy, Fraise (strawberry); Framboise (raspberry); Mûre (blackberry); Kirsch (cherry); Grappa (Italy); Marc (France) – from grape pomace, this grape brandy has 50 vol.% of alcohol (ethanol).

It is important to remember that brandy is flammable, and requires specialized (explosion proof) equipment for handling. Treat it with respect. Vapor. «Explosion proof» equipment. Treat it very gentle and carefully.

Table 1. Indicators of cellular metabolism in the Laboratory *Saccharomyces cerevisiae* yeast at grape must «Sauvignon» fermentation

Indicator of metabolism	3rd day of fermentation				5th day of fermentation			
Yeast culture	Y-3652	Y-3662	Y-3665	Y-3670	Y-3652	Y-3662	Y-3665	Y-3670
Total sugar in grape must (%)	23%	23%	23%	23%	23%	23%	23%	23%
pH	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Density (SG)	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03
Protein total (g/L)	21.40±0.02	22.10±0.60	21.60±0.40	22.30±0.30	15.80±0.50	14.70±0.20	12.60±0.40	13.50±0.40
Triglicеride (mmol/L)	28.20±0.40	27.40±1.10	21.80±0.40	20.50±0.30	57.80±1.60	59.40±1.60	52.70±0.70	62.90±1.20
Nitrogen (mmol/L)	0.16±0.02	0.05±0.01	1.74±0.20	0.06±0.05	0.21±0.01	0.15±0.02	0.18±0.02	0.12±0.01

The standard deviation and the statistical significance of differences here and further evaluated by Student t-test ($P \leq 0.05$).

Table 2. Concentration of macro- and micro-elements in the Laboratory *Saccharomyces cerevisiae* yeast at grape must «Sauvignon» fermentation

Macro- and micro-element	3rd day of fermentation				5th day of fermentation			
Yeast culture	Y-3652	Y-3662	Y-3665	Y-3670	Y-3652	Y-3662	Y-3665	Y-3670
Sodium (mmol/L)	23.70±0.50	42.61±1.0	38.86±3.20	43.28±0.50	54.36±1.10	56.71±2.10	48.26±0.50	55.82±0.70
Potassium (mmol/L)	23.50±0.40	19.50±0.50	21.63±0.20	21.70±0.50	28.52±0.70	29.46±0.30	28.16±0.30	28.36±1.0
Calcium (mmol/L)	2.85±0.20	2.38±0.20	2.37±0.10	2.24±0.06	2.16±0.10	3.18±0.10	2.84±0.20	2.47±0.10
Phosphorus (mmol/L)	5.47±0.10	5.24±0.10	5.47±0.15	5.36±0.10	5.38±0.20	5.58±0.05	5.27±0.20	5.84±0.10
Magnesium (mmol/L)	2.63±0.08	2.48±0.10	3.84±0.30	4.17±0.10	3.19±0.10	3.76±0.10	3.54±0.10	3.25±0.20
Iron (μmol/L)	4.70±0.40	5.40±0.20	5.50±0.40	4.90±0.20	20.80±0.60	18.50±0.50	21.60±0.50	21.30±0.30
Chloride (mmol/L)	12.10±0.40	12.30±0.30	12.60±0.40	12.20±0.20	3.20±0.30	3.50±0.30	3.30±0.20	3.40±0.20

Table 3. Cellular Enzyme activity in the Laboratory *Saccharomyces cerevisiae* yeast at grape must «Sauvignon» fermentation

Enzyme activity (μmol/min × 10^{-2} L)	3rd day of fermentation				5th day of fermentation			
Yeast culture	Y-3652	Y-3662	Y-3665	Y-3670	Y-3652	Y-3662	Y-3665	Y-3670
Lactate Dehydrogenase	6.15±0.20	6.07±0.20	4.10±0.20	5.01±0.10	1.34±0.10	1.04±0.06	0.92±0.04	1.14±0.10
Alanine aminotransferase	0.76±0.03	0.59±0.10	0.96±0.06	0.84±0.06	0.19±0.02	0.23±0.01	0.16±0.03	0.21±0.03
Aspartate aminotransferase	0.21±0.01	0.26±0.03	0.63±0.02	0.36±0.04	0.03±0.01	0.06±0.01	0.04±0.01	0.04±0.01
Phosphatase	1.07±0.05	1.08±0.06	0.48±0.10	0.76±0.04	0.06±0.01	0.08±0.01	0.08±0.01	0.03±0.01
Amylase	0.06±0.01	0.11±0.02	0.23±0.02	0.14±0.03	0.01±0.01	0.08±0.01	0.03±0.01	0.07±0.01

Table 4. Indicators of cellular metabolism in the Dry active commercial *Saccharomyces cerevisiae* yeast at grape must «Sauvignon» fermentation

Indicator of metabolism	3rd day of fermentation						5th day of fermentation					
Yeast culture	ICV D-47	ICV D-254	ICV DV-10	ICV QA-23	ICV D-47	ICV QA-23	ICV D-254	ICV DV-10	ICV QA-23	ICV D-47	ICV D-254	ICV DV-10
Total sugar in grape must (%)	23%	23%	23%	23%	23%	23%	23%	23%	23%	23%	23%	23%
pH	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Density (SG)	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03	1.03
Protein total (g/L)	22.40±0.30	20.8±0.80	21.5±0.50	21.70±0.50	15.70±0.50	14.50±1.50	12.30±0.90	13.20±0.80	22.40±0.30	20.8±0.80	21.5±0.50	21.70±0.50
Triglyceride (mmol/L)	30.10±0.80	27.3±1.80	16.2±0.30	20.40±0.40	58.90±5.70	57.60±2.60	52.80±2.00	62.70±1.20	30.10±0.80	27.3±1.80	16.2±0.30	20.40±0.40
Nitrogen (mmol/L)	0.14±0.02	0.12±0.01	0.82±0.04	0.15±0.04	0.23±0.02	0.16±0.02	0.20±0.02	0.15±0.01	0.14±0.02	0.12±0.01	0.82±0.04	0.15±0.04

Table 5. Concentration of macro- and micro-elements in the Dry active commercial *Saccharomyces cerevisiae* yeast at grape must «Sauvignon» fermentation

Macro- and micro-element	3rd day of fermentation						5th day of fermentation					
Yeast culture	ICV D-47	ICV D-254	ICV DV-10	ICV QA-23	ICV D-47	ICV QA-23	ICV D-254	ICV DV-10	ICV QA-23	ICV D-47	ICV D-254	ICV DV-10
Sodium (mmol/L)	43.70±0.90	42.50±0.90	42.90±0.70	45.10±0.70	54.20±0.80	52.40±1.90	48.60±1.50	54.80±1.50	43.70±0.90	42.50±0.90	42.90±0.70	45.10±0.70
Potassium (mmol/L)	35.60±0.40	19.60±0.70	20.60±0.40	21.80±0.60	28.60±0.80	29.60±1.20	28.50±1.00	29.30±0.30	35.60±0.40	19.60±0.70	20.60±0.40	21.80±0.60
Calcium (mmol/L)	3.56±0.10	2.83±0.10	2.68±0.10	2.17±0.10	2.52±0.20	3.84±0.20	2.16±0.10	1.68±0.07	3.56±0.10	2.83±0.10	2.68±0.10	2.17±0.10
Phosphorus (mmol/L)	5.84±0.20	5.47±0.10	5.73±0.20	5.72±0.20	5.69±0.20	5.58±0.10	5.24±0.04	5.24±0.10	5.84±0.20	5.47±0.10	5.73±0.20	5.72±0.20
Magnesium (mmol/L)	2.74±0.08	2.74±0.03	3.41±0.10	3.49±0.10	3.64±0.30	3.45±0.10	3.76±0.20	3.47±0.10	2.74±0.08	2.74±0.03	3.41±0.10	3.49±0.10
Iron (μmol/L)	4.80±0.50	5.10±0.10	5.30±0.20	5.60±0.30	19.10±1.80	19.20±1.70	20.70±0.50	18.90±0.30	4.80±0.50	5.10±0.10	5.30±0.20	5.60±0.30
Chloride (mmol/L)	14.20±0.50	11.80±0.50	12.80±0.50	13.70±0.70	3.50±0.30	3.70±0.20	3.50±0.20	3.50±0.40	14.20±0.50	11.80±0.50	12.80±0.50	13.70±0.70

Table 6. Cellular Enzyme activity in the Dry active commercial *Saccharomyces cerevisiae* yeast at grape must «Sauvignon» fermentation

Enzyme activity (μmol/min × 10^{-2} L)	3rd day of fermentation						5th day of fermentation					
Yeast culture	ICV D-47	ICV D-254	ICV DV-10	ICV QA-23	ICV D-47	ICV QA-23	ICV D-254	ICV DV-10	ICV QA-23	ICV D-47	ICV D-254	ICV DV-10
Lactate Dehydrogenase	6.34±0.60	5.94±0.30	4.19±0.20	5.59±0.10	1.31±0.04	1.11±0.10	0.96±0.02	1.19±0.10	6.34±0.60	5.94±0.30	4.19±0.20	5.59±0.10
Alanine aminotransferase	0.74±0.02	0.64±0.03	0.89±0.10	0.83±0.07	0.21±0.10	0.19±0.05	0.18±0.02	0.19±0.02	0.74±0.02	0.64±0.03	0.89±0.10	0.83±0.07
Aspartate aminotransferase	0.19±0.03	0.24±0.03	0.58±0.05	0.39±0.04	0.04±0.01	0.04±0.01	0.06±0.01	0.04±0.01	0.19±0.03	0.24±0.03	0.58±0.05	0.39±0.04
Phosphatase	1.09±0.10	1.06±0.20	0.44±0.03	0.74±0.08	0.08±0.01	0.06±0.01	0.08±0.01	0.05±0.01	1.09±0.10	1.06±0.20	0.44±0.03	0.74±0.08
Amylase	0.06±0.02	0.10±0.01	0.21±0.02	0.14±0.03	0.02±0.01	0.08±0.01	0.03±0.01	0.07±0.01	0.06±0.02	0.10±0.01	0.21±0.02	0.14±0.03

The quality and main characteristics of brandy have to be checked and confirmed by laboratories specially equipped to do such analyses and with legal capacity to issue valid certificates about brandy quality. The enzymatic activity in

the Laboratory selected *Saccharomyces cerevisiae* yeast culture and Dry Active commercial yeast at grape must «Sauvignon» fermentation presented in Table 7.

Table 7. **Dispersion analysis (ANOVA) represents enzymatic activity in the Laboratory selected *Saccharomyces cerevisiae* yeast culture and Dry Active commercial yeast at grape must «Sauvignon» fermentation**

Parameters of Enzyatic activity	Laboratory selected and Dry active commercial yeast culture		
	F	P	R_s
LDH 3 day fermentation (L/D)	1.51	0.30	0.95
LDH 5 day fermentation (L/D)	2.11	0.24	0.98
LDH 3/5 day fermentation (Lab)	96.87	0.01	0.69
LDH 3/5 day fermentation (Dry)	117.80	0.01	0.89
AlT 3 day fermentation (L/D)	0.12	0.75	0.88
AlT 5 day fermentation (L/D)	0.13	0.74	0.19
AlT 3/5 day fermentation (Lab)	42.19	0.01	- 0.30
AlT 3/5 day fermentation (Dry)	101.29	0.01	- 0.44
AsT 3 day fermentation (L/D)	0.90	0.41	0.98
AsT 5 day fermentation (L/D)	0.06	0.82	0.92
AsT 3/5 day fermentation (Lab)	11.69	0.04	- 0.07
AsT 3/5 day fermentation (Dry)	12.32	0.03	0.15
AlPh 3 day fermentation (L/D)	1.50	0.30	0.99
AlPh 5 day fermentation (L/D)	0.17	0.70	0.58
AlPh 3/5 day fermentation (Lab)	29.84	0.01	- 0.79
AlPh 3/5 day fermentation (Dry)	26.97	0.01	- 0.89
Amy/Amy 3 day fermentation (L/D)	1.12	0.36	0.99
Amy/Amy 5 day fermentation (L/D)	0.01	0.92	0.99
Amy/Amy 3/5 day fermentation (Lab)	5.10	0.10	0.06
Amy/Amy 3/5 day fermentation (Dry)	4.62	0.12	- 0.03

LDH – Lactate Dehydrogenese; AlT – Alanine aminotransferase; AsT – Aspartate aminotransferase; AlPh – Phosphatase; Amy – Amylase; 3/5 – comparation between 3rd and 5th day fermentation. R_s - Spearman`s rank correlation coefficient; F - Fisher criterion; P – Students P-value.

CONCLUSIONS

1. Laboratory selected *Saccharomyces cerevisiae* wine yeast showed high enzymatic activity with short lag phase.
2. Since of fermentation started on third day concentration of Triglicerides, Protein (total), Potassium and Sodium increased and then level of Protein (total) on the 5th day of fermentation twice decreased. Trigliceride concentration on the 5th day of fermentation continued to increase.

3. Concentration of Iron on the 5th day of fermentation increase in geometrical progression, concentration increase in 4-5 times. Contrary Chloride concentration on the 5th day of fermentation decreased in 3-4 times.
4. Enzymatic activity on 3rd day of fermentation maximal for Lactate Dehydrogenase, Alanine aminotransferase, Aspartate aminotransferase, Phosphatase.
5. Since of 5th day of fermentation Enzymatic activity for Lactate Dehydrogenase, Alanine aminotransferase, Aspartate aminotransferase 3-4 times. Especially level of Phosphatase activity very decreased in 6-7 times.
6. Comparative assessment between our Laboratory selected *Saccharomyces cerevisiae* yeast culture and Dry active commercial *Saccharomyces cerevisiae* yeast culture did not showed any difference in enzymatic activity. Both groups showed high enzymatic activity on the third day from the start of fermentation and decreasing on the fifth day since of fermentation started.

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