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SELECTION OF *SACCHAROMYCES CEREVISIAE* WINE YEAST FOR BIOTECHNOLOGY OF THE WINE INDUSTRY

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Key words: *yeast selection, organoleptic properties, wine yeast, Saccharomyces cerevisiae, sensory profiles, intensity of flavor and taste.*

In the article presented results of sensory characteristics of red dry wines received using wine yeast cultures perspective for winemaking. It is shown that the appearance of certain shades in the color, aroma and taste of dry wine largely connected due to the ratio of oxidized and reduced components including phenolics. Floral shades of flavor, fresh taste, dessert tone in aroma and flavor more pronounced in the case of the predominance of the reduced forms of components. Resinous-balsamic shades of flavors and spicy-balsamic, walnut-vanilla flavor tones appear stronger the higher ratio of oxidized forms of components. It was determined yeast culture that gave the best organoleptic characteristics of wines produced with the direct participation of the isolated and studied wine yeast, *Saccharomyces cerevisiae*.

Simple and effective procedure to select wine yeasts for industrial use based on biotechnological characteristics of the yeasts. Selection of wine yeast by results of organoleptic properties of samples obtained wine after fermentation is an integral part evaluation of the resulting product, its color, aroma, and flavor [1,2]. These criteria allow us to give the most complete sensory assessment, the overall quality of the product as a whole and its individual characteristics (muscat flavor). The physico-chemical characteristics of the wine, as addition to organoleptic studies [4,7].

The use of selected yeasts for wine-making has clear advantages over the traditional spontaneous fermentation. The selection procedure consists of just two steps. The first is a preselection based on resistance to sulfur dioxide, killer phenotype, growth at high temperature and low foam production. The second is a selection based on volatile acidity, ethanol production, and residual sugars. Most of the selected yeasts produced wines of greater acceptance than those from spontaneous fermentation [3,5].

The aim of this work is to establish correlations between flavor, taste, and aftertaste in red wines fermented in own *Saccharomyces cerevisiae* yeast cultures isolated from different industrial grape varieties in the Ukrainian and French selection. To determine organoleptic characteristics of flavor, taste and aftertaste in Red wines, we invited four experts in degustation of wines.

MATERIALS AND METHODS

Saccharomyces cerevisiae was isolated from different Ukrainian and French cultivars of grapes. The strains were cultivated in Wort Broth and Wort Agar. Yeast cultures were identified by PCR analysys. For each *Saccharomyces cerevisiae* yeast strain made morphological examination, biochemical and physiological tests.

Saccharomyces cerevisiae yeast cultures, with a high level of taste bouquet production, were isolated during the vintage season from the vineyards of the Agrocultural Company «Koblevo», located in the Nikolaev region and Tairov Research Institute for Viticulture and Enology, in the Odessa region of the Ukraine. The following yeast cultures were selected for the research.

I. Laboratory yeast cultures isolated from following fermentation grape must from the «Koblevo» Agricultural Company:

Y–3602 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Odessa Black.

Y–3603 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Isabella.

Y–3604 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Merlot.

Y–3605 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Bastardo.

Y–3606 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Cabernet – Sauvignon.

II. Laboratory yeast cultures isolated from following fermentation grape must from the Laboratory of Cloning Selection, Tairov Research Institute for Viticulture and Enology, Odessa region of Ukraine:

Y–2622 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Cabernet-Sauvignon, clone – 143141.

Y–2623 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Cabernet-Sauvignon, clone – 2043.

Y–3548 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Cabernet-Sauvignon, clone Fran VCR– 10.

Y–3549; MAFF-230184 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Cabernet-Sauvignon, clone Fran – 326.

Y–3550 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Cabernet-Sauvignon, clone Fran ICV – 101.

Y–3551; MAFF-230186 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety. Merlot, clone VCR–1.

Y–3552 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety. Merlot, clone VCR– 13.

Y–3553 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Merlot, clone 347.

Y–3558 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Pinot Gris, clone 1-84.

Y–3559 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Pinot Black, clone 872.

Y–3560 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Pinot Black, VCR– 9.

Y–3562 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Pinot Gris, clone 52.

Y–3588 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Ruby Jubilee, (Gerus L.V.)

Y–3590 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Tairov Ruby (Gerus L.V.)

Y–3591 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety No. 56–13–1 (Gerus L.V.)

Y–3592; MAFF-230227 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Golubok, (Gerus L.V.)

Y–3593 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Legend, (Gerus L.V.)

Y–3620 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Marseille Black.

Y–3621 *Saccharomyces cerevisiae* isolated from fermented grape must of the variety Cabernet-Sauvignon, clone Fran 441.

MAFF – Culture Collection of Microorganisms, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan. National Institute of Agrobiological Sciences, Genebank of Japan.

Pure yeast cultures were isolated from grapes and followed by fermentation by using traditional microbiological methods consisting of 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 culture media for yeast cultivation and isolation of the yeast strain.

The yeast cells were grown in 10 mL of glass test tubes of Wort Broth and then in Wort Agar containing (g/L) [6, 8]:

– Bacto malt extract – 15.0 g.
– Bacto Peptone – 0,78 g.
– Dextrin – 2,75 g.
– Bacto Agar – 15.0 g.
– Dipotassium Phosphate – 1.0g.
– Ammonium Chloride – 1.0g.
– Maltose Technical – 12,75 g.
– Purified by distillation water – 1 L.
– Bacto Glycerol – 2,35 g.
Adjusted at pH=4.8±0.1.

RESULTS AND DISCUSSION

The results of the organoleptic characteristic study of red grape varieties had specific differences in flavor, taste and aftertaste. The study showed that concentration of ethanol and residual sugar in wine stocks depends on enzymatic activity of *Saccharomyces cerevisiae* yeast strains which are used for winemaking. Investigation of the morphology of *Saccharomyces cerevisiae* yeast followed by fermentation illustrate that between red wine stocks of red grape varieties exist some differences in level of ethanol production. All the yeast cells were stained by Gram method, and Methylene Blue. Some cells of yeast are large and either rounded or oval shaped (Fig. 1–2).

When winemakers select a cultured yeast strain it largely done, because the winemaker wants a predictable fermentation taken by a strain that has a track record of dependability [8]. Among the particulars considerations that are often important to winemakers is a yeast's tendency to:

– Quickly start fermentation, out–competing other «wild yeasts» for nutrients in the must.

– Completely utilize all fermentable sugars with a predictable sugar to alcohol conversion rate.

– Have an alcohol tolerance up to 15% or even higher depending on the winemaking style.

– Have a high sulfur dioxide tolerance but low production of sulfur compounds such as hydrogen sulfide or dimethyl sulfide.

– Produce a minimum amount of residual pyruvate, acetic acid and acetaldehyde.

– Produce minimum foaming during fermentation which may create difficulties for cap management during maceration or cause bungs to pop out during barrel fermentation.

– Have high levels of flocculation and lees compaction that makes racking, fining and filtering of the wine easier [9, 10].

Inoculated (or pure cultured) yeasts are strains of *Saccharomyces cerevisiae* that have been identified and plated from wineries across the world (including notable producers from Ukrainian wine regions such as Odessa, Nikolaev, Cherson. These strains are tested in Laboratory to determine a strain's vigor, sulfur dioxide and alcohol tolerance, residual sugar, production levels of sulfur compounds, ability to re-ferment (positive for sparkling wine but a negative attribute for sweet late-harvest wines), enhancement of a wine's color or certain varietal characteristics by enzymes in the yeast cells and other metabolic products produced by the yeast, foaming and flocculation tendencies, yeasticidal properties (a trait known as "Killer yeast") and tolerance for nutritional deficiencies in a must that may lead to a stuck fermentation.

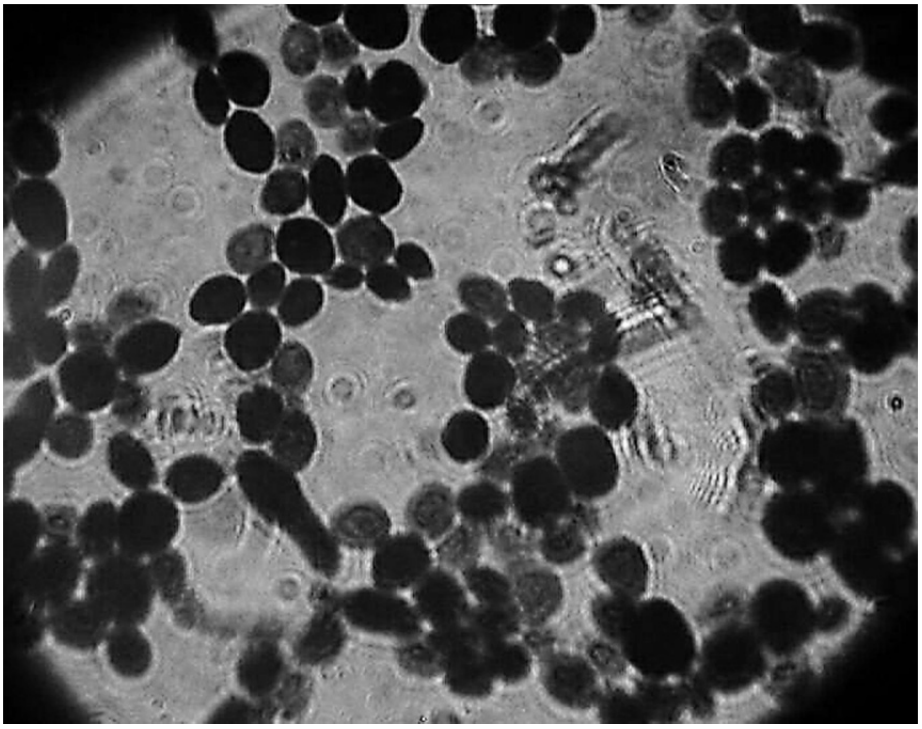

Fig. 1. Morphology of *Saccharomyces cerevisiae* yeast culture Y–3549; MAFF 230184 isolated from red grape variety «Cabernet–Sauvignon, clone Fran – 326». Stained by Gram method; magnification – × 900

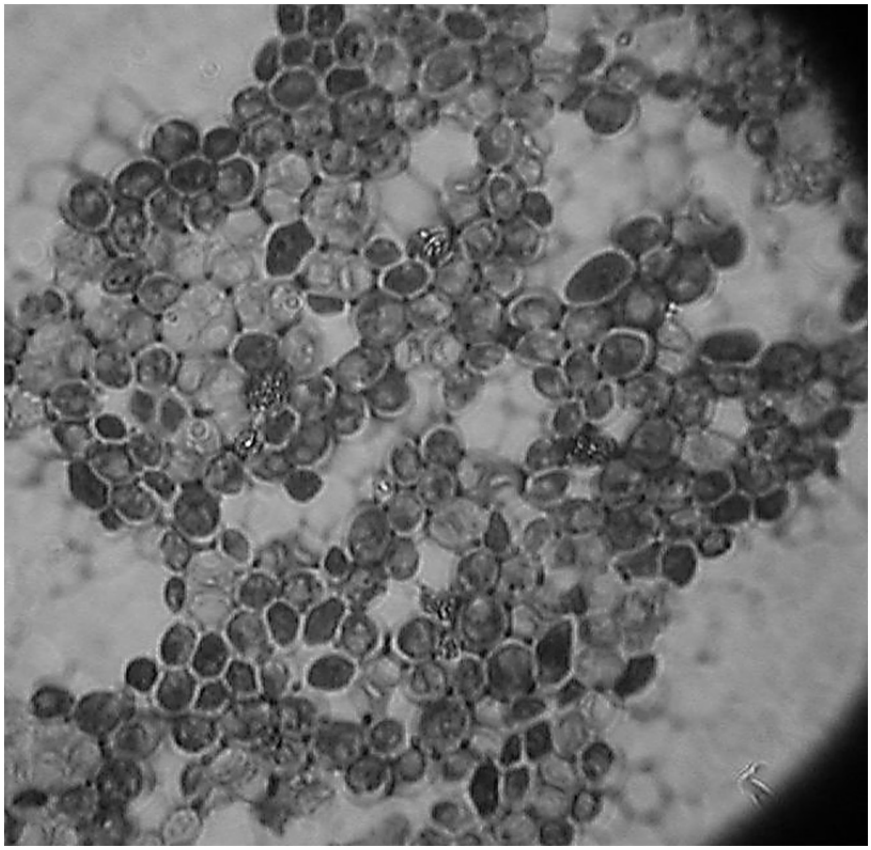

Fig. 2. Morphology of *Saccharomyces cerevisiae* yeast culture Y–3590 isolated from red grape variety «Tairov Ruby». Staining by Methylene Blue; magnification–×900

Table 1.

Organoleptic characteristics of flavor in Red wines following *Saccharomyces cerevisiae* fermentation

Grape cultivar *(source) Vineyards of Agricultural Company «Koblevo»	Wine characteristic	Yeast culture deposited in USRCB collection
Bastardo	Well flavor, with a berry and peach aroma. Harmonious wine with a good aftertaste. In the taste ethanol is felt. **Grade** – well.	Y–3605
Cabernet–Sauvignon	Good sample of wine in taste, aroma, and aftertaste. In the aroma present floral notes. In the taste ethanol is felt. **Grade** – well.	Y–3606
Isabella	In the taste mild tartness characteristic for wines produced from Red grape cultivars. In wine present aroma characteristic to grape variety Isabella, with the smell of strawberries. In the taste ethanol is felt. Harmonious wine with a good aftertaste. **Grade** – well.	Y–3603
Merlot	Slight hydrogen sulfide gassing. In the taste ethanol is felt. Sample of wine without defects. **Grade** – satisfactorily.	Y–3604
Odessa Black	In the color are present oxidized, bulbous tones. In the taste notes high acidity, and high level of volatile acids. **Grade** – unsatisfactorily.	Y–3602

** «Koblevo» Agricultural Company, Nikolaev region of Ukraine.*

Table 2.

Organoleptic characteristics of flavor in Red wines following *Saccharomyces cerevisiae* fermentation

Grape cultivar **(source) Vineyards of Tairov Research Institute, Department of Clonal Selection	Red wine characteristic	Yeast culture deposited in USRCB collection and MAFF collection in Japan
Cabernet-Sauvignon, clone – 2043	Color of pomegranate, felt varietal characteristics and properties. Taste felt like empty without tannins. **Grade** – unsatisfactorily.	Y–3623
Cabernet–Sauvignon, clone – 143141	Color of intens garnet. In taste felt acetone, and high volatile acidity. Also bitterness, dustiness, and aethereality. **Grade** – unsatisfactorily.	Y–3622
Cabernet–Sauvignon, clone Fran – 326	The wine with a high volatile acidity. **Grade** – unsatisfactorily.	MAFF 230184 Y–3549
Cabernet–Sauvignon, clone Fran ICV– 101	The wine with a high volatile acidity. The color is light and uncharacteristic, with aroma of herbs, dusty, empty, with a high acidity. **Grade** – unsatisfactorily.	Y–3550

Ending of the table 2

Cabernet–Sauvignon Fran, clone VCR– 10	Fatty wine. Unnatural, ethereal flavor. Strongly oxidized wine. **Grade** – unsatisfactorily.	Y–3548
Cabernet–Sauvignon, clone 441	In wine felt tannin taste. Aroma is herbal. Aftertaste with a murine tone. **Grade** – unsatisfactorily.	Y–3621
Golubok	Acetone in aroma, with a high level of volatile acids. **Grade** – unsatisfactorily.	MAFF 230227 Y–3592
Legend	Fatty wine. The wine is sick with a high acidity. **Grade** – unsatisfactorily.	Y–3593
Marseille Black	Sweet of milk caramel flavor. Sour of wine. **Grade** – unsatisfactorily.	Y–3620
Merlot, clone VCR– 13	Felt acetone, and high volatile acidity. Also bitterness, dustiness, and aethereality. **Grade** – unsatisfactorily.	Y–3552
Merlot, clone VCR– 1	Felt acetone, and high volatile acidity. Also bitterness, dustiness, and aethereality. **Grade** – unsatisfactorily.	MAFF 230186 Y–3551
Merlot, clone 347	Wine with the smell of cinnamon. Aftertaste with a murine tone. **Grade** – unsatisfactorily.	Y-3553
Pinot Black, VCR– 9	Aroma of plants. In the taste also present herbal notes. **Grade** – unsatisfactorily.	Y–3560
Pinot Black, clone 872	In aroma present dustiness, etheric fats, a lot of tannins and bitterness, and high level of volatile acids. Murine tone in the aftertaste. **Grade** – unsatisfactorily.	Y–3559
Pinot Gris, clone 1–84	In aroma present dustiness, etheric fats, a lot of tannins and bitterness, and high level of volatile acids. Murine tone in the aftertaste. **Grade** – unsatisfactorily.	Y–3558
Pinot Gris, clone 52	Color of wine is marshy. Fatty, oxidized, with a high level of volatile acids. In taste bitterness and acidity. **Grade** – unsatisfactorily.	Y–3562
Ruby Jubilee	Color is dark ruby. Specific odor, characteristic, in the taste with signs of walnut. **Grade** – satisfactorily.	Y–3588
Tairov Ruby	In aroma present lactic acid tone like kefir. **Grade** – unsatisfactorily.	Y–3590
56–13–1	Smells like sweetmeats, with a good flavor, and floral bouquet, with well taste, and moderate acidity. **Grade** – well.	Y–3591

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CONCLUSIONS

We studied five samples of red wines, where grapes obtained from vineyards of Agricultural Company «Koblevo», Nikolaev region of Ukraine.

Y–3603 «Isabella», in wine present aroma characteristic to grape variety «Isabella», with the smell of strawberries. Harmonious Red wine with a good aftertaste. Grade – well. Y–3605 «Bastardo», well flavor, with a berry and peach aroma. Harmonious wine with a good aftertaste. Grade – well. Y–3606

«Cabernet–Sauvignon», good sample of wine in taste, aroma, and aftertaste. In the aroma present floral notes. Grade – well.

Y–3591 «56–13–1», smells like sweetmeats, with a good flavor, and floral bouquet, with well taste, and moderate acidity. Grade – well. Those samples which received well grade of wine in taste, aroma, and aftertaste could be recommended for winemaking. Many studied samples of red wines, where grapes obtained from vineyards of the Laboratory Cloning Selection, Tairov Research Institute of Viticulture and Enology, most of them received unsatisfactorily grade due to their unpleasant aroma, taste, and aftertaste.

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Байрактар В.Н.

СЕЛЕКЦИЯ *SACCHAROMYCES CEREVISIAE* ВИННЫХ ДРОЖЖЕЙ ДЛЯ БИОТЕХНОЛОГИИ ВИНОДЕЛИЯ

Ключевые слова: *селекция дрожжей, органолептические свойства, Saccharomyces cerevisiae, сенсорные профили, интенсивность аромата и вкуса.*

В статье показаны результаты сенсорных характеристик красного сухого вина, полученного с использованием винных культур дрожжей перспективных для виноделия. Показано, что появление некоторых оттенков в цвете, аромате и вкусе сухого вина в значительной мере связано с соотношением в них окисленных и восстановленных компонентов включая фенольные вещества. Цветочные оттенки аромата, свежий вкус, десерные тона в аромате и вкусе более выражены в случае преобладания восстановленных форм компонентов. Смолисто-бальзамические оттенки вкуса и пряно-бальзамические, орехово-ванильные тона аромата проявляются тем сильнее, чем большее соотношение окисленных форм компонентов. Установлены дрожжевые культуры, которые дали наилучшие органолептические характеристики вин полученных при непосредственном участии выделенных и изученных винных дрожжей, *Saccharomyces cerevisiae*.

Байрактар В.М.

СЕЛЕКЦІЯ *SACCHAROMYCES CEREVISIAE* ВИННИХ ДРІЖДЖІВ ДЛЯ БІОТЕХНОЛОГІЇ ВИНОРОБСТВА

Ключові слова: *селекція дріжджів, органолептичні властивості, Saccharomyces cerevisiae, сенсорні профілі, інтенсивність аромату та смаку.*

У статті показані результати сенсорних характеристик червоного сухого вина, отриманого з використанням винних культур дріжджів перспективних для винобробства. Показано, що поява деяких відтінків в кольорі, ароматі й смаку сухого вина значною мірою пов'язані із співвідношенням в них окислених і відновлених компонентів включаючи фенольні речовини. Квіткові відтінки аромату, свіжий смак, десерність тону в ароматі і смаку більш виражений у разі переважання відновлених форм компонентів. Смолисті й бальзамічні відтінки смаку і пряно-бальзамічні, горіхово-ванільні тони аромату проявляються тим сильніше, чим більше співвідношення окислених форм компонентів. Встановлено дріжджові культури, які дали найкращі органолептичні характеристики вин отриманих при безпосередній участі виделених і вивчених винних дріжджів, *Saccharomyces cerevisiae*.