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# THE INFRARED LASER EFFECT ON CARBOHYDRATE FERMENTATION BY *Saccharomyces cerevisiae* YEAST

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Combined treatment of yeast cultures using infrared laser gives an ability to select yeast cultures with new properties for wine industry. Infrared laser action during 10 min with wavelength from 800 to 900 nm at total basic power of 10 Wt laser output exposure and combination with magnetic field induction within 10–40 mT (millitesl) makes laser treatment efficient for yeast biostimulation process. Power density for infrared laser treatment at 5 mWt/cm<sup>2</sup> was used. Such parameters as content of protein (total), carbohydrate (glucose), triglycerides and fermentative activity in yeast cultures were determined. Analysis of these parameters clearly showed that after infrared laser treatment the fermentation of grape must proceeded without pauses and delays, with good fermentation of carbohydrates and maximal quantity of ethyl alcohol production at the level of 8–14%. Also the following parameters of culture solution were studied: protein (total), glucose, ergosterol, triglycerides, urea, calcium, phosphorus, magnesium, iron, chlorides. Following enzyme activity was detected: glucose-6-phosphate dehydrogenase, lactate dehydrogenase, gamma-glutamyltransferase, alkaline phosphatase, alpha-amylase, esterase.

At biochemical testing increasing of enzyme activity, triglyceride level and urea concentration were determined. During fermentation glucose concentration had the tendency to decrease.

**Key words:** infrared laser, yeast cultures, enzymes, carbohydrates, fermentation.

Spontaneous fermentation of grape must is always accompanied with complex biochemical reactions such as carbohydrates transformation into ethyl alcohol. The mechanism of such processes starts up due to the yeast enzymes produced during their reproduction in cultural solution. Together with bacteria and fungi the yeasts are located on the skin of a ripe grape. In anaerobic conditions yeast enzymes participate in the process of grape must transformation into ethyl alcohol, carbon dioxide and other products [14–16]. All of this plays an important role in the technology of making high-quality natural wine. Taste and wine delicate aroma depend on the yeast culture used in the production.

In nature, there are different wild yeast species that are able to participate in the process of grape must fermentation as well. Therefore our task was to determine the isolated yeast species. Pure yeast culture makes biotechnological process more controllable and for this reason it is more preferable in wine industry.

Pure yeast cultures resistant to ethyl alcohol, acid and sulphites provide possibility for fermentation of grape must even in unusual, extreme conditions [19, 7].

Specific difference in yeast cultures plays an important role in receiving high quality product. The must possess yeast strains with high zymotic activity [17, 9, 10].

The objective of this research was to study the effect of infrared laser on yeast cultures activation of enzymes. Selection of yeast cultures for further industrial use from isolated wild yeast cultures out of ripe grapes and grape must after anaerobic fermentation was done.

## Materials and methods

Identification of the isolated yeast cultures was performed by PCR analysis using universal primers for yeasts. After the identification of yeast species by PCR analysis, isolated cultures were deposited in NRRL Culture Collection, Peoria, USA (Nord Regional

Research Laboratory) and in British National Collection of Yeast Culture (NCYC), Norwich, UK.

Samples from industrial grape were collected during the season of vintage from vineyard of winery Company «Shabo», Odesa region. Other table varieties of grapes were purchased from private farmers who cultivated vines.

The total amount of the varieties which were selected for the research was thirty.

The following industrial varieties of vine were selected for the research: Chardonnay, Cabernet, Merlot, Sauvignon, Wrestling Rhenish, Aligote, Rkatsiteli. They were cultivated on the sandy loam soils in the district located between Black Sea and Dniester estuary.

The following varieties were selected for the research from the table varieties of vine: Keshu, Kishmish, Karaburnu, Lidia, Odesa souvenir, Dniester pink, Original, Muscat white, Isabela, Crimean black, Moldova, Doina, Queen of vineyards, Arcadia, Bako, Suruchenian, Sucholimanskoe white.

It was also selected the following hybrid varieties of vine: Noa (black novak), One thousand first hybrid, Twenty eighth hybrid, Seibel white, Seibel black, Kudrek white.

The grape must from different grape varieties was placed into the sterile glass flasks to half 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 a result of active anaerobic fermentation processes in grape must. At the end of grape must fermentation pure yeast cultures were isolated using traditional microbiological methods [1–3] by consistent inoculation of a sample into Petri dish with a few modifications of nutrient selective agar for yeast isolation and cultivation.

Primary yeast isolation was carried out on Inhibitory Mold Agar medium, (Becton Dickinson Company, USA). The yeast culture morphological properties were checked after the primary isolation. Yeasts were identified by polymerase chain reaction (PCR) using universal yeast primers [20, 11]. After yeast cultures identification the next yeast cultivation was carried out on Wort Agar medium (Becton Dickinson Company, USA).

Each isolated yeast culture after identification was deposited in the NRRL Culture Collection (Nord Regional Research Laboratory), Peoria, USA and in British National Collection of Yeast Culture (NCYC), Norwich, UK.

It was used industrial wine-making yeast culture *Saccharomyces cerevisiae* species

called — Oenoferm Bouquet yeast for white wine making as a control. It was made for the development of fresh fruit aromas. Oenoferm Bouquet is a pure yeast culture produced by the ERBSLOH Company, Geisenheim AG, Germany.

For yeast isolate identification it was used amplification of ITS1-5.8S-ITS1-2b and D1-D2 26S genome locus fragments that code ribosomal RNA with the next direct sequencing of received DNA fragments [20, 11]. Genome DNA was isolated from 20–60 mg of yeast biomass using yeast cells lysis in the buffer contained Guanidinium thiocyanate, and with further adsorption in Silica gel cleaning with spirit and DNA desorption in the buffer with low ionic strength.

Polymerase chain reaction was carried out in the final volume of 50 mL which contained 65 mM Tris-HCl (pH 8.9), 16 mM Ammonium sulfate, 3.5 mM Magnesium chloride; 0.5% Tween-20; 0.2 mM dNTP (Deoxyribonucleotide triphosphate); 0.2 mM solution oligonucleotide primers; 1–10 ng (nanogram) DNA and 1 Unit activity of Taq-DNA polymerase. Samples were tested using Eppendorf amplifier, with initial denaturation at 95 °C for 3 min, then during 33 cycles with denaturation at 95 °C for 10 seconds, primers annealing (see table 1) for 10 seconds at 60 °C and elongation for 15 seconds at 72 °C. Final elongation was at 72 °C for 5 minutes.

Amplification products were analyzed using 6% polyacrylamide gel. The gel was stained with the Ethidium bromide, DNA visualization was carried out in ultra violet light.

DNA sequencing was carried out in the Sequencing Center of Research Institute of Chemical Biology and Fundamental Medicine, Novosibirsk.

Test samples for nucleotide sequence determination were sequenced using Sanger's method using a test kit of BigDye Terminator Cycle Sequencing Kit v 3.1 and genetic analyzer ABI PRISM 3100 (Applied Biosystems, USA) in accordance with the producer's protocol.

PCR amplification of ITS1-5.8S-ITS2 locus was performed using two primers 5'-TCCG-TAGGTGAACCTGCGG-3' or 5'-TCCTCCGCT-TATTGATATGC-3' and D1-D2 locus analysis was performed using one primer 5'-GCATAT-CAATAAGCGGAGGAAAAG-3' [20, 11]. These oligonucleotides correspond to sequences described by White et. al, 1990 and Dr. Kletus Kutzman et. al, 1997. The primers were constructed by Dr. Maxim Filipenko, Laboratory of Pharmacogenomics, Research Institute of Chemical Biology and Fundamental Medicine, Novosibirsk.

After PCR identification of yeast species as *Saccharomyces cerevisiae*, each isolated yeast culture was tested for mating type by PCR in cooperation with Dr. Yoshinobu Kaneko, Department Biotechnology, Osaka University, Japan.

As soon as it was specified, the mating type for each yeast culture, their morphological, physiological and biochemical properties were studied.

Each yeast culture technological characteristics such as growth resistance at high temperature +42 °C and low temperature 6–8 °C growth at low pH 2.6–3.0 (acid resistance), growth at the presence of 5, 10, 15% of ethyl alcohol (ethyl alcohol resistance), growth at the presence of high sulfite concentration (sulfite resistance), hydrosulfide synthesis (production, gassing) were studied as well.

Magnetic treatment of yeast *Saccharomyces cerevisiae* was made at magnitude induction density from 10 to 40 mT and duration of action for 1; 3; 5; 10; 15; 20; 30 minutes. Total number of conducted magnetic yeast treatments was performed with an interval of 3–4 days between the treatments.

Infrared laser treatment used for yeasts was held with semiconductor laser on different electromagnetic wavelengths near infrared diapason or with a laser that corresponded to different light-spectra such as 610–650 nm; 750–780 nm; 800–900 nm with the total power rate at the output not less as 10 Wt. Biostimulating infrared laser used in the research worked at the streaming operation in infrared spectrum with wavelength of 800–900 nm of total exposure rate 10 Wt. A semiconductor laser with a light-emitting diode was based on Gallium arsenide crystals — GaAs (Gallium arsenide laser). Emitting source was a diode of LDN-7model.

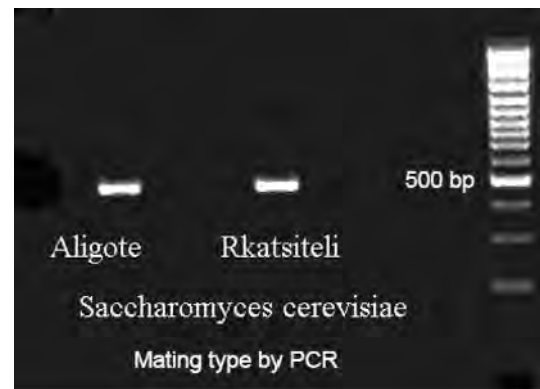
Time of laser exposures for yeast cultures was 1; 3; 5; 10; 15; 20 minutes. Total number of conducted infrared laser yeast treatments was 10 with an interval of 3–4 days between yeast laser treatments.

## Results and discussion

Analysis results of mating types of yeasts isolated from grape variety Aligote and Rkatsiteli are shown in the Fig 1.

Experimental research was focused on such carbohydrates as Glucose, Sucrose, Maltose, Fructose, Lactose and Galactose for fermentation completeness confirmation in grape must. The article is intentionally

focused on glucose fermentation followed by infrared laser treatment of yeast cultures and these results are described in details and showed in the tables.



**Mating types of *Saccharomyces cerevisiae* yeast isolated from grape must and grape variety Aligote and Rkatsiteli**

Refreshed yeast cultures were inoculated into glass test tubes contained pure 20% solution of D-glucose and after 10 hours of cultivation yeast suspensions were checked for the following parameters: Protein (total), Ergosterol, Triglycerides, Urea, Glucose, Calcium, Phosphorus, Magnesium, Iron, Chlorides. Activity of such enzymes as Dehydrogenase, Transferases, Alkaline phosphatase,  $\alpha$ -Amylase, Esterase, Glucose-6-phosphate dehydrogenase was studied as well.

All the above listed parameters are based on a principle of spectrophotometric analysis. All the tests were conducted using specific test kit for each parameter. The kits were made by the BioSystems Company S.A. Costa Brava, Spain. It was used the kits made by the Sentinel Company, Italy for Glucose-6-phosphate dehydrogenase testing, and by Pliva-Lachema Diagnostika, Brno, Czech Republic for Chlorides and Esterase testing.

The most perspective yeast cultures used for biotechnology of wine making were chosen for the further research associated with their graded adaptation to ultra violet, magnetic, infrared and red laser treatment. Its purpose was the inducted selection for further using in biotechnology of wine making.

It was found out that laser treatment of *Saccharomyces cerevisiae* yeast stimulated the reproduction process of yeast cells isolated from different grape varieties. Fermentation speed was higher and wine quality became better with pleasant aroma bouquet and taste.

It was detected that laser action on yeast cells stimulated processes of cell reproduction,

activated cell division, and fermentation processes proceeded smoothly and freely as a result of small strength laser using with exposition at 5 mWt/cm<sup>2</sup>.

Laser treatment effectiveness showed also the activity fermentation in yeast cultures for better ethyl alcohol production.

Enzymes activity before infrared laser treatment and 10 hours after treatment of yeast cultures isolated after spontaneous fermentation of grape must gave a positive comparison (table 1).

Glucose-6-Phosphate Dehydrogenase activity was tested 10 hours after infrared laser treatment of the yeast suspension. The range of enzyme parameters varied from 0.149  $\mu\text{mol}/\text{sec}\cdot\text{L}$  to 0.232  $\mu\text{mol}/\text{sec}\cdot\text{L}$  during fermentation. Alkaline phosphatases were the most effective in an alkaline environment [5, 18].

Alkaline phosphatase activity was tested 10 hours after magnetic infrared laser exposure of the yeast suspension. The range of enzyme parameters were varied from

0.0166  $\mu\text{mol}/\text{sec}\cdot\text{L}$  to 0.398  $\mu\text{mol}/\text{sec}\cdot\text{L}$  during fermentation.

At high concentrations of lactate, lactate dehydrogenase showed feedback inhibition and the rate of conversion of pyruvate to lactate was decreased. It also catalyzed the dehydrogenation of 2-Hydroxybutyrate, but it was considerably poorer substrate than lactate. There was little to no activity with beta-hydroxybutyrate [22, 21].

Lactate Dehydrogenase activity was tested 10 hours after infrared laser treatment of the yeast suspension. The range of enzyme parameters varied from 0.0166  $\mu\text{mol}/\text{sec}\cdot\text{L}$  to 0.415  $\mu\text{mol}/\text{sec}\cdot\text{L}$  during fermentation.

Aspartate aminotransferase activity was tested 10 hours after magnetic infrared laser exposure of the yeast suspension. The range of enzyme parameters varied from 0.0166  $\mu\text{mol}/\text{sec}\cdot\text{L}$  to 0.68  $\mu\text{mol}/\text{sec}\cdot\text{L}$  during fermentation.

$\alpha$ -Amylase (EC 3.2.1.1) is an enzyme that hydrolyses alpha-bonds of large alpha-linked polysaccharides such as starch and glycogen,

Table 1. Glucose fermentation index 10 hours after infrared laser treatment of *Saccharomyces cerevisiae* yeast cultures isolated from different varieties of grape must

No.	The grape variety	LDH, $\mu\text{mol}/\text{sec}$	CHE, $\mu\text{mol}/\text{sec}$	G6PDH, $\mu\text{mol}/\text{sec}$	$\gamma$ -GT, $\mu\text{mol}/\text{sec}$	ALT, $\mu\text{mol}/\text{sec}$	AST, $\mu\text{mol}/\text{sec}$	ALP, $\mu\text{mol}/\text{sec}$	$\alpha$ AmL, $\mu\text{mol}/\text{sec}$
1	Aligote	0.06 $\pm$ 0.007	0.41 $\pm$ 0.008	0.19 $\pm$ 0.002	0.016 $\pm$ 0.001	0.016 $\pm$ 0.001	0.016 $\pm$ 0.001	0.016 $\pm$ 0.001	0.05 $\pm$ 0.001
2	Bako	0.016 $\pm$ 0.001	0.05 $\pm$ 0.001	0.15 $\pm$ 0.001	0.09 $\pm$ 0.002	0.016 $\pm$ 0.001	0.016 $\pm$ 0.001	0.016 $\pm$ 0.001	0.16 $\pm$ 0.007
3	Cabernet	0.215 $\pm$ 0.01	0.34 $\pm$ 0.003	0.16 $\pm$ 0.002	0.016 $\pm$ 0.001	0.16 $\pm$ 0.002	0.016 $\pm$ 0.001	0.39 $\pm$ 0.007	0.066 $\pm$ 0.001
4	Chardonnay	0.09 $\pm$ 0.007	0.066 $\pm$ 0.001	0.16 $\pm$ 0.002	0.19 $\pm$ 0.002	0.016 $\pm$ 0.001	0.30 $\pm$ 0.019	0.016 $\pm$ 0.001	0.016 $\pm$ 0.001
5	Lidiya	0.083 $\pm$ 0.001	0.348 $\pm$ 0.001	0.182 $\pm$ 0.003	0.016 $\pm$ 0.002	0.016 $\pm$ 0.001	0.99 $\pm$ 0.002	0.016 $\pm$ 0.001	0.033 $\pm$ 0.002
6	Oenopherm Bouquet (Control)	0.215 $\pm$ 0.001	0.415 $\pm$ 0.001	0.232 $\pm$ 0.002	0.05 $\pm$ 0.002	0.016 $\pm$ 0.001	0.315 $\pm$ 0.002	0.016 $\pm$ 0.001	0.05 $\pm$ 0.001
7	Suruchanskiy	0.166 $\pm$ 0.002	0.215 $\pm$ 0.001	0.19 $\pm$ 0.01	0.016 $\pm$ 0.001	0.016 $\pm$ 0.001	0.232 $\pm$ 0.002	0.016 $\pm$ 0.001	0.083 $\pm$ 0.001
8	Rkatsiteli	0.049 $\pm$ 0.001	0.166 $\pm$ 0.001	0.232 $\pm$ 0.002	0.35 $\pm$ 0.012	0.016 $\pm$ 0.001	0.132 $\pm$ 0.002	0.016 $\pm$ 0.001	0.033 $\pm$ 0.001
9	Sucholimanskiy	0.415 $\pm$ 0.001	0.132 $\pm$ 0.002	0.19 $\pm$ 0.002	0.09 $\pm$ 0.002	0.116 $\pm$ 0.002	0.68 $\pm$ 0.015	0.016 $\pm$ 0.001	0.016 $\pm$ 0.001
10	Sauvignon	0.149 $\pm$ 0.003	0.116 $\pm$ 0.001	0.215 $\pm$ 0.002	0.016 $\pm$ 0.001	0.033 $\pm$ 0.001	0.481 $\pm$ 0.001	0.016 $\pm$ 0.001	0.016 $\pm$ 0.001

Standard deviation was calculated, and statistical significance of difference was evaluated by Student's t-test ( $P < 0.05$ ).

LDH — Lactate Dehydrogenase.

CHE — Cholinesterase.

G6PDH — Glucose-6-phosphatdehydrogenase.

$\gamma$ -GT — gamma glutamyltransferase.

ALT — Alanine aminotransferase.

AST — Aspartate aminotransferase.

ALP — Alkaline phosphatase.

$\alpha$ -AmL — Alpha amylase.

1  $\mu\text{mol}$  — it is enzyme activity catalized transformation substrate during 1 minute ( $\mu\text{mol}/\text{min} \times \text{L}$ ) = 1  $\mu\text{kat}/\text{L}$ .

yielding glucose and maltose. Alpha-amylase is used in ethanol production to break starches in grains into fermentable sugars [12, 13].

$\alpha$ -Amylase activity was tested 10 hours after infrared laser treatment of the yeast suspension. The range of enzyme parameters varied from 0.0166  $\mu\text{mol}/\text{sec}\cdot\text{L}$  to 0.166  $\mu\text{mol}/\text{sec}\cdot\text{L}$  during fermentation.

The Cholinesterase (EC 3.1.1.8) is a family of enzymes that catalyze the hydrolysis of the acetylcholine into choline and acetic acid.

Cholinesterase activity was tested 10 hours after infrared laser treatment of the yeast suspension. The range of enzyme parameters varied from 0.049  $\mu\text{mol}/\text{sec}\cdot\text{L}$  to 0.415  $\mu\text{mol}/\text{sec}\cdot\text{L}$  during fermentation.

Gamma-glutamyl transferase activity was tested 10 hours after magnetic infrared laser treatment of the yeast suspension. The range of enzyme parameters varied from 0.0166  $\mu\text{mol}/\text{sec}\cdot\text{L}$  to 0.348  $\mu\text{mol}/\text{sec}\cdot\text{L}$  during fermentation.

As it can be seen from Table 2, concentration of calcium, phosphorus, magnesium and iron in yeast suspension 10 hours after infrared laser treatment was low. Only chloride parameters were high and varied from 14.5 to 21.8 mmol/L.

Table 3 shows that the basic concentration of glucose was high; protein concentration (total) was low, the amount of urea was low as a result of yeast fermentation, the amount of ergosterole was low and triglyceride concentrations were high.

Our research showed that in combination with magnetic action on yeast suspension laser had an essential influence on fermentative processes. Infrared laser action on yeasts with wavelength of 800–900 nm led to complete fermentation of carbohydrates. It was confirmed by the information given in the tables 2 and 3. Yeast suspension samples were tested during six days treatment with laser. It was found

**Table 2. Concentration of macro and microelements in yeast suspension 10 hours after infrared laser treatment of yeast cultures isolated after spontaneous fermentation of grape must**

No.	The grape Variety	Calcium, mmol/L	Phosphorus, mmol/L	Magnesium, mmol /L	Iron, $\mu\text{mol}/\text{L}$	Chlorides, mmol /L
1	Aligote	0.31±0.01	0.51±0.01	0.11±0.01	4.0±0.11	21.3±0.17
2	Bako	0.49±0.01	0.01±0.002	0.10±0.01	1.0±0.06	17.1± 0.14
3	Cabernet	0.40±0.02	0.01±0.002	0.09± 0.01	6.4±0.16	18.4±0.08
4	Chardonnay	0.69±0.01	0.01±0.002	0.11±0.01	14.0±1.49	16.6±0.15
5	Lidiya	0.26±0.01	0.40±0.01	0.11±0.01	3.1±0.10	21.8±0.12
6	Oenopherm Bouquet	0.74±0.01	0.02±0.001	0.07±0.003	7.2±0.07	18.1±0.10
7	Rkatsiteli	0.72±0.01	0.02±0.001	0.12±0.01	2.0±0.05	17.4±0.13
8	Suruchanskiy	0.10±0.01	0.01±0.002	0.04±0.002	10.3±0.12	14.5±0.12
9	Sucholimanskiy	0.21±0.01	0.01±0.002	0.11±0.01	3.1±0.14	17.8±0.12
10	Sauvignon	0.66±0.01	0.37±0.01	0.13±0.01	5.0±0.10	21.0±0.16

**Table 3. Total parameter concentration 10 hours after infrared laser treatment of yeast cultures isolated after spontaneous fermentation of grape must**

No.	The grape Variety	Protein (total), g/L	Glucose, mmol/L	Urea, mmol/L	Triglycerides, mmol/L
1	Aligote	0.1±0.01	4.85±0.08	0.10±0.01	6.02±0.10
2	Bako	0.9±0.01	4.75±0.01	0.30±0.01	4.90±0.10
3	Cabernet	0.1±0.01	4.32±0.01	0.70±0.01	5.44±0.09
4	Chardonnay	0.9±0.01	4.94±0.10	0.23±0.01	5.85±0.02
5	Lidiya	0.1±0.01	2.81±0.01	1.00±0.12	6.10±0.06
6	Oenopherm Bouquet	0.8±0.01	4.25±0.03	0.19±0.007	4.90±0.10
7	Rkatsiteli	0.8±0.01	4.10±0.06	0.45±0.01	5.19±0.02
8	Suruchanskiy	0.1±0.01	3.26±0.02	0.39±0.01	5.48±0.07
9	Sucholimanskiy	0.1±0.01	4.01±0.01	0.11±0.01	5.20±0.02
10	Sauvignon	0.4±0.01	3.38±0.01	0.10±0.01	6.11±0.06

that carbohydrate fermentation was 82.8–84.3% completed. Concentration of ethyl alcohol in all tested samples was higher as compared to the control samples. Concentration of ethyl alcohol corresponded to the amount of fermented carbohydrates.

Results of the research showed that optimal time for infrared laser treatment at wavelength 800–900 nm was 10 minutes at 5 mWt/cm<sup>2</sup> (power density of infrared laser). In such mode of infrared laser treatment intensive carbon dioxide production in yeasts during the fermentation of 20% glucose solution was observed.

Enzyme activity index was decreased noticeably after stabilization and fermentation completion. Table 4 clearly shows that some yeasts displayed almost zero enzyme activity after completion of fermentation. Glucose-6-phosphatdehydrogenase fermentative activity in different grape variety yeasts was in a range from 0.029 to 0.068 mmol/sec\*L and so after fermentation completion it was 2–4 times lower compared to fermentation start.

Triglycerides concentration became normal after fermentation and ranged on the average from 2.43 to 4.68 mmol/L comparing

to triglycerides fermentation at the start that ranged from 4.90 to 6.11 mmol/L.

Glucose concentration significantly decreased after fermentation and ranged on the average from 0.01 to 0.12 mmol/L compared with fermentation at the beginning of the process with glucose index ranging from 2.81 to 4.94 mmol/L. Final glucose concentration level at the end of fermentation process was low and confirmed that glucose transformed into carbon dioxide, ethyl alcohol and other products.

Wine contained a small amount of Ergosterol in a range from 0.02 to 0.06 mmol/L after fermentation shown in the Table 6.

Laser beams had the following properties: coherence, monochromaticity, polarization. These defined biological characteristics of biostimulating action on yeasts.

The experimental data showed that comparatively low doses (5 Wt/cm<sup>2</sup>) and short period (10 min) of yeast treatment caused a long lasting macroeffect. Yeasts' stimulation exhibited that the 3–4 days interval between the infrared laser treatment procedures was optimal.

The *Saccharomyces cerevisiae* yeast cultures were treated by infrared laser and incubated

Table 4. Enzyme activity of wine after fermentation completion using *Saccharomyces cerevisiae* yeast without infrared laser treatment

No.	The grape variety	LDH, $\mu\text{mol}/\text{sec}$	CHE, $\mu\text{mol}/\text{sec}$	G6PDH, $\mu\text{mol}/\text{sec}$	$\gamma$ -GT, $\mu\text{mol}/\text{sec}$	ALT, $\mu\text{mol}/\text{sec}$	AST, $\mu\text{mol}/\text{sec}$	ALP, $\mu\text{mol}/\text{sec}$	$\alpha\text{AmL}$ , $\mu\text{mol}/\text{sec}$
1	Bako	0.016± 0.001	0.415± 0.002	0.046± 0.001	0.016± 0.001	0.199± 0.001	0.149± 0.002	0.63± 0.012	0.111± 0.002
2	Lidiya	0.016± 0.001	0.348± 0.001	0.029± 0.001	0.033± 0.001	0.298± 0.001	0.232± 0.001	0.116± 0.001	0.039± 0.001
3	Rkatsiteli	0.016± 0.001	0.332± 0.001	0.044± 0.001	0.016± 0.001	0.116± 0.001	0.166± 0.001	0.531± 0.002	0.33± 0.098
4	Sucholimanskiy	0.232± 0.001	0.016± 0.001	0.068± 0.001	0.049± 0.001	0.249± 0.001	0.365± 0.001	0.149± 0.002	0.081± 0.001
5	Suruchenskiy	0.016± 0.001	0.016± 0.001	0.064± 0.001	0.016± 0.001	0.066± 0.011	0.049± 0.001	0.199± 0.001	0.28± 0.009

Table 5. Concentration of macro and microelements after fermentation completion using native pure *Saccharomyces cerevisiae* yeast cultures without infrared laser treatment

No.	The grape variety	Calcium, mmol/L	Phosphorus, mmol/L	Magnesium, mmol/L	Iron, $\mu\text{mol}/\text{L}$	Chlorides, mmol/L
1	Bako	3.56±0.01	4.02±0.01	1.80±0.01	21.0±0.01	14.0±0.14
2	Lidiya	2.12±0.01	2.33±0.03	0.98±0.02	2.0±0.1	4.6±0.07
3	Rkatsiteli	1.86±0.01	1.89±0.01	1.29±0.02	17.0±0.14	5.7±0.02
4	Sucholimanskiy	2.67±0.02	3.81±0.03	1.85±0.01	43.0±0.12	9.3±0.04
5	Suruchenskiy	2.76±0.01	3.11±0.01	1.21±0.01	1.0±0.01	5.0±0.12

Table 6. Concentration of protein, triglycerides and carbohydrate after completion of fermentation using native pure *Saccharomyces cerevisiae* yeast cultures without magnetic infrared laser treatment

No.	The grape variety	Protein (total), g/L	Glucose, mmol/L	Ergosterol, mmol/L	Triglycerides, mmol/L
1	Bako	7.4±0.02	0.12±0.008	0.06±0.001	2.43±0.01
2	Lidiya	0.1±0.01	0.04±0.02	0.06±0.001	3.34±0.002
3	Rkatsiteli	0.1±0.01	0.08±0.001	0.05±0.002	3.04±0.002
4	Sucholimanskiy	4.0±0.14	0.08±0.001	0.02±0.001	3.50±0.01
5	Suruchenskiy	1.8±0.01	0.01±0.001	0.05±0.002	4.68±0.002

for 10 hours at 24 °C. Activity of some enzymes was determined after incubation. The obtained data showed that growth stimulation was accompanied by increasing of respiration activity (with no accumulation of toxic intermediates of oxygen metabolism) and by synthetic processes predominance over degenerative ones in a cell.

These data indicated that the infrared laser treatment caused a cell metabolism rearrangement under light playing a role of a trigger controller of a cell metabolism.

The above mentioned complex absorbed at wavelength about 800–900 nm. Thus a distinct correlation existed between the action spectra and the absorption bands of respiratory chain components corresponding to yeasts.

Another argument in favor of the suggestion that the primary photoacceptors could be the respiratory chain components is an intimate correlation between oxygen consumption activity and culture growth which is especially appreciable for yeasts. For instance in our experiments with yeast cultures *Saccharomyces cerevisiae* correlations were found between the respiration intensity of an intact culture and its ability to activate itself after stimulation of yeast biomass production with infrared laser treatment.

The following conclusions as a result of the research of *Saccharomyces cerevisiae* yeast culture selection with the use of magnetic and infrared laser treatment could be drawn: optimal infrared laser exposure intensity that is 10 min at 5 mWt/cm<sup>2</sup> with basic power at 10 Wt. in regime of constant CW laser was specified. Semi-conductive laser based on crystals of gallium arsenide (GaAs) worked in infrared spectrum with wavelength of 800–900 nm; optimal intensity for magnetic treatment of yeast *Saccharomyces cerevisiae* in a range from 10 to 40 mT (milli Tesl) with action duration of 15 minutes was determined; significant increase of triglycerides concentration in the samples where fermentation

process was already completed was determined; it was found that in the samples with completed fermentation high concentration of such enzyme as alpha amylase existed yet in the samples where fermentation was already completed there existed low residual glucose concentration in a range from 0.01 to 0.08 mM/L showing that fermentation had been already finished and process of fermentation stabilized. Such types of macro and microelements as calcium, phosphorus, magnesium, iron, chlorides concentrations before and after infrared laser treatment, during and after completion of 20% glucose solution fermentation were determined. Selective criteria of yeast cultures perspective for biotechnology of wine making industry were specified.

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**ВПЛИВ ІНФРАЧЕРВОНОГО ЛАЗЕРА  
НА ФЕРМЕНТАЦІЮ ВУГЛЕВОДІВ  
ДРІЖДЖАМИ**  
*Saccharomyces cerevisiae*

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Комбінована обробка дріжджів з використанням інфрачервоного лазера дає можливість селекціонувати культури з новими властивостями для винної індустрії. Комбінація інфрачервоного лазерного опромінювання з довжиною хвилі 800–900 нм за основної вихідної потужності 10 Вт протягом 10 хв з індукцією магнітного поля в межах 10–40 мТ (мілітесл) приводить до ефективної біостимуляції дріжджових культур. Використовували енергетичну щільність інфрачервоного лазерного опромінювання 5 мВт/см<sup>2</sup>. У культурах дріжджів визначали такі параметри: вміст протеїну (загального), вуглеводів (глюкози), тригліцеридів та ензиматичну активність. Аналіз цих параметрів показав, що ферментація виноградного сусла після інфрачервоного лазерного опромінювання проходить без пауз і затримок, з ефективним бродінням вуглеводів і максимальною кількістю утворюваного етилового спирту на рівні 8–14%. Були також визначені параметри культурального розчину: вміст протеїну (загального), глюкози, ергостеролу, тригліцеридів, сечовини, кальцію, фосфору, магнію, заліза, хлоридів. Установлена активність таких ензимів: глюкозо-6-фосфатдегідрогенази, лактатдегідрогенази, гамма-глутамілтрансферази, лужної фосфатази, альфа-амілази, естерази.

Під впливом опромінювання за наслідками біохімічного тестування було виявлено збільшення ензиматичної активності, вмісту тригліцеридів і концентрації сечовини. Протягом ферментації концентрація глюкози мала тенденцію до зниження.

**Ключові слова:** інфрачервоний лазер, культури дріжджів, ензими, вуглеводи, ферментація.

**ВЛИЯНИЕ ИНФРАКРАСНОГО ЛАЗЕРА  
НА ФЕРМЕНТАЦИЮ УГЛЕВОДОВ  
ДРОЖЖАМИ**  
*Saccharomyces cerevisiae*

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Комбинированная обработка дрожжей с использованием инфракрасного лазера дает возможность селекционировать культуры с новыми свойствами для винной индустрии. Комбинация инфракрасного лазерного облучения с длиной волны 800–900 нм при основной выходной мощности 10 Вт в течение 10 мин с индукцией магнитного поля в пределах 10–40 мТ (миллитесл) приводит к эффективной биостимуляции дрожжевых культур. Использовали энергетическую плотность инфракрасного лазерного облучения 5 мВт/см<sup>2</sup>. В культурах дрожжей определяли следующие параметры: содержимое протеина (общего), углеводов (глюкозы), триглицеридов и энзиматическую активность. Анализ этих параметров показал, что ферментация виноградного сусла после инфракрасного лазерного облучения протекает без пауз и задержек, с эффективным брожением углеводов и максимальным количеством образуемого этилового спирта на уровне 8–14%. Были также определены параметры культурального раствора: содержание протеина (общего), глюкозы, эргостерола, триглицеридов, мочевины, кальция, фосфора, магния, железа, хлоридов. Установлена активность следующих ферментов: глюкозо-6-фосфатдегидрогеназы, лактатдегидрогеназы, гамма-глутамилтрансферазы, щелочной фосфатазы, альфа-амилазы, эстеразы.

Под влиянием облучения по результатам биохимического тестирования было обнаружено увеличение энзиматической активности, содержания триглицеридов и концентрации мочевины. В течение ферментации концентрация глюкозы имела тенденцию к снижению.

**Ключевые слова:** инфракрасный лазер, культуры дрожжей, энзимы, углеводы, ферментация.