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MAGNETIC FIELD EFFECT ON YEAST *Saccharomyces cerevisiae* ACTIVITY AT GRAPE MUST FERMENTATION

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Treatment of yeast cultures using magnetic fields enables us to gain a better understanding of the magnetic fields' action on enzyme activity and the fluctuation of macro- and micro-element concentrations within yeast cultures. For this purpose, the two following groups of yeast were studied: laboratory yeast cultures isolated from regional grape must and commercial yeast cultures that are commonly used in the wine industry. Both yeast groups were biochemically tested with and without magnetic field treatment exposure. We used the following parameters of magnetic field frequency: 160 Hz with an intensity of 5 mT and an exposure time of 30 minutes. Based on our laboratory tests, the yeast cultures that were not exposed to magnetic field treatment had a high correlation between the activities of alcohol dehydrogenase and cocarboxylase. The groups of yeast that underwent magnetic field treatment had a high correlation between the activities of alcohol dehydrogenase, alanine aminotransferase, amylase and phosphatase.

Study of the morphology of *Saccharomyces cerevisiae* yeast followed by magnetic field treatment illustrated that between 30 and 70% of the magnetic field treated yeast died. The surviving yeast cultures in the grape must (confirmed by Gram staining) revealed increased enzymatic activity and a high correlation between levels of potassium and calcium, as well as between levels of potassium and magnesium.

Key words: *Saccharomyces cerevisiae*, enzyme activity, macro- and micro-elements, wine biotechnology.

Magnetic field treatment is an interesting and promising field that is currently being investigated by the wine and food industries. According to the results of a number of researchers, exposure to magnetic fields causes inhibition of microorganism growth and reproduction, including that of yeast cultures [1, 2]. On application of a magnetic field of 50 tesla (T), with a frequency of between 5–500 kHz, the number of microorganisms was reduced by at least 2 cycles [3, 4]. The ability of magnetic field treatment to inactivate yeast cultures is an effective process [5, 6]. Once this process is better refined through further study and experimentation, it could be proposed that it is of potential value to the wine and food industries [7, 8].

Magnetic field treatment using non-ionizing electromagnetic effects, therefore, can be used to inhibit certain target processes in wine biotechnology.

Spontaneous fermentation of grape must is invariably accompanied by complex biochemical reactions such as the transformation of carbohydrates into ethanol, releasing carbon dioxide and other products of primary fermentation. The mechanisms of these processes are initiated by yeast enzymes produced during their reproduction in grape must.

Yeast is found, together with bacteria, on the skin of ripe grapes, and, as a result, the yeast becomes active in a short lag phase on interacting with grape must. In anaerobic conditions, yeast enzymes participate in the process of grape must transformation into ethanol, carbon dioxide and other products [9, 10]. This plays an important role in the technology of making high quality, natural wine. Wine's taste and delicate bouquet (or aroma) depends on the yeast culture that is used in the fermentation process during winemaking.

The products of primary fermentation are aromas and flavors, carbon dioxide, and released heat. The heat production during fermentation (an exothermic process) means that, during fermentation, the temperature near the fermentation vessel rises and requires cooling to an optimal temperature [11, 12].

In nature, there are varied wild yeast species that are able to participate in the process of grape must fermentation. Our task, therefore, was to determine enzyme activity, fluctuation of macro- and micro-elements and some other parameters of cellular metabolism

in the isolated yeast species. In comparison with wild yeast cultures, pure yeast cultures make biotechnological processes more controllable and, for this reason, pure yeast cultures are preferable for use within the wine industry. Pure yeast cultures resistant to ethanol, acid and potassium bisulfite provide qualities that aid in the fermentation of grape must even in unusual, extreme conditions [13, 14].

Specific differences in yeast cultures play an important role in the production of high quality wine. The grape must possesses yeast strains with high zymotic activity [15, 16].

The aim of this research was to study the effect of magnetic field treatment on the yeast culture *Saccharomyces cerevisiae*. For this purpose, the activity of selected enzymes, concentration of macro- and micro-elements and some other parameters of yeast metabolism were determined.

Sugar degradation includes three distinct pathways: alcoholic fermentation under anaerobic conditions, glyceropyruvic fermentation, and respiration under aerobic conditions [17, 18].

Glucose-6-phosphate dehydrogenase (G6PD) plays an important role in the mechanism of fermentation. G6PD is a cytosolic enzyme in the pentose phosphate alcohol dehydrogenase pathway. During wine-making, 8% of glucose follows this pathway. The fermentation is glyceropyruvic and is important at the beginning of the alcoholic fermentation of grape must, when the concentration of alcohol dehydrogenase is low.

Cocarboxylase, thiamine pyrophosphate (TPP), is an enzyme for the decarboxylation of pyruvic acid to carbon dioxide and acetaldehyde. Cocarboxylase was found to be the only growth-promoting factor. It increases the rate of alcoholic fermentation. TPP plays an important role in yeast metabolism, wherein it is a coenzyme for α-ketoacid dehydrogenase [19].

Lactate dehydrogenase plays an important role in lactic acid fermentation, which converts carbohydrates such as glucose, fructose and sucrose into cellular energy and the metabolic byproduct, lactate.

Phosphatase, an enzyme that removes a phosphate group from its substrate by hydrolyzing phosphoric acid monoesters into phosphate ions and a molecule with a free hydroxyl group, participates in the dephosphorylation process. Phosphate groups can activate or deactivate an enzyme. Phosphates, therefore, are integral to many signal transduction pathways. Phosphates are important in signal transduction as they regulate the proteins to which they are attached. To reverse the regulatory effect, the phosphate is removed. This occurs as a result of hydrolysis, or is mediated by protein phosphates. Protein phosphorylation plays a crucial role in biological functions and controls nearly every cellular process, including metabolism, gene transcription and translation, cell-cycle progression, cytoskeletal rearrangement, protein-protein interactions, and protein stability.

Aminotransferases catalyze the reversible reaction, and there are multiple transaminase enzymes which vary in substrate specificity. Some show a preference for particular amino acids or classes of amino acids as amino group donors and/or for particular α-keto acid acceptors. Aspartate donates its amino group, becoming the α-keto acid oxaloacetate. Similarly, alanine becomes pyruvate as the amino group is transferred to α-ketoglutarate. The single (cytosolic) aspartate aminotransferase was purified in high yield from yeast *Saccharomyces cerevisiae*, which confirms that this enzyme exists in yeast and plays an important role.

Magnetic field treatment for yeast cultures is common, because it can be applied to sterilization and the inhibition of growth of yeast cultures [20]. Additionally, magnetic field treatment activates some yeast enzymes.

Materials and methods

Samples from different industrial grape species were collected during the vintage season from the vineyard of the winery (the Koblevo Company, located in the Nikolaev region, Ukraine). The total number of species selected for the research was fourteen. The following industrial species of grapes were selected for the research: Chardonnay, Cabernet Sauvignon, Merlot, Sauvignon, Riesling Rhenish, Aligote, Rkatsiteli, Bastardo, Traminer, Irshai Oliver, Muscat Ottonel, Hamburg`s Muscat, Fetyaska, and Isabella.

The grape species were cultivated on the soils in the district located between the Black Sea and the Tiligul estuary.

Yeast strains:

I. Laboratory yeast cultures isolated from grape must of the «Koblevo» winery:

– Y-3362; *MAFF-230073. *Saccharomyces cerevisiae* isolated from grape must of the cultivar Aligote;

– Y-3366; MAFF-230075. *Saccharomyces cerevisiae* isolated from grape must of the cultivar Rkatsiteli;

– Y-3367; MAFF-230076. *Saccharomyces cerevisiae* isolated from grape must of the cultivar Sauvignon;

– Y-3394; MAFF-230086. *Saccharomyces cerevisiae* isolated from grape must of the cultivar Sucholimansky white;

– Y-3368; MAFF-230077. *Saccharomyces cerevisiae* isolated from grape must of the cultivar Chardonnay;

– Y-3442; MAFF-230104. *Saccharomyces cerevisiae* isolated from grape must of the cultivar Odessa Muscat.

The designated yeast culture numbers were derived from those deposited in the MAFF Collection,* National Institute of Agrobiological Sciences, Genbank of Japan.

II. Commercial (for wine industry use) *Saccharomyces cerevisiae* yeast cultures:

– Y-3436 *Saccharomyces cerevisiae* Lalvin ICV D-47;

– Y-3437 *Saccharomyces cerevisiae* Lalvin ICV D-254;

– Y-3438 *Saccharomyces cerevisiae* Lalvin QA-23;

– Y-3439 *Saccharomyces cerevisiae* Uvaferm CS-2;

– Y-3440 *Saccharomyces cerevisiae* Anchor Vin-13;

– Y-3441 *Saccharomyces cerevisiae* Anchor Vin-2000.

Note: *MAFF — Collection of microorganisms, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan.

Grape must derived from different grape species was placed into sterile glass flasks to half of the 250ml 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, identified yeast culture was deposited in the NRRL Culture Collection (Nord Regional Research Laboratory, Peoria, USA) and in the British National Collection of Yeast Culture (NCYC), Norwich, UK. The following methods were used for yeast isolate identification: 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. Amplification of yeast cultures was carried out in cooperation with the Laboratory of Pharmacogenomics, Research Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russian Federation.

Following PCR identification of yeast species as *Saccharomyces cerevisiae*, their morphological, physiological and biochemical properties were determined.

Each yeast culture was tested for 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 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 (production, gassing) was studied in addition. Magnetic field treatment for the yeast, *Saccharomyces cerevisiae*, was introduced with a magnetic field frequency of 160 Hz, an intensity of 5 Milli-Tesla and an exposure time of 30 minutes, with streaming conditions. This basic power rating of magnetic induction makes magnetic field treatment efficient for yeast research. For research purposes, the magnetic field device «Alimp-1» was utilised. The total number of magnetic field treatments conducted was 10, with an interval of 48 hours between each treatment. Total magnetic field exposure time was 30 minutes for a single procedure. Before and after magnetic field treatment, yeast suspension parameters of nitrogen, protein, carbohydrate, lipid metabolism, enzymatic activity and total sugar (which included glucose, fructose, and sucrose) were determined. Macro- and micro-element concentrations in fermented grape must, which contained pure yeast culture, was determined prior to and after magnetic field treatment. These included: potassium (ion-selective method), sodium (ion-selective method), calcium (color change of methylthymol blue in alkaline media method), phosphorus (formation of phosphomolibdate complex in acidic media method), magnesium (calmagite in alkaline media forms a stable chromogen), iron (ferrozine method), chlorides (mercury thiocyanate in acid media react with trivalent ferric ions and form a red complex) and some parameters of yeast cellular metabolism, such

as: protein (total) (biuret method), glucose (glucose oxidase/peroxidase), triglycerides (glycerol phosphate oxidase / peroxidase method), nitrogen of urea (urease/glutamate dehydrogenase).

All biochemical parameters were tested in the fermenting grape must on the 5th day.

The following enzyme activity was determined: glucose-6-phosphate dehydrogenase (the method of determination was measurement of the amount of reduced NADPH formed during oxidation of glucose-6-phosphate into phosphor gluconolactone), alcohol dehydrogenase (Ethanol + b-NAD $\xrightarrow[\text{Dehydrogenase}]{\text{Alcohol}}$ Acetaldehyde + b-NADH), cocarboxylase (splitting the pyruvic acid to acetaldehyde and CO_2 method), lactate dehydrogenase (recovery of pyruvate by NADH, thus forming lactate and NAD+), phosphatase (conversion of phosphate groups with 4-nitrophenyl phosphate in 2-amino-2-methyl-1-propanol), amylase (catalytic concentration was determined by the formation of 4-nitrophenol), and, in addition, some aminotransferases by alanine and aspartate amino acids were determined (catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm by means of the malate dehydrogenase coupled reaction).

The parameters listed above were based on the principle of spectrophotometric analysis and determined using the Cobas biochemical analyzer, produced by Hoffman La Roche Company, Switzerland. All tests were conducted using specific test kits for each studied parameter. The kits for the enzyme activity, and macro- and micro-element concentration testing were made by the BioSystems Company S.A. (Costa Brava, Spain). The Glucose-6-phosphate Dehydrogenase kit was made by the Sentinel Company, Italy. The Chloride testing kit was made by Pliva Lachema Diagnostika (Brno, Czech Republic). Electrolyte concentrations of sodium and potassium were tested by an ion-selective electrode measurement analyzer, produced by the Instrumentation Laboratory Company (Bedford, MA, USA).

The volume fraction of alcohol and pH were determined by the spectroscopy method using the Bacchus-II spectrometer (Microdom Company, France).

Statistical deviation and significance were evaluated using Student`s t-test with P — value: $P < 0.1$; $P < 0.05$; $P < 0.01$. We calculated Spearman's rank correlation coefficient for the tested biochemical parameters between tested groups of yeast prior to, and following, magnetic field treatment. Each biochemical test was repeated on three occasions to confirm the result. For the groups prior to and following magnetic field treatment, the dispersion analysis (ANOVA), based on Fisher-Snedecor test (unifactorial model), was used, wherein F-distribution arises in frequency as the null distribution of a test statistic, most notably in the analysis of variance.

Results and discussion

Yeast cultures isolated from various cultivars of grape must were identified by PCR analysis, e.g. Fig.1. For commercial (industrial yeast cultures) it was not necessary to determine species, as these cultures were identified previously.

24 hours following magnetic field treatment, the morphology of the yeast cultures (stained by Gram method) was analyzed. Cultures were examined microscopically, under immersion, at a magnification of ×900. The results confirmed that, in each yeast culture, dead yeast existed at a level of between 20% and 90% of the total number, and the same result applied to both laboratory and commercial samples. The percentage of dead yeast depended on the type of culture and the results are as follows: Y-3366 — 70%, Y-3394 — 60%, Y-3437 — 20%, Y-3440 — 50%. Fig. 2. — Morphology of *Saccharomyces cerevisiae* isolated from grape cultivar «Sauvignon» Y-3367, 24 hours following magnetic field treatment. 10% of the yeast cells, stained violet, were alive and active. 90% of the cells, unstained, represented dead cells, resembling empty honeycombs. Fig. 3. — Morphology of *Saccharomyces cerevisiae* isolated from grape cultivar «Chardonnay» Y-3368. 80% of the cells, stained violet, were alive and active. 20% of the cells that remained unstained represented dead cells, again resembling empty honeycombs. Fig. 4. —Morphology of yeast culture *Saccharomyces cerevisiae* isolated from grape cultivar «Sucholimansky white» Y-3394. All yeast cells were stained violet in color, and were large, and round or oval shaped. Fig. 5 — Morphology of commercial (industrial) yeast culture *Saccharomyces cerevisiae* «Vin-13» produced by the Anchor Company, Y-3440. All yeast cells stained violet in color; cells were large and oval shaped.

The yeast cultures isolated from Sauvignon and Chardonnay grape varieties were identified by PCR analysis as *Saccharomyces cerevisiae*. These clearly demonstrated specific bands corresponding to *Saccharomyces cerevisiae*.

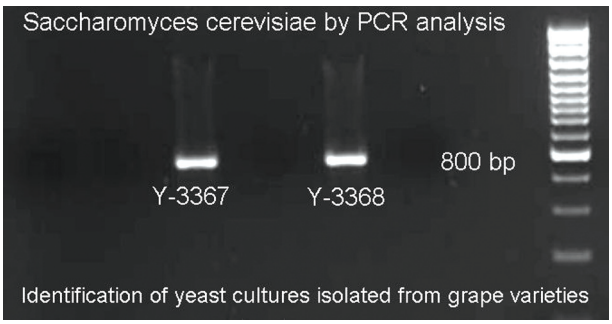

Fig. 1. **Electrophoregram for identification of yeast cultures by PCR analysis using universal yeast primers**

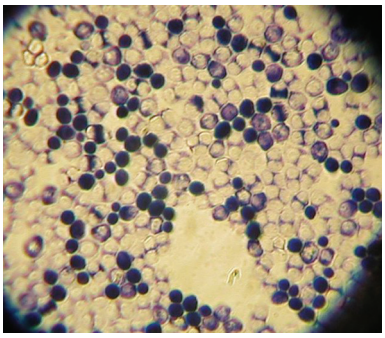

Fig. 2. **Morphology of yeast culture *Saccharomyces cerevisiae* isolated from grape cultivar «Sauvignon» Y-3367**

Yeast cells of *Saccharomyces cerevisiae*, 24 hours following magnetic field treatment.

Stained by Gram method. Magnification — ×900.

Yeast cells that stained violet in color represent live and active cells (10% of the overall number of yeast cells). Cells that are not stained are dead (90% overall) and resemble empty honeycombs.

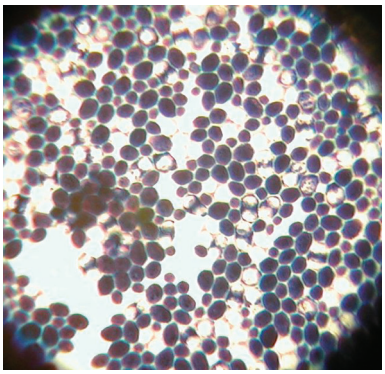

Fig. 3. **Morphology of yeast culture *Saccharomyces cerevisiae* isolated from grape cultivar «Chardonnay» Y-3368**

Yeast cells of *Saccharomyces cerevisiae* 24 hours following magnetic field treatment.

Stained by Gram method. Magnification — ×900.

Yeast cells that stained violet in color represent live and active cells (80% overall). Cells that are not stained are dead (20% overall) and resemble empty honeycombs.

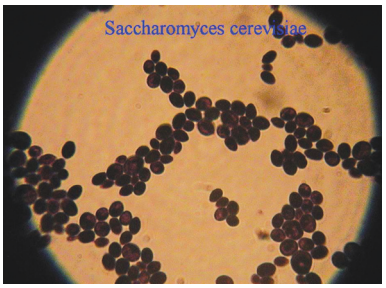

Fig. 4. **Morphology of yeast culture *Saccharomyces cerevisiae* isolated from grape cultivar «Sucholimansky white» Y-3394**

Yeast cells of *Saccharomyces cerevisiae*, after cultivation for 24 hours without magnetic field treatment.

Stained by Gram method. Magnification — ×900.

All yeast cells are stained violet in color. Cells are large and either round or oval shaped.

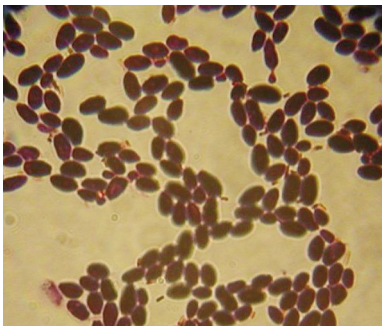

Fig. 5. **Morphology of commercial yeast culture *Saccharomyces cerevisiae* «Vin-13» produced by the Anchor Company, Y-3440**

Yeast cells of commercial (industrial) *Saccharomyces cerevisiae*, after cultivation for 24 hours without magnetic field treatment.

Stained by Gram method. Magnification — ×900.

All cells are stained violet in color. Cells are large and oval-shaped.

The impact of magnetic field treatment impact on *Saccharomyces cerevisiae* yeast cultures manifested in enzyme activity, the concentration of macro- and micro-elements, and some parameters of cellular metabolism including nitrogen, protein, carbohydrate, and lipid metabolism of yeast cells. This was studied using dispersion analysis (ANOVA) as illustrated in tables 5 and 6.

The morphology of yeast cultures, both without magnetic field exposure and 24 hours following magnetic field treatment, are shown in Fig. 2–5. Both groups of yeast cultures *Saccharomyces cerevisiae*, including wild yeast cultures isolated from different grape must cultivars after spontaneous fermentation and commercial (industrial) yeast cultures, were tested for the following biochemical parameters: protein (total), glucose,

Table 1. **Enzymatic activity of yeast cultures *Saccharomyces cerevisiae* isolated from vineyards of the «Koblevo» winery**

Enzyme activity (μmol/min ×10^{-2} L)	Grape must fermentation on the 5th day without magnetic field treatment (control)						Grape must fermentation on the 5th day following magnetic field treatment					
USRCB collection number	Y-3362	Y-3366	Y-3367	Y-3394	Y-3368	Y-3442	Y-3362	Y-3366	Y-3367	Y-3394	Y-3368	Y-3442
Alanine aminotransferase	37.6± 2.4	46.4± 1.2	24.9± 0.6	29.9± 0.6	49.8± 0.6	68.0± 0.3	39.7± 0.2	41.6± 0.4	26.6± 0.4	32.8± 0.4	37.2± 0.2	51.3± 0.6
Alcohol dehydrogenase	46.2± 0.4	28.9± 0.5	32.7± 0.3	24.8± 0.5	18.4± 0.4	35.4± 0.3	64.8± 0.2	59.2± 0.2	63.1± 0.6	57.4± 0.4	62.7± 0.6	83.9± 0.2
Aspartate aminotransferase	24.9± 0.5	*13.2 ± 0.1	39.8± 0.2	3.3± 0.3	14.9± 0.5	74.7± 0.4	365.2 ± 8.7	129.4 ± 1.7	202.5 ± 1.9	439.9 ± 2.5	207.5± 1.9	126.6 ± 0.4
Amylase	3.9± 0.3	13.6± 0.5	6.06± 0.2	7.4± 0.3	6.8± 0.3	7.3± 0.2	226.2 ± 0.9	99.6± 0.6	16.6± 0.9	116.2 ± 0.7	135.2± 1.2	16.6± 0.4
Glucose-6-phosphate dehydrogenase	20.4± 0.4	26.7± 0.3	28.2± 0.2	22.5± 0.2	*24.6± 0.1	27.8± 0.3	14.7± 0.2	12.6± 0.3	16.8± 0.2	*15.2± 0.1	*10.8± 0.1	14.7± 0.2
Cocarboxylase	47.4± 1.0	38.2± 0.2	42.7± 0.2	35.1± 0.2	27.2± 0.4	39.6± 0.4	65.2± 0.7	74.8± 1.1	68.5± 1.0	77.3± 0.9	64.6± 0.8	72.3± 0.4
Lactate dehydrogenase	28.2± 0.2	21.5± 0.2	11.6± 0.3	14.9± 0.3	38.1± 0.2	23.2± 0.2	126.6 ± 0.7	124.5 ± 0.3	89.6± 0.6	48.1± 0.9	117.8± 0.6	44.9± 0.7
Phosphatase	71.3± 0.4	83.0± 0.2	96.2± 0.4	78.0± 0.3	66.4± 0.3	72.9± 0.5	285.5 ± 1.1	197.5 ± 0.5	123.6 ± 0.6	132.8 ± 1.2	122.8± 1.0	106.2 ± 0.4

Note: standard deviation was calculated; statistical significance of difference was evaluated using Student's t-test; *P*-value * $P \leq 0.1$.

Table 2. **Concentration of macro- and micro-elements in yeast cultures *Saccharomyces cerevisiae* isolated from vineyards of the «Koblevo» winery**

Macro- and microelements (mmol/L)	Grape must fermentation on the 5th day without magnetic field treatment (control)						Grape must fermentation on the 5th day following magnetic field treatment					
USRCB collection number	Y-3362	Y-3366	Y-3367	Y-3394	Y-3368	Y-3442	Y-3362	Y-3366	Y-3367	Y-3394	Y-3368	Y-3442
Potassium (mmol/L)	35,57 ±0,3	*35,61 ±0,1	*34,03 ±0,1	33,56 ±0,2	34,83 ±0,4	*34,53 ±0,1	46,66 ±0,2	44,24 ±0,2	*38,47 ±0,1	35,1± 0,2	41,2± 0,2	36,16 ±0,2
Sodium (mmol/L)	*28,9 ±0,1	*16,0 ±0,1	20,2± 0,3	22,6± 0,4	27,8± 0,6	21,7± 0,2	*30,0 ±0,1	*16,5 ±0,1	*19,4 ±0,1	*17,8 ±0,1	*16,6 ±0,1	*22,0 ±0,1
Calcium (mmol/L)	5,44± 0,08	**4,32 ±0,02	4,85± 0,08	**4,68 ±0,05	*5,19 ±0,1	*3,09 ±0,1	*6,56 ±0,1	8,28± 0,08	*5,89 ±0,1	**4,03 ±0,02	**7,91 ±0,02	*5,94 ±0,1
Phosphorus (mmol/L)	**1,7 ±0,05	1,40,± 0,06	***0,85 ±0.01	1,49± 0,07	1,99± 0,08	*3,12 ±0,1	**0,61 ±0,03	*1,2± 0,1	0,7±0, 06	2,4±0, 2	1,32± 0,02	*3,2± 0,1
Magnesium (mmol/L)	1,48± 0,06	***1,4 2±0,01	*1,58± 0,1	***1,5 1±0,01	1,56± 0,09	1,40± 0,07	**3,94 ±0,05	2,73± 0,08	**3,93 ±0,05	*1,1± 0,1	**3,22 ±0,03	**1,38 ±0,05
Iron (μmol/L)	1,0±0, 08	1,0±0, 09	2,0±0, 08	1,0±0, 04	*3,0± 0,1	*8,0± 0,1	27,0± 0,6	14,0± 0,8	16,0± 0,3	12,0± 0,3	29,0± 0,7	9,0±0, 4
Chlorides (mmol/L)	*16,8 ±0,1	14,1± 0,2	*16,3 ±0,1	12,8± 0,3	14,7± 0,6	14,5± 0,6	35,7± 0,4	27,6± 0,4	93,8± 0,5	10,2± 0,2	46,7± 0,7	5,5±0, 2
Protein (total) (g/L)	3.9± 0.3	3.0± 0.2	3.9± 0.3	2.4± 0.2	*2.6± 0.1	*2.1± 0.1	*12.1 ± 0.1	14.7± 0. 2	11.8± 0.2	*9.4± 0.1	12.6± 0.2	*10.2 ± 0.1
Glucose (mmol/L)	*6.24 ±0.1	*6.91 ± 0.1	**5.73 ±0.02	6.64± 0.08	5.28± 0.07	**6.36 ±0.04	**2.68 ±0.04	**2.25 ±0.04	*3.20 ±0.1	**2.86 ±0.05	**2.32 ±0.03	**3.47 ±0.03
Triglycerides (mmol/L)	4.39± 0.06	5.65± 0.08	*5.37 ±0.1	**5.22 ±0.02	4.67± 0.08	4.75± 0.06	4.05± 0.09	4.14± 0.07	4.41± 0.07	2.39± 0.06	**3.36 ±0.03	**0.27 ±0.02
Nitrogen of Urea (mmol/L)	**0.16 ±0.02	***0.21 ±0.01	***0.18 ±0.01	***0.05 ±0.01	**0.26 ± 0.02	**0.79 ± 0.05	**0.71 ± 0.02	***0.05 ±0.01	**0.65 ± 0.02	***0.02 ±0.006	**1.06 ±0.04	***0.13 ±0.01

Standard deviation was calculated; statistical significance of difference was evaluated using Student's t-test.
Note: P-value * $P \leq 0.1$; ** $P \leq 0.05$; *** $P \leq 0.01$.

Table 3. **Enzymatic activity for commercial (industrial) yeast cultures *Saccharomyces cerevisiae***

Enzymatic activity (μmol/min×10^{-2} L)	**Grape must fermentation on the 5th day without magnetic field treatment (control)**						**Grape must fermentation on the 5th day following magnetic field treatment**					
USRCB collection number	Y-3436	Y-3437	Y-3438	Y-3439	Y-3440	Y-3441	Y-3436	Y-3437	Y-3438	Y-3439	Y-3440	Y-3441
Designation of yeast	D-47	D-254	QA-23	CS-2	Vin-13	Vin-2000	D-47	D-254	QA-23	CS-2	Vin-13	Vin-2000
Alanine aminotransferase	32.3± 0.8	33.8± 0.6	29.4± 0.5	24.9± 0.5	35.6± 0.4	43.1± 0.3	27.4± 0.3	32.7± 0.4	38.2± 0.5	27.9± 1.2	42.8± 1.0	24.5± 0.4
Alcohol dehydrogenase	27.4± 0.4	28.9± 0.2	32.7± 0.3	35.4± 0.3	26.5± 0.4	24.8± 0.6	57.2± 0.4	62.7± 1.1	63.1± 0.4	58.9± 0.7	64.8± 1.3	72.5± 2.0
Aspartate aminotransferase	24.6± 0.4	33.4± 0.3	31.5± 0.5	28.6± 0.4	26.3± 0.4	37.9± 0.3	285.2 ± 0.7	248.4 ± 0.9	239.8 ± 3.0	275.3 ± 1.0	207.5 ± 1.9	248.6 ± 2.5
Amylase	52.4± 0.3	43.9± 0.6	73.7± 0.7	50.5± 0.3	82.5± 0.4	60.3± 0.4	265.2 ± 0.7	99.6± 1.2	127.6 ± 0.6	116.2 ± 1.3	135.2 ± 2.2	198.6 ± 3.0
Glucose-6-phosphate dehydrogenase	23.1± 0.7	25.4± 0.4	27.3± 0.3	28.2± 0.4	22.5± 0.4	24.6± 0.6	16.8± 0.4	14.6± 0.4	13.7± 0.5	19.4± 0.4	12.3± 0.3	15.9± 0.6
Cocarboxylase	36.4± 0.6	28.7± 0.5	34.6± 0.4	26.8± 0.5	32.1± 0.2	35.9 ± 0.6	72.3 ± 0.4	68.5± 0.8	77.4± 0.8	65.2± 0.8	75.8± 1.3	73.2± 0.5
Lactate dehydrogenase	14.8± 0.5	23.7± 0.4	19.2± 0.2	23.8± 0.5	25.4± 0.3	26.9± 0.2	86.3± 0.4	127.4 ± 0.8	95.7± 0.4	128.6 ± 1.1	117.3 ± 1.4	123,2 ± 1.2

Standard deviation was calculated; statistical significance of difference was evaluated using Student's t-test.
Note: P-value * $P \le 0.1$; ** $P \le 0.05$; *** $P \le 0.01$.

Table 4. **Concentration of macro- and micro-elements in commercial (industrial) yeast *Saccharomyces cerevisiae***

Macro- and microelements	**Grape must fermentation on the 5th day without magnetic field treatment (control)**						**Grape must fermentation on the 5th day following magnetic field treatment**					
USRCB collection number	Y-3362	Y-3366	Y-3367	Y-3394	Y-3368	Y-3442	Y-3362	Y-3366	Y-3367	Y-3394	Y-3368	Y-3442
Potassium (mmol/L)	*34.01 ± 0.1	36.93 ± 0.3	35.2± 0.7	*33.94 ± 0.1	37.8± 0.2	36.97 ± 0.2	40.2± 0.3	*40.85 ± 0.1	39.17 ± 0.2	*41.26 ± 0.1	39.21 ± 0.2	39.27 ± 0.3
Sodium (mmol/L)	11.3± 0.2	23.6± 0.6	20.5± 0.4	16.9± 0.6	22.8± 0.6	22.6± 0.4	27.5± 0.3	28.3± 0.4	22.9± 0.7	25.5± 0.7	28.7± 0.6	29.2± 0.5
Calcium (mmol/L)	2.77± 0.07	*2.95 ± 0.1	2.92± 0.09	2.94± 0.04	*5.08 ± 0.1	*3.12 ± 0.1	3.07± 0.06	*2.21 ± 0.1	**2.63 ± 0.04	2.41± 0.06	3.0± 0.3	*3.36 ± 0.1
Phosphorus (mmol/L)	3.7± 0.2	*1.3± 0.1	*4.74 ± 0.1	*3.3± 0.1	**2.09 ± 0.03	2.01± 0.06	2.46± 0.09	1.68± 0.07	2.32± 0.07	**2.27 ± 0.02	*2.82 ± 0.1	**2.04 ±0.04
Magnesium (mmol/L)	**1.32 ± 0.03	*1.2± 0.1	1.07± 0.09	**1.03 ± 0.03	**1.81 ± 0.04	**1.06 ± 0.02	**1.03 ± 0.03	***0.94 ±0.01	**0.98 ± 0.02	**0.87 ± 0.02	**0.99 ± 0.02	***0.86 ±0.01
Iron (μmol/L)	2.0± 0.06	2.6± 0.3	*1.2± 0.1	5.0± 0.2	6.4± 0.2	1.8± 0.2	*1.4± 0.1	1.9± 0.08	1.5± 0.2	1.7± 0.2	**1.4 ± 0.05	1.6± 0.2
Chlorides (mmol/L)	*11.6 ± 0.2	24.4± 0.3	17.1± 0.3	12.1± 0.3	16.2± 0.2	14.7± 0.4	*11.6 ± 0.1	9.3± 0.2	6.7± 0.2	*6.5± 0.1	10.0± 0.3	6.4± 0.2
Protein (total) (g/L)	*1.5± 0.1	1.6± 0.2	*1.4± 0.1	*1.2± 0.1	*1.4± 0.1	*1.5± 0.1	2.7± 0.2	*3.4± 0.1	*2.6± 0.1	3.8± 0.2	*4.5± 0.1	2.9± 0.3
Glucose (mmol/L)	5.1± 0.02	5.05± 0.09	*5.12 ± 0.1	*4.85 ± 0.1	*5.82 ± 0.1	**4.93 ± 0.04	2.75± 0.1	*3.29 ± 0.1	*3.84 ± 0.1	2.27± 0.09	2.35± 0.08	2.41± 0.06
Triglycerides (mmol/L)	*5.1± 0.1	5.05± 0.08	**5.12 ± 0.02	4.65± 0.08	5.80± 0.2	4.93± 0.07	4.05± 0.09	**4.14 ±0.02	4.41± 0.07	*2.34 ± 0.1	*3.36 ± 0.1	3.27± 0.04
Nitrogen of Urea (mmol/L)	***0.16 ±0.01	**0.57 ±0.02	0.18± 0.5	**0.44 ± 0.02	**0.52 ± 0.02	***0.48 ±0.01	***0.71 ±0.01	***0.12 ±0.01	**0.65 ±0.03	0.02± 0.003	1.06± 0.5	**0.13 ±0.02

Standard deviation was calculated; statistical significance of difference was evaluated using Student's t-test.
Note: P-value * $P \le 0.1$; ** $P \le 0.05$; *** $P \le 0.01$.

Table 5. **Dispersion analysis (ANOVA) represents the influence of isolated laboratory yeast cultures with and without magnetic field treatment and their action upon enzyme activity, concentration of macro- and micro-elements and some other parameters of cellular metabolism**

Parameter	Standard deviation		F	P	r
Alanine aminotransferase, μmol/(min×10^{-2} L)	M_1=15.56	M_2=8.36	0.4	*0.54	0.92
Alcohol dehydrogenase, μmol/(min×10^{-2} L)	M_1=9.53	M_2=9.56	38.28	0.0001	0.36
Amylase, μmol/(min×10^{-2} L)	M_1=3.24	M_2=79.14	8.49	0.015	-0,26
Aspartate aminotransferase, μmol/(min×10^{-2} L)	M_1=25.80	M_2=128.86	16.32	0.002	-0.54
Glucose-6-phosphate dehydrogenase, μmol/(min×10^{-2} L)	M_1=3.11	M_2=2.11	50.28	<0.0001	0.10
Cocarboxylase, μmol/(min×10^{-2} L)	M_1=6.88	M_2=5.19	82.98	<0.0001	-0.06
Lactate dehydrogenase, μmol/(min×10^{-2} L)	M_1=9.51	M_2=37.60	18.99	0.001	0.49
Phosphatase, μmol/(min×10^{-2} L)	M_1=10.59	M_2=68.53	8.68	0.014	-0.16
Potassium (mmol/L)	M_1=0.82	M_2=4.56	8.79	0.014	0.91
Sodium (mmol/L)	M_1=4.82	M_2=5.14	0.74	*0.40	0.54
Calcium (mmol/L)	M_1=0.83	M_2=1.54	6.59	0.028	0.13
Phosphorus (mmol/L)	M_1=0.76	M_2=1.02	0.13	*0.72	0.74
Magnesium (mmol/L)	M_1=0,07	M_2=1.23	5.89	0.035	0.48
Iron (μmol/L)	M_1=2.73	M_2=8.23	18.35	0.001	-0.36
Chlorides (mmol/L)	M_1=1.47	M_2=31.99	2.76	*0.127	-0.005
Protein (total) (g/L)	M_1=0.76	M_2=1.86	114.27	<0.0001	0.39
Glucose (mmol/L)	M_1=0.59	M_2=0.48	117.26	<0.0001	-0.83
Triglycerides (mmol/L)	M_1=0.47	M_2=1.56	8.11	0.01	0.27
Nitrogen of Urea (mmol/L)	M_1=0.26	M_2=0.43	0.62	*0.44	-0.17

Note: M_1 = values for yeast cultures without magnetic field treatment;
M_2 = values for yeast cultures following magnetic field treatment;
r — Spearman`s rank correlation coefficient;
* $P > 0.05$ — this means that, on the indicated parameter, differences between the investigated groups existed.

triglycerides, nitrogen of urea, potassium, sodium, calcium, phosphorus, magnesium, iron, and chlorides. As a result of the biochemical evaluation of grape must samples during active fermentation, it was determined that magnetic field treatment of yeast cultures *Saccharomyces cerevisiae* inhibited enzyme activity and halted reproduction. Those yeast cultures remaining alive following magnetic field treatment in Agar in a Petri dish, however, were activated. The results for yeast cultures, prior to and following magnetic field treatment, cultivated in Wort Agar in a Petri dish are shown in Fig. 2–5.

Increasing enzyme activity in both groups of yeast cultures that remained alive was noted post magnetic field treatment. Yeast cultures were inoculated in grape must at a concentration of 5×10^9 cells/ml, and were tested during cultivation on the 5th day, wherein the activity of enzymes increased in alcohol dehydrogenase by 200%, cocarboxylase activity increased by 200%, lactate dehydrogenase activity increased by 300%, phosphatase enzyme activity increased by 200%, aspartate aminotransferase enzyme activity increased by 1000%, and Amylase activity increased by 250%. Contrary to this, magnetic field treatment, comparing the groups without and following magnetic field treatment, decreased the activity of glucose-6-phosphate dehydrogenase by 30%, and decreased Alanine aminotransferase enzyme activity by almost the same amount.

On examining the concentration of macro- and micro-elements between the aforementioned groups (prior to and following magnetic field treatment) in commercial yeast cultures (Table 2), the following was observed: Potassium concentration increased by 15%, sodium concentration increased by 10%, calcium concentration didn't change, phosphorus concentration decreased by 25%, magnesium concentration decreased by 20%, concentration of iron decreased by 40%, and chloride concentration decreased by 50%. Results of enzyme activity for the laboratory isolated yeast cultures without, and following, magnetic field treatment are illustrated in Table 3. Enzyme activity for aspartate aminotransferase enzyme activity increased by 800%, alcohol dehydrogenase activity increased by

Table 6. **Dispersion analysis (ANOVA) represents the influence of commercial (industrial) yeast cultures with and without magnetic field treatment and their action upon enzyme activity, concentration of macro- and microelements and some other parameters of cellular metabolism**

Parameter	Standard deviation		F	P	R
Alanine aminotransferase, μmol/(min×10^{-2} L)	M_1= 6.13	M_2=7.06	0.06	*0.81	-0.15
Alcohol dehydrogenase, μmol/(min×10^{-2} L)	M_1=4.01	M_2=5.36	153.77	<0.0001	-0.56
Amylase, μmol/(min×10^{-2} L)	M_1=14.82	M_2=62.82	13.41	0.004	-0.08
Aspartate aminotransferase, μmol/(min×10^{-2} L)	M_1=4.90	M_2=27.53	372.74	<0.0001	-0.15
Glucose-6-phosphate dehydrogenase, μmol/(min×10^{-2} L)	M_1=2.25	M_2=2.50	50	<0.0001	0.47
Cocarboxylase, μmol/(min×10^{-2} L)	M_1=3.95	M_2=4.55	259.4	<0.0001	0.74
Lactate dehydrogenase, μmol/(min ×10^{-2} L)	M_1=4.49	M_2=17.80	146.63	<0.0001	0.89
Phosphatase, μmol/(min×10^{-2} L)	M_1=4.36	M_2=11.66	149.16	<0.0001	-0.44
Potassium (mmol/L)	M_1=1.65	M_2=0.91	29.41	0.0002	-0.48
Sodium (mmol/L)	M_1=4.73	M_2=2.39	11.65	0.006	0.23
Calcium (mmol/L)	M_1=0.88	M_2=0.43	1.66	*0.22	0.27
Phosphorus (mmol/L)	M_1= 1.27	M_2=0.38	1.18	*0.30	0.38
Magnesium (mmol/L)	M_1= 0.29	M_2=0.06	5.98	0.03	0.57
Iron (μmol/L)	M_1=2.06	M_2= 0.19	3.51	*0.09	-0.10
Chlorides (mmol/L)	M_1= 4.64	M_2=2.19	13.11	0.004	0.03
Protein (total) (g/L)	M_1=0.13	M_2=0.73	37.98	0.0001	-0.36
Glucose (mmol/L)	M_1=0.34	M_2=0.62	63.48	<0.0001	-0.12
Triglycerides (mmol/L)	M_1=0.38	M_2=0.76	18.94	0.001	0.30
Nitrogen of Urea (mmol/L)	M_1=0.17	M_2=0.41	0.1	*0.75	-0.35

Note: M_1 = values for yeast cultures without magnetic field treatment;
M_2 = values for yeast cultures following magnetic field treatment;
r — Spearman`s rank correlation coefficient;
* $P > 0.05$ — this means that, on the indicated parameter, there are differences between the investigated groups.

200%, cocarboxylase activity increased by 170%, lactate dehydrogenase activity increased by 850%, phosphatase enzyme activity increased by 200%, amylase activity increased by 300%, and alanine aminotransferase activity remained the same between groups.

The following enzyme activity was tested: glucose-6-phosphate dehydrogenase, alcohol dehydrogenase, lactate dehydrogenase, phosphatase, amylase, transferase to amino acids such as alanine, and, in addition, aspartic acid activity was determined.

The data illustrated in Fig. 2-3 indicated that, following magnetic field treatment, yeast cultures were being inhibited, and some yeast cells were dying (at a death rate of between 20% and 70%). After inoculation into grape must, however, yeast cultures remained alive following magnetic field treatment. The activation level depended on the frequency and intensity of the magnetic field [21, 22], see Tables 1 and 3. The enzyme activity between groups with and without magnetic field treatment shown for the group of yeast cultures *Saccharomyces cerevisiae* isolated from the vineyards of the Koblevo winery showed that those without magnetic field treatment had a high correlation between glucose-6-phosphate dehydrogenase and cocarboxylase (r = 0.94, P = 0.1). The biological significance of such a high correlation between these enzymes is natural, because glucose-6-phosphate dehydrogenase is a key enzyme in the pentose phosphate pathway. Cocarboxylase is an enzyme for decarboxylation of pyruvic acid into acetaldehyde and increases the speed of alcoholic fermentation.

The activity of enzymes for the laboratory yeast cultures *Saccharomyces cerevisiae* following magnetic field treatment showed a high correlation between alcohol dehydrogenase and alanine aminotransferase (r = 0.73, P = 0.004), and a high correlation between amylase and phosphatase (alcaline) (r = 0.79, P=0.1). Laboratory yeast cultures *Saccharomyces cerevisiae* in groups with and without magnetic field treatment indicated a high correlation of the enzyme alanine aminotransferase (r = 0.92, P = 0.5), which is a natural correlation, showing high enzyme activity in anaerobic conditions during grape must fermentation. According to the correlation analysis between the activity of enzymes, direct, weak, but consistent connec-

tions exist for alcohol dehydrogenase ($r = 0.36$, $P = 0.0001$), as this enzyme catalyses the reversible reaction formation of ethanol from acetaldehyde. Lactate dehydrogenase ($r = 0.49$, $P = 0.001$) catalyzes the transfer of electrons from L-lactate to cytochrome C.

The correlation analysis indicates negative, but statistically significant connections for aspartate aminotransferase ($r = -0.54$, $P = 0.002$). Synthesis of amino acids occurs by transamination reactions from keto acids, glutamic acid, and is catalyzed by aminotransferases. High activity of aspartate aminotransferase is noted during anaerobic fermentation with an Amylase correlation coefficient ($r = -0.26$, $P = 0.015$). Activity and synthesis of this enzyme in grapes increases at the end of July, in the period before grape maturation. An especially strongly expressed synthetic activity of amylase was noted in the shoots, wherein synthesis of starch was about 18–20 mg per 1.0g of crude material. Amylase is a calcium dependent enzyme. Cocarboxylase ($r = -0.06$, $P = 0.0001$), its coenzyme, which is required for many reactions of decarboxylation, especially for alcoholic fermentation for decarboxylation of pyruvic acid to acetaldehyde, showed a phosphatase correlation coefficient ($r = -0.16$, $P = 0.01$). Phosphatase participates in the process of dephosphorylation. Glucose easily penetrates into cells and its transport occurs by passive diffusion and active transport due to enzymes. In the cells, glucose takes part in phosphorylation with phosphatase and then participates in the process of alcoholic fermentation.

The initial stages of sugar splitting (to pyruvate) are the same for both energy generation pathways:

Oxidation of pyruvate to CO_2 and H_2O takes place through a series of intermediate products in the tricarbonic acid cycle and the respiratory chain, and recovery of ethanol and CO_2 takes place during alcoholic fermentation.

Concentration of macro- and micro-elements and some parameters of cellular metabolism for isolated yeast cultures Saccharomyes cerevisiae, with and without magnetic field treatment, highlighted a high correlation for Potassium ($r = 0.91$, $P = 0.01$). Potassium is necessary for carbohydrate metabolism, especially in the process of assimilation via ion pumps, and is responsible for pH adjustment. Potassium is needed by yeast, not only as a nutrient element, but also as a stimulator of yeast reproduction. The stimulating effect confers potassium an important role in oxidative phosphorylation and the process of glycolysis. The movement of inorganic phosphorus into cells is stimulated by potassium. Potassium activates the yeast enzyme aldolase, which is necessary for the action of the enzyme pyruvate carboxylase, and acts in the same manner as nitrogen and sulfur in yeast cell lipid metabolism. Phosphorus concentration showed a high correlation between groups with and without magnetic field treatment ($r = 0.74$, $P = 0.7$). Phosphorus is required for synthesis of ATP, creation of the cytoplasmic membrane, and support of a buffer (against pH fluctuation). Insufficient concentrations of phosphorus result in poor fermentation and cellular growth in yeast. According to the correlation analysis for concentrations of Magnesium ($r = 0.48$, $P = 0.03$) direct, weak, but consistent connections were observed for Sodium ($r = 0.54$, $P = 0.4$), for Calcium ($r = 0.13$, $P = 0.02$), for Chlorides ($r = 0.64$, $P = 0.1$), and for Protein (total) ($r = 0.39$, $P = 0.0001$). Moreover, between groups, negative, but statistically significant relationships were observed for Glucose ($r = -0.005$, $P = 0.0001$), for Iron concentration ($r = -0.36$, $P = 0.001$), and for nitrogen of urea ($r = -0.17$, $P = 0.4$).

The enzyme activity for commercial (industrial) cultures of *Saccharomyces cerevisiae* showed a high correlation between groups for cocarboxylase ($r = 0.74$, $P = 0.0001$), and lactate dehydrogenase ($r = 0.89$, $P = 0.0001$). According to the correlation analysis between activity of enzymes, direct, weak, but consistent connections exist for glucose-6-phosphate dehydrogenase ($r = 0.47$, $P = 0.0001$). At the same time, between groups, it indicated negative, but statistically significant connections for alcohol dehydrogenase ($r = -0.56$, $P = 0.0001$), for aspartate aminotransferase ($r = -0.15$, $P = 0.0001$), for phosphatase ($r = -0.44$, $P = 0.0001$), and for amylase ($r = -0.08$, $P = 0.004$).

Concentrations of macro- and micro-elements for commercial (industrial) cultures *Saccharomyces cerevisiae,* between groups with and without magnetic field treatment, do not indicate high correlations. According to the correlation analysis between concentrations of macro- and micro-elements, however, direct, weak, but consistent connections existed for sodium ($r = 0.23$, $P = 0.006$), and for calcium ($r = 0.27$, $P = 0.2$), slowing yeast cell degeneration, and promoting flocculation. Deficiency of calcium was compensated for by magnesium or manganese. The high ratio of magnesium and calcium increases initial fermentation rate, increases the amount of alcohol, and increases viability of yeast at the end of fermentation. For phosphorus, the correlation coefficient is weak

(r = 0.38, $P = 0.3$), as it is for magnesium (r = 0.57, $P = 0.03$), and for chlorides (r = 0.03, $P = 0.004$). Chloride ions participate in maintaining the osmotic balance and have an optimum radius to penetrate via cell membranes. This explains joint participation with potassium and sodium ions in the establishment of permanent osmotic pressure and regulation of the water-electrolyte metabolism. Triglycerides (r = 0.3, $P = 0.001$), in lipid metabolism, function as cell energetic protection.

Furthermore, negative, but statistically significant connections were observed for the concentration of Potassium (r = −0.48, $P = 0.0002$), and for Iron (r = −0.1, $P = 0.09$). Iron is present in cytochromes, in enzymes, cytochrome oxidase, peroxidase, catalase and other enzymes that participate in the processes involved in yeast respiration. Iron also participates in the functions of other enzymes such as: zymogenase, and pyrophosphatase. Protein (total) correlation is (r = −0.36, $P = 0.0001$), for Glucose correlation is (r = −0.12, $P = 0.0001$), and for Nitrogen of Urea concentration correlation is (r = −0.35, $P = 0.75$). Urea is necessary for yeast growth (respiration); the amino acid content required for this is 100 mg/L, and that required for fermentation is 150–200 mg/L of urea. Almost all yeasts use urea as a source of nitrogen, splitting it into CO_2 and NH_3. Ascomycete yeast cultures initially exhibit carboxylisation of urea with formation of allophanate, which is then gradually hydrolyzed, forming ammonia bound in organic compounds.

In the cell, transaminase systems remove enzymes from the amino acid molecule, NH_2, with carbon residue being directed to the oxoacid pool (keto acids). In the oxoacid pool, precursors of aldehydes and higher alcohols accumulate. This also forms and determines the final product flavor.

Spirits, even in low concentrations (of 3–4%), inhibit the budding yeast, including *Saccharomyces cerevisiae*. In continuous stream, however, yeast cultures are able to reproduce at a high concentration of spirit (of between 7–8 v/v %) and to continue the fermenting of sugar up to higher spirit concentrations (of 10–12 v/v %). Reproduction of yeast cultures depends mostly upon nutrient content, but not upon the content of spirit in nutrient media.

The ethanol concentration in yeast cultures after fermentation was:

I. Laboratory yeast cultures *Saccharomyces cerevisiae* isolated from grape must of the «Koblevo» winery after magnetic field treatment: Y-3362, pH = 3.35, volume fraction of alcohol 10.76 v/v%; Y-3366, pH = 3.36, volume fraction of alcohol 11.25 v/v%; Y-3367, pH = 3.38, volume fraction of alcohol 11.96 v/v%; Y-3394, pH = 3.31, volume fraction of alcohol 10.64 v/v%; Y-3368, pH = 3.34, volume fraction of alcohol 10.98 v/v%; Y-3442 , pH = 3.41, volume fraction of alcohol 11.40 v/v%.

II. Commercial (for wine industry) yeast cultures of *Saccharomyces cerevisiae* after magnetic field treatment: Y-3436, pH = 3.31, volume fraction of alcohol 11.34 v/v%. Y-3437, pH = 3.29, volume fraction of alcohol 11.46 v/v%. Y-3438, pH = 3.35, volume fraction of alcohol 11.54 v/v%. Y-3439, pH = 3.33, volume fraction of alcohol 11.49 v/v%. Y-3440, pH = 3.32, volume fraction of alcohol 11.33 v/v%. Y-3441, pH = 3.35, volume fraction of alcohol 11.23 v/v%.

1. Magnetic field treatment inhibits reproduction, enzyme activity and damages varying amounts of yeast cells up to, and including, death.

2. Magnetic field treatment inhibits yeast cell growth, which was confirmed morphologically by yeast cell damage and death. Differing yeast strains exhibited different percentages of dead cells.

3. It was determined that active fermentation proceeds in yeast cultures that were not treated with magnetic field treatment. In the case of laboratory yeast cultures, there was a very high correlation between the enzymes alcohol dehydrogenase and cocarboxylase (r = 0.94, $P < 0.1$).

4. Laboratory yeast cultures exposed to magnetic fields indicated a high correlation between the enzymes alanine aminotransferase and alcohol dehydrogenase (r = 0.73, $P < 0.0004$), in addition to a high correlation between the enzymes amylase and phosphatase (r = 0.79, $P < 0.1$).

5. Laboratory yeast cultures with and without magnetic field treatment indicated a very high correlation of potassium (r = 0.91, $P < 0.01$) and phosphorus (r = 0.74, $P < 0.7$).

6. Commercial (industrial) yeast cultures with and without magnetic field treatment indicated a high correlation of cocarboxylase (r = 0.74, $P < 0.0001$) and lactate dehydrogenase (r = 0.89, $P < 0.8$).

7. It was found that yeast cells that remained alive following magnetic field treatment and inoculation into grape must, had a high fermentative activity.

8. On comparing yeast culture groups with and without magnetic field treatment, there was no difference in pH level, which was around pH = 3.3 for all yeast strains.

9. Comparison of alcohol concentration on completion of fermentation between groups, in isolated laboratory yeast cultures with and without magnetic field treatment and commercial (industrial) yeast cultures, in v/v % between groups is ($r = 0.38$, $P < 0.2$).

10. The yeast cultures that demonstrated high fermentative activity and high macro- and micro-element concentrations, as well as indicating a high correlation between enzyme activity, macro- and micro-elements and some parameters of cellular metabolism will be recommended for the biotechnology of wine making.

11. Magnetic field treatment could be recommended for cases wherein it is necessary to urgently inhibit wine fermentation in bottles and in cases with excessive fermentation of sparkling wines. It could be easily utilized by exposing bottled wines to a magnetic field for 30 minutes.

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ВПЛИВ МАГНІТНОГО ПОЛЯ НА АКТИВНІСТЬ ДРІЖДЖІВ *Saccharomyces cerevisiae* ПРИ БРОДІННІ ВИНОГРАДНОГО СУСЛА

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Оброблення дріжджових культур з використанням магнітного поля дає змогу краще з'ясувати дію магнітного поля на активність ензимів, коливання концентрації макро- і мікроелементів у дріжджових культур. Вивчали дві групи дріжджів: лабораторні дріжджові культури, виділені з регіональних виноградних сусел, та комерційні (виробничі) культури дріжджів, які зазвичай використовують у виноробстві. Обидві групи дріжджів було досліджено за біохімічними показниками під впливом магнітного поля і без такого впливу. Використовували такі параметри частоти магнітного поля: 160 Гц, інтенсивність — 5 мТл, час експозиції 30 хв. Ґрунтуючись на наших лабораторних дослідженнях, дріжджові культури які не піддавали обробленню магнітним полем, мали високу кореляцію між активністю ензимів алкогольдегідрогенази і кокарбоксилази. Групи дріжджів, які було піддано обробленню магнітним полем, мали високу кореляцію між активністю алкогольдегідрогенази, аланінамінотрансферази, амілази і фосфатази. Морфологія дріжджів *Saccharomyces cerevisiae* показала, що через 24 год після оброблення магнітним полем виявляли від 30 до 70% загиблих дріжджових клітин (підтверджено фарбуванням за Грамом). Однак ті дріжджові культури, які залишилися живі й були вміщені у виноградне сусло, показували посилення ензиматичної активності та високу кореляцію між концентраціями калію і кальцію, а також калію і магнію.

Ключові слова: *Saccharomyces cerevisiae*, активність ензимів, макро-і мікроелементи, біотехнологія вина.

ВЛИЯНИЕ МАГНИТНОГО ПОЛЯ НА АКТИВНОСТЬ ДРОЖЖЕЙ *Saccharomyces cerevisiae* ПРИ БРОЖЕНИИ ВИНОГРАДНОГО СУСЛА

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Обработка дрожжевых культур с использованием магнитного поля позволяет лучше понять действие магнитного поля на активность энзимов, колебания концентрации макро- и микроэлементов у дрожжевых культур. Изучали две группы дрожжей: лабораторные дрожжевые культуры, выделенные от региональных виноградных сусел, и коммерческие (производственные) культуры дрожжей, которые обычно используют в виноделии. Обе группы дрожжей были исследованы по биохимическим показателям под воздействием магнитного поля и без такого воздействия. Использовали следующие параметры частоты магнитного поля: 160 Гц, интенсивность — 5 мТл, время экспозиции — 30 мин. Основываясь на наших лабораторных исследованиях, дрожжевые культуры, не подвергавшиеся обработке магнитным полем, имели высокую корреляцию между активностью энзимов алкогольдегидрогеназы и кокарбоксилазы. Группы дрожжей, которые были подвергнуты обработке магнитным полем, отличались высокой корреляцией между активностью алкогольдегидрогеназы, аланинаминотрансферазы, амилазы и фосфатазы. Морфология дрожжей *Saccharomyces cerevisiae* показала, что через 24 ч после обработки магнитным полем выявлялось от 30 до 70% погибших дрожжевых клеток (подтверждается окрашиванием по Граму). Однако те дрожжевые культуры, которые остались живы и помещались в виноградное сусло, показывали усиление энзиматической активности и высокую корреляцию между концентрациями калия и кальция, а также калия и магния.

Ключевые слова: *Saccharomyces cerevisiae*, активность энзимов, макро- и микроэлементы, биотехнология вина.