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The influence of fermentation activity and ester-producing capacity of yeast strains on the chemical composition and organoleptic characteristics of Gamza wines

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ABSTRACT
The influence of the two technological parameters, temperature (20 °C, 24 °C, 28 °C) and inoculum amount of yeast culture (2%, 3%, 4%) on the duration of the alcoholic fermentation and the ester-producing capacity of the strains *Badachoni* and *24-6*, of *Saccharomyces cerevisiae* species was studied. The yeast exhibited high fermentation activity. The intensity with which they initiated the process and the time for its completion were in correlation with the studied factors. The strains showed the best activity at 28 °C/4%. Neural networks were applied and mathematical models were derived, properly describing the fermentation process and the change in the total amount of esters in its course, depending on the technological conditions. The rate in the dynamics of the esters during the process was monitored in 4 stages - onset, rapid fermentation, quiet fermentation and after malolactic fermentation. The maximum quantity of esters was synthesized in the middle of the process. During the alcoholic fermentation, the esters followed a tendency to increase with decreasing the temperature, increasing the amount of yeast culture and the time of the process. Both strains demonstrated the highest ester-producing capacity under the temperature of 20 °C and with the 4% inoculum of the yeast culture. The strain *Badachoni* quantitatively produced more esters than the strain *24-6*. No strict correlation was found between the amount of esters and the organoleptic properties of the wines. The sample containing the most esters had the highest tasting score only in the variants of the strain *24-6*.

Introduction*

Esters were the largest and the most diverse group of substances involved in the aroma and bouquet development of wine. They had a different origin and were formed chemically (esterification) and microbiologically in the process of grape ripening, during the alcoholic fermentation and the aging of wines. Ester formation began in the process of grape ripening, but their ratio was small (10 - 30 mg/l). Eleven different esters had been identified in grapes of different varieties. Wine contained much more esters, both in terms of representatives and quantity. Over 80 different representatives were identified. Esters belonged to two large groups, ethyl and acetate (Bisson and Karpel, 2010; Chobanova, 2012; Mina and Tsales, 2017).

The aromatic composition of wine depended on the variety and quality of the grapes. The application of various agricultural practices might enhance the typical varietal aroma or emphasize the aromas formed by the terroir (Styger, 2011). The ester content was significantly affected by the stage of grape ripeness. There was a correlation between the synthesis of esters and the composition of grapes, the method of its processing, yeast metabolism, and the fermentation conditions (Antalick et al., 2015).

Enological practices during the processing of the grapes, such as maceration or use of aromatic enzymes, as well as various technological operations

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in the vinification process, were also of importance for the content of the aromatic components (Herraiz et al., 1990; Styger, 2011; Guerrini et al., 2020; Filimon et al., 2021).

The main amount of esters in wine was produced by the yeast during the alcoholic fermentation. Their formation depended on the species and strain of the yeast, its fermentation activity, propagating capacity, and the inoculum amount of the used yeast culture. The esters were a product of their metabolism and played an essential role in the wine aroma formation. The volatile aromatic components might be synthesized directly by the yeast or released from their non-volatile precursors by the glucosidases produced by them. The biosynthesis of esters was mainly catalyzed by two types of yeast enzymes – esterases and lipases (Bisson and Karpel, 2010; Mina and Tsales, 2017; Rodrigues et al., 2017; Yang et al., 2017; Miñoz-Gonzalez et al., 2019; Delgado et al., 2020).

The yeast exhibited different ester-producing capacity. It was specific for the different species and strains causing the alcoholic fermentation that affected the level of esters in the wine. The yeast *Saccharomyces* produced larger amounts of pleasant-smelling esters (Chobanova, 2012; http://wineserver.ucdavis.edu/industry-info/enology/fermentation-management-guides). The spontaneous fermentation might add new flavors. Non-*Saccharomyces* yeasts could often produce larger amounts of esters and other aromatic substances because they produced enzymes, missing or present in small amounts, in *Saccharomyces cerevisiae*. However, they also synthesized components that negatively affected the wine aroma (Bisson and Karpel, 2010; Styger, 2011).

The alcoholic fermentation conditions were of prime importance for ester formation – temperature, oxygen, pH, content of assimilated nitrogen substances, etc. The maximum rate of esters was synthesized in the middle of the fermentation, in the presence of 9 - 12 vol. % ethanol. Ethyl esters were formed mainly during the first phase of the process. It was believed that a temperature within the range of 15 – 25 °C was the most beneficial for yeast development. More ethyl esters were synthesized at low temperatures and low oxygen levels. The low fermentation temperatures were more favorable for ester production. Most of the representatives, giving a pleasant fruity aroma to the wine, were formed then. The ratio of the esters was lower at higher temperatures. The rapid fermentation at higher temperatures resulted in the loss of the aromatic components. Under these conditions, the yeast produced enzymes that hydrolyzed them, leading to a reduction in their amount. At higher temperatures, more ethyl octanoate and ethyl decanoate were produced, adding heavy aromas, as well as 2-phenylacetate, adding the rose aroma. The nitrogen sources in the grape must were organic (amino acids) and inorganic (ammonium salts). The aromatic profile of wine might be modified by an appropriate ratio between them. The content of acetate esters that added the fruit aroma depended on and was favored by the higher concentration of amino acids, while the presence of a higher ratio of ammonium salts increased the amount of ethyl esters (Bisson and Karpel, 2010; Sumby et al., 2010; Antalick et al., 2014; Mina and Tsales, 2017; Kim et al., 2018 (http://wineserver.ucdavis.edu/industry-info/enology/fermentation-management-guides).

The malolactic fermentation in red wines was a biological process and as a result of their development, the malolactic bacteria also produced volatile aromatic metabolites or modified the components of grapes and those metabolites formed by the yeast. The content of ethyl acetate and ethyl lactate increased after the malolactic fermentation (Sumby et al., 2010; Styger, 2011; Antalick et al., 2014; Kim et al., 2018).

The ester composition of wines also changed during their aging in bottles or barrels, as then the esters were formed mainly chemically. The amount of esters increased depending on the duration of aging (Styger, 2011; Chobanova, 2012).

The wine aroma depended on the ratio of the components involved in its formation and their threshold of perceiving. The esters had a positive effect on the wine aroma and could give it fruity or floral notes, as well as notes of herbs and green grass, which was especially important for young wines. Ethyl acetate, isoamyl acetate, isobutyl acetate, ethyl caproate, ethyl octanoate, ethyl hexanoate, and ethyl decanoate and 2-phenylacetate had the greatest contribution. In low concentrations (below 100 g/l), they added a pleasant fruity aroma to the wine, while in high ratios they had a negative effect and could mask other specific aromas typical for the variety. Wines containing over 90 g/l of ethyl acetate or 200 g/l of total esters were considered defective. As a result of the malolactic fermentation, the fruit and oily notes in the aroma increased, but the plant and herbal aromas decreased (Bisson and Karpel, 2010; Sumby et al., 2010; Styger, 2011; Mina and Tsales, 2017; Rodrigues et al., 2017; Yang et al., 2017; Kim et al., 2018; Miñoz-Gonzalez et al., 2019; Delgado et al., 2020; Lee, 2020; http://wineserver.ucdavis.edu/industry-info/enology/fermentation-management-guides).

The objective of the study was to investigate the influence of the technological factors yeast strain, temperature and quantity of the inoculum yeast culture on the synthesis of esters during the alcoholic

fermentation and in the obtained wines, and their impact on the organoleptic profile of Gamza red wines.

Materials and methods

Processing and composition of the grapes

The experiments were carried out at the Institute of Viticulture and Enology (IVE) – Pleven, with grapes of the local variety Gamza, typical for the region of Pleven, Central Northern Bulgaria (the Danube plain). The grapes were harvested at suitable technological maturity (Table 1) and processed according to the standard technology for red wine production in the conditions of micro-vinification (Yankov, 1992). Each variant was crushed and strained off separately and the uniformity of the raw material was ensured by equal distribution of the clusters. The analyzed indicators were determined in accordance with the following methods: dry matter, % - Abbe refractometer; sugar, % - hydrometer of Dujardin; glucose and fructose, g/l - iodometric method; titratable acids, g/l - titration with NaOH; glucoacidimetric index – calculation method as the ratio of sugars, % and titratable acids, g/l; pH - pH-meter (Ivanov et al., 1979).

Alcoholic fermentation

The alcoholic fermentation occurred under the following conditions:

- substrate - 4.0 kg of grape pomace, sulfated with 50 mg/kg SO_2, with chemical composition presented in Table 1.
- inoculum - 48-hour active yeast culture from *Badachoni* and *24-6* strains of *Saccharomyces cerevisiae* species, in the quantity of 2%, 3%, 4% (the strains were provided from the yeast collection of the Department of Wine and Beer Technology, University of Food Technologies (UFT) – Plovdiv, Bulgaria);
- temperature – 20 ºC, 24 ºC, 28 ºC.

The course of the alcoholic fermentation was monitored daily through the dry matter change, measured with an Abbe refractometer to a constant value. The rate of change of the total esters in the course of the process was determined by recordings in the following stages: onset (day 1), rapid fermentation (day 5), quiet fermentation (day 10) and after the malolactic fermentation (day 20). Their quantity was determined by the method of saponification with NaOH (Ivanov et al., 1979). The determination of esters was based on their ability to be saponified in the presence of alkaline hydroxide.

Neural networks

The experimental results were modelled through neural networks of the Statistica 8 software package using a second-order quasi-Newton algorithm describing the influence of time, fermentation temperature, and the amount of the yeast culture on the synthesis of the esters. For each model, the number of neurons in the input layer was 3 (temperature, amount of yeast culture, time) and 1 for the output layer. The number of neurons in the hidden layer was set to be changed from 3 to 15. As the result, the network that gave the highest correlation ratio with the experimental data was chosen. The results were also presented in the form of surfaces describing the experimental data with high precision (Cichoski and Unbehauen, 1993; Nicoletti et al., 2009).

Chemical composition and organoleptic profile of the wines

After the completion of the alcoholic and malolactic fermentation, all experimental wines were decanted and analyzed for the following indicators: alcohol, vol.% and total extract, g/l (DEE Distillation Unit with Densimat and Alcomat, Gibertini); sugar, g/l (Schoorl's method); sugar free extract, g/l (calculation method as the difference between the total extract and the residual sugars in wine); titratable acids, g/l (titration with NaOH); volatile acids, g/l (distillation method with subsequent titration with NaOH); pH (pH-meter); total esters, mg/l (method of saponification with NaOH) (Ivanov et al., 1979). The results obtained from the chemical analysis were the arithmetic mean value of two parallel samples. When a significant difference in the rates of the studied indicator was found, a third sample was made and the two closest values were taken into account. Statistical data processing was represented by the mean value and the standard deviation (± SD) (Excel 2016 Microsoft Office).

The organoleptic characteristics of the experimental wines were evaluated by a 5-member tasting committee from the Department of Wine and Beer Technology (UFT – Plovdiv), by the 100-score scale and by the method of the main indicators (Tsvetanov, 2001; Prodanova, 2008). The tasting results represented an average of the committee members' scores, as the highest and the lowest were discarded.

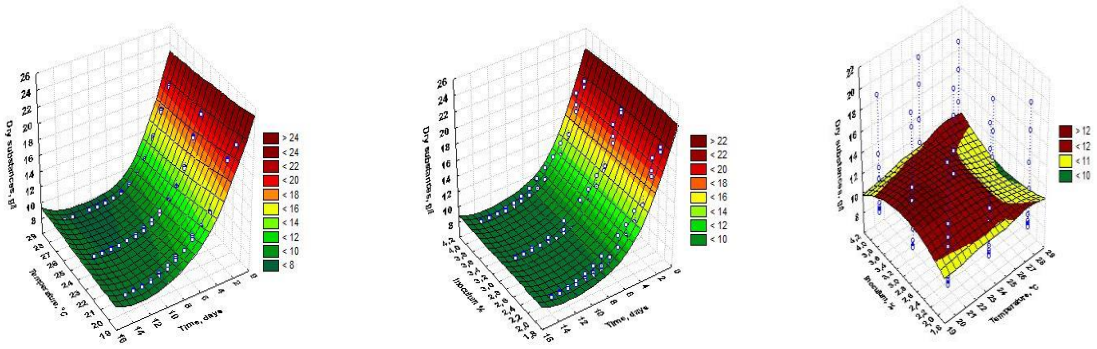
Table 1. Chemical composition of the grapes from Gamza variety

Indicators						
Dry matter, %	**Sugar, %**	**Glucose, g/l**	**Fructose, g/l**	**Titratable acids, g/l**	**Glucoacidimetric index**	**pH**
21.60 ±0.84	21.10 ±0.57	95.86 ±0.11	114.14 ±0.11	6.80 ±0.58	3.62 ±0.11	3.31 ±0.06

Results and discussion

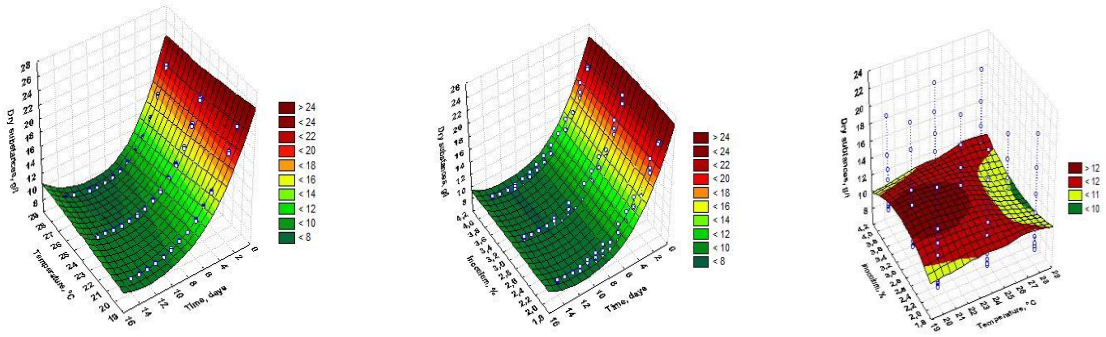
The influence of the temperature and the amount of inoculum yeast culture on the duration of the alcoholic fermentation with the studied strains of *Saccharomyces cerevisiae* was studied. A similarity in the change of the dry matter under the created experimental conditions was observed. The differences in the speed of initiating and running the process were due to the impact of the technological factors. The studied strains exhibited high fermentation activity, as the intensity with which the alcoholic fermentation started and the time for its completion were in correlation with the temperature and the amount of yeast culture. At 20 °C, the fermentation started and proceeded more slowly. The strains *Badachoni* and *24-6* showed the highest activity at 28 °C. The duration of the fermentation process was 3-4 days shorter compared to the lowest temperature (20 °C), at which the yeast multiplied more slowly. With the increase in the amount of yeast (2%, 3%, 4%), within one temperature range, the fermentation began and ended earlier, due to the greater number of active yeast cells in the medium. The results from the change in the dry matter during the fermentation of the grape pomace were modelled by neural networks. The depicted surfaces described the course of the process under the individual and overall impact of the studied fermentation factors and confirmed the established tendency (Figure 1, Figure 2). The fermentation started more intensively with the strain *Badachoni* and after the first 24 hours, the dry matter decreased by 1.4% to 2.0%, while the strain *24-6* showed a slightly slower start of the process. Despite the different initial speeds, no significant differences were subsequently found in its course with both strains of yeast. The reduction of the dry matter was more intensive with increasing the temperature and the amount of yeast culture, as that was more pronounced for the factor temperature. At 20 °C, the process ended completely on days 11 and 12, and at 28 °C on day 8. The variants with the 4% yeast culture fermented faster due to the presence of more active cells in the medium (Figure 1, Figure 2).

The dynamics in the total ester ratio during the alcoholic fermentation, depending on the changing technological factors, was monitored. For all variants, neural networks were prepared, describing the influence of time, fermentation temperature, and the amount of inoculum yeast culture on their change. The presented models of the obtained results were characterized by high accuracy of the description of the experimental data and revealed the stronger influence of the temperature as a parameter of the fermentation process. The impact of the amount of yeast culture of the studied strains was similar and relatively weaker. Under the experimental conditions, the ester-producing capacity of the yeast depended more significantly on the temperature (Sumby et al., 2010; Antalick et al., 2015). The results obtained for the synthesis of the esters during the alcoholic fermentation confirmed the fact that a greater amount was synthesized at lower temperatures (Beltran and Casellas, 2005; Molina et al., 2007; Bisson and Karpel, 2010; Antalick et al., 2014). The production of esters during the alcoholic fermentation occurred mainly biologically by the cellular enzymes, and the yeast exhibited better esterase activity under these conditions (Sumby et al., 2010; Mina and Tsaltas, 2017). The strains *Badachoni* and *24-6* produced more esters at 20 °C and 24 °C than at 28 °C. It was also observed that in the variants fermented with a higher ratio of the yeast culture, the ester content was higher due to the presence of a larger number of cells with active esterase. The results modelled through the neural networks confirmed the established trend. Figure 3 and Figure 4 represented the synthesis of the total esters during the alcoholic fermentation with the studied strains, under the individual and overall impact of the studied fermentation factors. The esters followed a tendency to rise with decreasing the temperature, increasing the amount of yeast culture and fermentation time. This tendency confirmed the research of other authors (Balik et al., 2002; Kolarik et al., 2004; Bisson and Karpel, 2010; Antalick et al., 2014). The depicted surfaces described the change in the synthesis of esters from the beginning of the alcoholic fermentation to its end and in the obtained wines, after the completion of the malolactic fermentation. The rise of their concentration in the course of the process was due to their production, not only biologically, but also chemically, i.e., as a result of the esterification. That process was determined by the increasing amount of alcohols formed in the medium and their interaction with the organic acids.



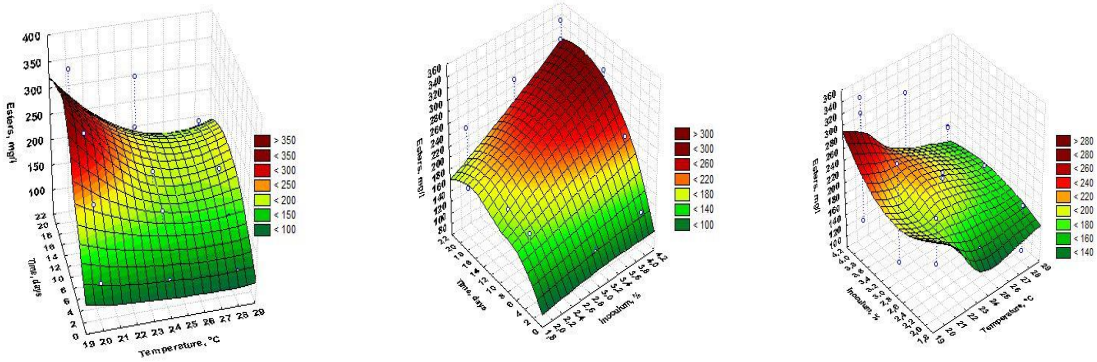
a) b) c)

Figure 1. Change in the dry matter during the alcoholic fermentation with the strain *Badachoni*
a) influence of temperature
b) influence of the inoculum amount of yeast culture
c) influence of temperature and the inoculum amount of yeast culture



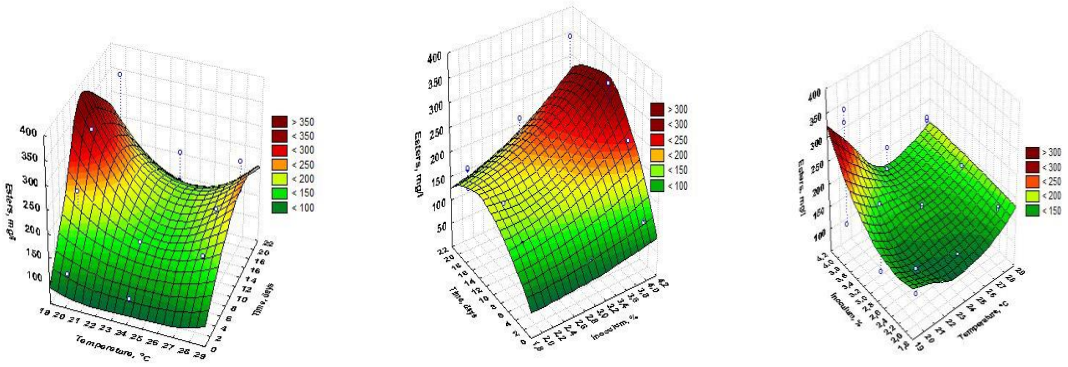
a) b) c)

Figure 2. Change in the dry matter during the alcoholic fermentation with the strain *24-6*
a) influence of temperature
b) influence of the inoculum amount of yeast culture
c) influence of temperature and the inoculum amount of yeast culture



a) b) c)

Figure 3. Change in the total ester ratio during the alcoholic fermentation with the strain *Badachoni*
a) influence of temperature
b) influence of the inoculum amount of yeast culture
c) influence of temperature and the inoculum amount of yeast culture



a) **b)** **c)**

Figure 4. Change in the total esters ratio during the alcoholic fermentation with the strain *24-6*.
a) influence of temperature
b) influence of the inoculum amount of yeast culture
c) influence of temperature and the inoculum amount of yeast culture

Both strains exhibited the highest ester-producing capacity at the temperature of 20 °C and with the 4% inoculum of the yeast culture. From the beginning of the fermentation, the strain *Badachoni* showed better ester-producing capacity compared to *24-6*. In the variants of the strain *Badachoni*, the ester ratio during the rapid fermentation varied from 123.20±22.73 (28 °C/2%) to 286.40±43.52 mg/l (20 °C/4%), while at the end of the process – from 193.60±31.26 (28 °C/2%, 3%, 4%) to 316.80±54.38 mg/l (20 °C/4%). In the variants of the strain *24-6* during the rapid fermentation, the esters were within the range from 96.80±28.12 (28 °C/2%) to 246.40±33.72 mg/l (20 °C/4%), and at the end of the process – from 176.00±31.56 (28 °C/2%) to 254.80±43.41 mg/l (20 °C/4%). The chemical composition of the obtained experimental wines in terms of the main indicators and the total ester content was presented in Table 2 and Table 3. The amount of residual sugars in the variants confirmed the full course of the alcoholic fermentation and the high activity of the strains under the created experimental conditions. The samples obtained with *Badachoni* were distinguished for their higher alcohol ratio, due to the better alcohol-forming ability of the strain. The differences in the alcohol content between the variants of one strain were insignificant. For *Badachoni*, the difference between the minimum and the maximum quantity was 0.28 vol. %, while for *24-6* – 0.35 vol. %. In the samples fermented at 28 °C, the alcohol ratio was higher due to the better fermentation activity shown by the yeast. In the wines Gamza, the sugar free extract ratio was not high, due to the specifics of the variety. Its content in the variants of both strains was similar and correlated with the increase of the fermentation temperature. The samples fermented at 28 °C had the highest rates because the higher temperature favored the extraction of a larger number of extract substances from the grapes during the fermentation. There were no significant differences in the content of titratable acids in the wines fermented with both yeast strains. The difference between the minimum and maximum value in the variants of both strains was 0.75 g/l. From the samples of *Badachoni* strain with higher acidity were these obtained at 20°C, and from those of *24-6* strain – at 28 °C. That showed it was possible to choose the fermentation conditions in order to regulate the acids in the wine. The volatile acidity of all samples was within the normal range. From the variants of the strain *Badachoni*, higher values were obtained at 24 °C, with the maximum found rate of 0.57±0.42 g/l. The strain *24-6* produced more volatile acids at 28 °C, with the maximum of 0.42±1.33 g/l. These ratios represented a good property of the studied strains. The alcoholic fermentation conditions significantly affected the synthesis of metabolites from yeast and the organoleptic profile of the obtained wines, respectively. Some studies reported higher concentrations of fresh and fruity aromas after fermentation at 15 °C, as opposed to a 28 °C fermentation, which produced higher concentrations of compounds with a flowery aroma (Molina et al., 2007; Sumby et al., 2010). The trend found in the course of the alcoholic fermentation was preserved in the finished wines. The strain *Badachoni* exhibited better ester-producing capacity and overall synthesized more esters than *24-6*. A correlation was observed between the amount of total esters formed and the percentage of inoculum, which was due to the presence of more viable cells in the medium. The results in Table 2 revealed that the strain *Badachoni* synthesized significantly fewer esters at 28 °C than at 20 °C and 24 °C. At 28 °C, the ester ratio in wines was from 176.00±36.64 to 211.20±39.24 mg/l. The strain exhibited the highest ester-producing capacity at 20 °C. In these samples, their concentration varied from

246.40±26.09 to 352.00±28.05 mg/l. The peak was found at 20 °C/4%. The amount of esters had a positive effect on the organoleptic qualities of wines, but the higher content in the variants was not always associated with a higher tasting score (Figure 5). That was explained by the ratio between the individual representatives and the activity of the specific esterases, which determined their synthesis depending on the conditions. The same trend was observed in the strain *24-6* (Table 3). The strain synthesized more esters at 20 °C, the peak being established under the conditions of 20 °C/4%, (272.00±48.62 mg/l). That variant also received the highest tasting score (Figure 5). The lowest ratio of esters was found in the wine obtained under the conditions of 28 °C/2% - 176.00±26.18 mg/l. The results from the analyzed indicators of the composition of Gamza wines showed that the wines fermented with the strain *Badachoni* had better organoleptic features and tasting scores compared to the variants of *24-6* (Figure 5, Figure 6). The samples fermented with the strain *Badachoni* at the temperature of 24°C had the best organoleptic indicators. The variant obtained under the conditions of 24 °C/2%, characterized by an intense fruity (raspberry) aroma and balance between the taste components, was distinguished by the highest tasting evaluation (78.00 points). From the variants of the strain *24-6*, the one with the best organoleptic characteristics was obtained at the temperature of 20 °C and with the 4% inoculum of the yeast culture. It was evaluated with 77.44 points, due to the pure varietal aroma, good harmony in taste, with sufficient freshness and density.

Table 2. Chemical composition of the experimental Gamza wines fermented with the strain *Badachoni*

Variants	20 °C			24 °C			28 °C		
Indicators	**2%**	**3%**	**4%**	**2%**	**3%**	**4%**	**2%**	**3%**	**4%**
Alcohol, vol. %	12.57 ±0.44	12.60 ±0.50	12.65 ±0.62	12.42 ±0.48	12.50 ±0.55	12.67 ±0.53	12.62 ±0.60	12.70 ±0.62	12.70 ±0.54
Sugar, g/l	1.37 ±1.24	1.54 ±1.31	1.54 ±1.48	1.47 ±1.29	2.01 ±1.66	1.78 ±1.23	1.81 ±2.11	1.40 ±1.88	1.54 1.52
Sugar free extract, g/l	20.33 ±3.71	20.26 ±2.67	20.96 ±4.10	20.63 ±3,18	20.79 ±3,77	21.02 ±2,45	21.18 ±4.26	21.00 ±3.79	21.27 ±3.05
Titratable acids, g/l	5.93 ±1.31	6.00 ±1,65	5.93 ±2.33	5.78 ±3.12	5.55 ±1.83	5.25 ±1.54	5.70 ±2.56	5.85 ±2.83	5.48 ±3.11
Volatile acids, g/l	0.45 ±0.81	0.30 ±0.33	0.48 ±1.05	0.54 ±0.14	0.57 ±0.42	0.54 ±1.28	0.48 ±1.31	0.42 ±0.76	0.42 ±1.23
pH	3,25 ±0.00	3,18 ±0.05	3,19 ±0.08	3,17 ±0.02	3,23 ±0.02	3,26 ±0.05	3,22 ±0.07	3,21 ±0.09	3,25 ±0.06
Total esters, mg/l	246.40 ±26.09	299.20 ±31.94	352.00 ±28.05	193.60 ±22.78	246.00 ±25.14	316.80 ±44.15	176.00 ±36.64	188.00 ±33.18	211.20 ±39.24

Table 3. Chemical composition of the experimental Gamza wines fermented with the strain *24-6*

Variants	20 °C			24 °C			28 °C		
Indicators	**2%**	**3%**	**4%**	**2%**	**3%**	**4%**	**2%**	**3%**	**4%**
Alcohol, vol. %	12.38 ±0.26	12.26 ±0.32	12.58 ±0.38	12.23 ±0.53	12.51 ±0.56	12.48 ±0.41	12.53 ±0.39	12.58 ±0.55	12.57 ±0.52
Sugar, g/l	1.74 ±2.17	1.61 ±2.38	1.54 ±3.68	1.71 ±4.12	1.74 ±4.33	1.50 ±2.18	1.74 ±3.18	1.57 ±1.38	1.88 ±1.33
Sugar free extract, g/l	20.06 ±4.67	20.00 ±3.18	20.16 ±5.11	20.29 ±3.24	20.26 ±4.31	20.70 ±2.17	20.96 ±2.88	21.03 ±3.62	21.00 ±3.81
Titratable acids, g/l	5.73 ±3.22	5.40 ±1.61	5.40 ±1.83	5.10 ±2.90	5.30 ±3.44	5.85 ±4.26	5.78 ±2.28	5.78 ±2.63	5.85 ±3.11
Volatile acids, g/l	0.39 ±1.13	0.36 ±1.47	0.36 ±0.88	0.28 ±1.00	0.40 ±0.53	0.40 ±0.48	0.42 ±1.33	0.40 ±1.72	0.42 ±1.11
pH	3,27 ±0.03	3,19 ±0.04	3,22 ±0.00	3,21 ±0.05	3,23 ±0.10	3,17 ±0.08	3,19 ±0.00	3,19 ±0.06	3,15 ±0.06
Total esters, mg/l	176.00 ±38.12	246.40 ±52.77	272.00 ±48.62	158.40 ±33.21	158.40 ±28.14	228.80 ±44.10	176.00 ±26.18	193.00 ±24.44	246.40 ±31.45

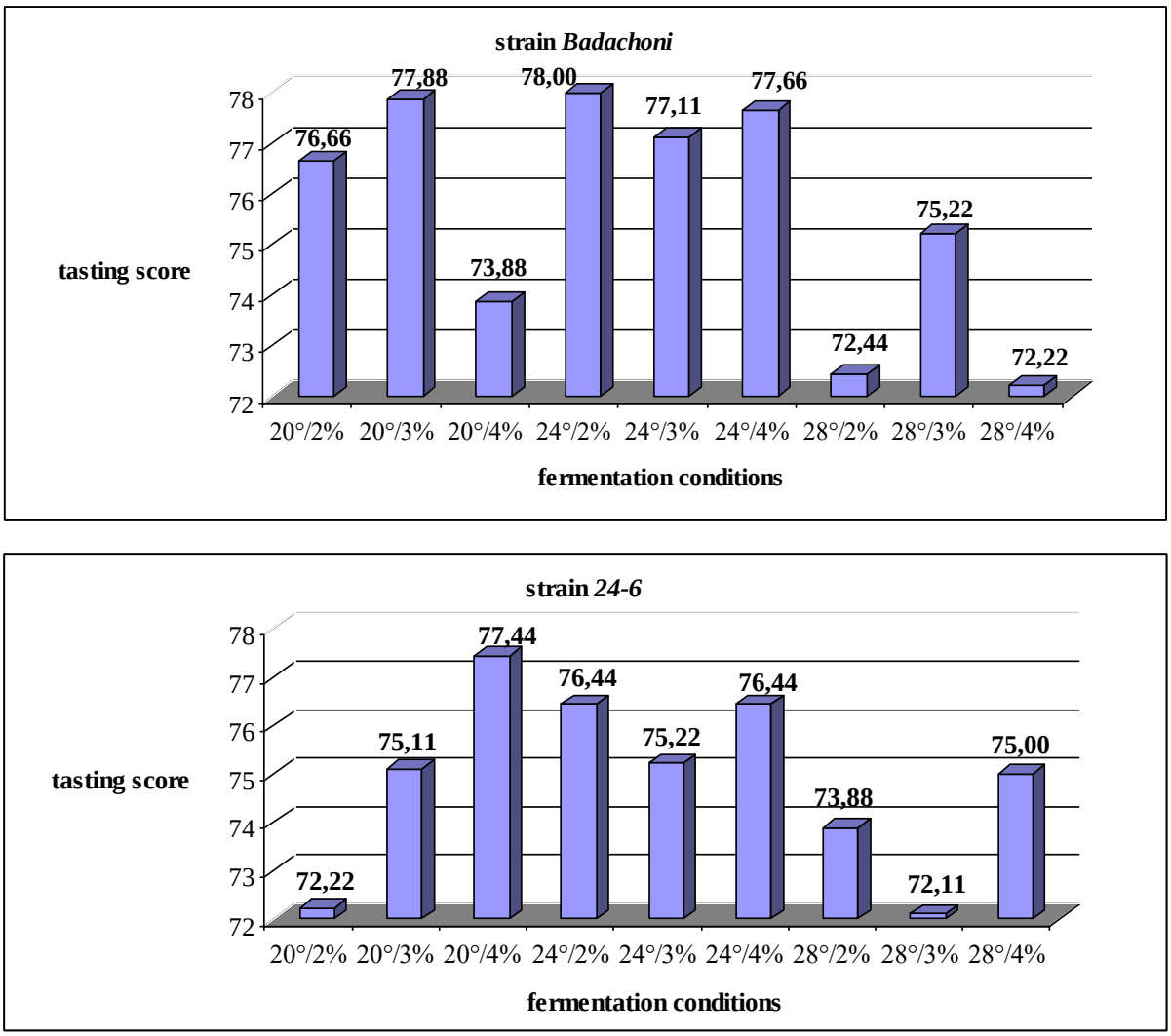


Figure 5. Tasting scores of the experimental Gamza wines

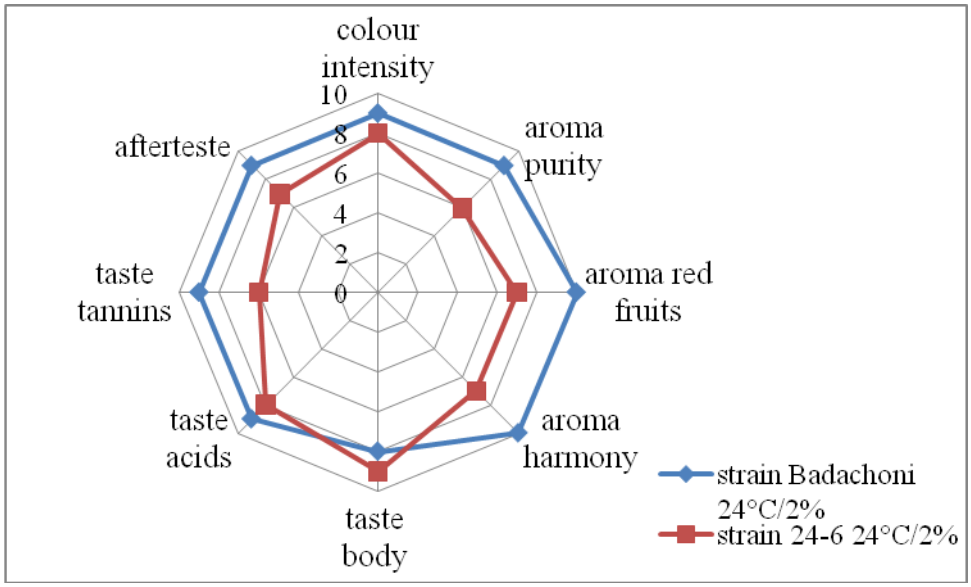


Figure 6. Organoleptic profile of the experimental Gamza wines

Conclusions

On the basis of the results obtained under the conditions of the experiment, the following might be summarized:

- both studied strains exhibited higher fermentation activity. The intensity at the beginning of the alcoholic fermentation and the time for its completion were in correlation with the temperature and the amount of inoculum yeast culture. The strains *Badachoni* and *24-6* showed the best activity at 28 °C/4%.
- the applied neural networks and the derived mathematical models adequately described the fermentation process and the change in the total amount of esters in its course, depending on the technological conditions.
- during the alcoholic fermentation, the esters followed a tendency to increase with decreasing the temperature, increasing the amount of yeast culture and the time of the process.
- The strains *Badachoni* and *24-6* showed the highest ester-producing capacity at the temperature of 20 °C and with 4% inoculum of the yeast culture. The strain *Badachoni* quantitatively produced more esters than *24-6*.
- no significant differences were observed in the ratio of the main chemical indicators from the composition of the obtained wines fermented with both strains. As the fermentation temperature became higher, the content of alcohol and the sugar free extract in the experimental samples increased.
- there was no strict correlation between the amount of esters and the organoleptic features of the wines. From the variants of the strain *24-6*, with the highest score (77.44 points) was the sample containing the most esters and obtained at 20 °C/4%. From the variants of the strain *Badachoni*, the sample obtained at 24 °C/2% received the highest score (78.00 points).

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