

COMPARATIVE STUDY OF THE INFLUENCE OF YEASTS ON PHYSICO-CHEMICAL AND BIOCHEMICAL INDEXES OF DRY WHITE WINES

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Summary: Alcoholic fermentation is a basic factor influencing on the formation of the wine, and yeast strains are responsible for this process. In this comparative study the influence of 10 selected yeast strains: from the National Collection of Microorganisms for Winemaking (CNMIV) and three dry active **Maria Antohi**, strains and endogenous microflora on physical, chemical and biochemical parameters was studied. Amino acid composition of experimental wines and volatile aromatic complex were analyzed.

It can be noted that yeast strain has the influence on the synthesis of fermentation byproducts and amino acid composition (about 12 amino acids), being emphasized some selected yeast strains (No. 29, 30 and 55 – collection strains and a active dry yeast–Oenoferm F3) and spontaneously fermented wine. It was found a vast aromatics composition of tested samples of wine, allowing to identify about 38 aromatic components, each of them is dependent on the strain of yeast used in the process of alcoholic fermentation, and the variation of their concentration determines the organoleptical specificity taste of each studied wine samples

Key words: yeasts, alcoholic fermentation, volatile aromatic complex

Introduction

The determinant factor in the process of wine formation is assigned to alcoholic fermentation, responsible for which are yeast strains. A rapid metabolism of amino acids in must by yeasts (except proline, arginine, alanine) occurs during the alcoholic fermentation, others are metabolized at a rate of 70–90% [1]. The decrease of the concentration of amino acids in the fermentation is followed by an increase during the yeast autolysis. The wines concentration of amino acids is much lower than in must and is about 700–1300 mg/dm³ for red wines and for white wines – 350–650 mg/dm³ or 30–50% of the red grapes amino acids and 20 to 30% of white grapes. [2].

One of the most important roles in the formation of the wine quality index is assigned to microorganisms, in particular to yeasts, the use of which for carrying out the fermentation process results in obtaining ethyl alcohol and carbon dioxide named key product. Another capacity of yeasts is the ability to synthesize certain byproducts that contribute to the formation of wine's bouquet and taste. Glycerol, acetoin substances (2,3 butanediol, acetoin, diacetyl), higher alcohols (aliphatic and with aromatic character), aldehydes, organic acids, fatty acids, ester compounds, etc. are some of these byproducts.

The research goals

The research was carried out to study the influence of yeast strains on physico-chemical contents, on free amino acids content and volatile aromatic compounds of dry white wines.

Materials and Methods

During the research, by capillary electrophoresis, was studied the comparative influence of yeast strains selected from the National Collection of Microorganisms for Wine Industry (NCMWI) and 2 strains of dry active imported yeast. The sample fermented by endogenous microflora served as a control. Must from the Chardonnay grape variety obtained by the classical scheme served as feedstock. The physico-chemical indices were identified in the experimental wines and the content of amino acid was determined after their maintenance on the yeast sediment for one month.

Determination of amino acids was performed by capillary electrophoresis method. The method is based on the cleavage of the samples by acid or base hydrolyzes with the passage into the free amino acid forms, derivatives obtaining, phenylzotiocarbamyl, and their future separation with the quantitative determination in the ultraviolet spectrum at a wavelength of 254 nm.

Analysis of the aromatic compounds of experimental wines was performed by gas-liquid chromatography method.

The results of the physico-chemical analyzes are shown in Table 1.

Physico-chemical analysis found that alcoholic concentration of experimental wines ranging from 12.2% vol in strain 81, Siha Activehefe and with endogenous fermentation (sample no. 1, 9 and 13), till 13.3% vol, indicating the higher concentration in the strain no. 1 (sample no. 2).

The value of pH index varies in the range 2.2–2.3, indicating a very acid environment. The concentration of titratable acidity varies slightly from 6.5 to 7.2 g/dm³, is characterized by a pronounced acidity in wines fermented with strains 29, 64 and control (no. 4, 8 and 1). The presence of moderate concentrations of malic acid in the range of 2.24 to stem no. 29 (no. 4) and 3.49 g/dm³ after fermentation by endogenous microflora (no. 1) was observed, which indicates the freshness in taste. Noted that in some samples was determined the presence of malic acid too, the highest value of about 0.28 g/dm³ being registered on wine fermented with Siha Activehefe active dry strain (no. 13), due probably to the strains' metabolic activity.

After sensory appreciation of the Chardonnay experimental wines highlighted the wine fermented with strain no. 30 (no. 5), characterized by clean and floral aroma, clean, mining, full, and harmonious taste; gained the highest mark of 8.00 points.

In order to determine the yeast strains assimilation of nitrogen sources and their metabolism in the amino acids form in the Chardonnay experimental wines was performed the analysis of free amino acids content [4UTM rom]. The results are shown in Table 2.

From the 7 amino acids encountered in big quantities in wine (proline, arginine, lysine, phenylalanine, glutamic acid, histidine and asparagine), the analysis of experimental wines were determined only two amino acids with higher concentrations – proline (which is not involved in wine's microflora metabolism) and arginine. [1, 2] The

proline concentration varies very large: from 69.32 (sample no. 9) up to 364.7 mg/dm³ (sample no. 1), that is, about 5 times.

By the mechanism of their involvement in the fermentation process amino acids which are consumed in the fermentation process and don't turn into wine in the autolysis as arginine, phenylalanine and cistidina are distinguished. Thus, the arginine is found in the experimental wines in a concentration of up to 15.65 mg/dm³ (sample no. 1 – endogenous microflora), so that the diversity of endogenous microflora's microorganisms don't consume this amino acid. From strain collection the most consumed by strains no. 55 (sample no. 7), 81 (sample no. 9) and 47 (sample no. 6). Phenylalanine, the phenethyl acid precursor that prints honey flavor to wines, was identified only in 3 experimental wines and 4.77 mg/dm³ concentration was determined in sample no. 1 spontaneously fermented (on the endogenous microflora). It follows that other yeast strains studied, consume completely the phenylalanine and cistidina, which was not determined in any sample of wine.

The leucine and methionine were identified from the tioamino acid representatives. Methionine was determined at concentrations of up to 11.61 mg/dm³, in the sample wine no. 7 (strain 55), and in sample no. 11.0 mg/dm³. 4 (strain 29).

Thus we see that by metabolic action of yeast's microflora, the threonine was determined in concentration of 15.36 mg/dm³ in sample no.1 and 9.87 mg/dm³ in collection strain no. 89 (sample no.11) and 9.12 mg/dm³ respectively for dry active imported yeast strain IOC 18–2007 (sample no. 12).

Table 1. Physicochemical indices of Chardonnay wines fermented with different yeast strains, vintage 2013

No.	Experimental sample of wine	Alcoholic concentration, % vol	pH index	Potential OR, mV	The concentration in mass:									Organoleptic mark, points
					sugars, g/dm ³	titratable acidity, g/dm ³	volatile acidity, g/dm ³	sulphurous acid, total / free g/dm ³	dry unreduced extract, g/dm ³	tartaric acid, g/dm ³	malic acid, g/dm ³	lactic acid, g/dm ³	citric acid, g/dm ³	
1	Chardonnay control (endogenous fermentation)	12,2	3,22	218,0	1,4	7,2	0,33	75/13	18,2	3,4	3,49	0,003	0,45	7,78
2	Chardonnay strain no. 1	13,3	3,29	212,1	2,3	6,5	0,36	34/14	17,8	2,9	2,57	–	0,35	7,86
3	Chardonnay strain no. 11	13,2	3,24	216,3	1,8	6,7	0,34	72/18	17,2	3,1	2,73	–	0,4	7,85
4	Chardonnay strain no. 29	12,6	3,2	213,3	1,7	7,2	0,37	70/16	17,9	3,3	2,24	–	0,41	7,78
5	Chardonnay strain no. 30	12,9	3,23	217,3	1,9	7,0	0,46	59/15	17,7	3,2	3,00	0,01	0,4	8,00
6	Chardonnay strain no. 47	12,9	3,26	216,9	2,3	6,5	0,24	71/10	17,5	3,1	2,36	0,1	0,41	7,84
7	Chardonnay strain no. 55	12,4	3,23	217,2	1,6	6,8	0,31	48/15	17,4	3,3	3,22	–	0,42	7,97
8	Chardonnay strain no. 64	12,7	3,25	216,8	1,4	7,2	0,35	98/15	18,4	3,1	3,38	–	0,45	7,88
9	Chardonnay strain no. 81	12,2	3,22	214,5	1,5	6,8	0,26	59/15	16,8	3,0	2,84	0,13	0,41	7,74
10	Chardonnay strain no. 88	12,8	3,26	208,6	1,4	6,6	0,40	58/13	17,1	3,1	2,80	–	0,38	7,73
11	Chardonnay strain no. 89	12,4	3,23	217,2	1,5	6,9	0,33	80/10	17,8	3,2	3,90	–	0,44	7,79
12	Chardonnay strain IOC 18–2007	12,6	3,25	216,3	1,4	7,1	0,53	99/12	18,2	3,0	3,38	0,05	0,45	7,83

Continuation <i>Table 1.</i>														
13	Chardonnay strain Siha Activehefe	12,2	3,25	209,8	1,3	6,9	0,40	61/18	17,5	3,1	2,90	0,28	0,37	7,70
14	Chardonnay strain Oenoferm F3	12,6	3,25	214,6	2,1	6,9	0,26	58/17	18,2	3,1	3,17	–	0,44	7,83

Table 2. Free amino acid content of dry white wines fermented with different yeast strains

No.	Wine name	The concentration in mass: , mg/dm ³												The total amount of amino acids mg/dm ³
		arginine	β-phenyl alanine	histidine	leucine	metionine	valine	proline	threonine	ryptophan	serine	α-alanine	glycine	
1	Chardonnay martor	15,65	4,77	2,36	3,22	0,32	2,05	364,7	15,36	5,51	3,39	2,6	2,65	422,6
2	Chardonnay tulpina nr. 1	12,53	0,87	–	0,25	1,76	0,01	138,3	2,27	–	0,43	2,54	0,49	159,4
3	Chardonnay suşa nr. 11	9,90	0,37	0,9	1,71	7,5	0,33	94,32	1,10	1,00	–	–	–	117,2
4	Chardonnay tulpina nr. 29	8,91	–	–	2,09	11,03	0,44	140,4	4,46	2,72	0,74	3,55	0,16	174,4
5	Chardonnay tulpina nr. 30	9,32	–	–	1,14	6,98	2,15	111,3	2,05	1,34	0,51	2,78	0,56	138,2
6	Chardonnay tulpina nr. 47	8,33	–	–	0,21	0,75	0,87	135,7	1,45	2,06	–	–	0,73	150,1
7	Chardonnay tulpina nr. 55	7,09	–	–	1,52	11,61	1,98	169,9	4,31	1,63	1,17	1,31	1,57	202,1
8	Chardonnay tulpina nr. 64	12,71	–	–	0,13	2,38	0,43	175,7	3,84	1,44	0,95	0,34	0,47	198,4
9	Chardonnay tulpina nr. 81	7,83	–	–	1,17	2,45	1,12	69,32	1,47	0,36	–	–	–	83,71

Continuation *Table 2.*

10	Chardonnay tulpina nr. 88	9,25	–	–	1,5	6,62	2,00	115,5	5,2	3,75	0,19	1,10	0,31	145,4
11	Chardonnay tulpina nr. 89	4,30	–	–	1,09	7,68	1,95	218,5	9,87	6,27	0,77	6,46	0,99	257,9
12	Chardonnay tulpina IOC 18–2007	14,52	–	–	0,53	6,77	1,12	207,1	9,12	1,10	1,17	2,74	0,50	249,5
13	Chardonnay tulpina Siha Activehefe	2,16	–	–	0,66	0,20	–	128,5	4,76	0,08	0,19	0,36	0,08	137,0
14	Chardonnay tulpina Oenoferm F3	13,87	–	–	1,07	1,89	0,23	161,0	1,63	1,19	0,38	2,54	0,65	184,5

Table 3. Aromatics content of wines fermented with different yeast strains, mg/dm³

Aromatic compound	Chardonnay control	Chardonnay strain no. 1	Chardonnay strain no. 11	Chardonnay strain no.29	Chardonnay strain no. 30	Chardonnay strain no.47	Chardonnay strain no.55	Chardonnay strain no.64	Chardonnay strain no.81	Chardonnay strain no. 88	Chardonnay strain no. 89	Chardonnay strain IOC 18–2007	Chardonnay strain Siha Activehefe	Chardonnay strain Oenoferm F3
Acetaldehyde	37,11	20,63	12,31	13,62	28,57	5,53	20,97	23,14	32,83	20,3	32,4	25,62	28,4	27,37
Methyl	–	–	7,82	–	–	–	–	1,54	–	–	–	–	–	–
Diacetyl	–	–	–	–	–	–	–	–	–	4,91	–	–	–	5,94
Acetoin	14,39	8,52	–	18,75	20,05	–	–	36,56	–	8,50	19,54	11,43	28,29	7,55
The amount of aldehydes	51,5	29,15	20,1	32,27	49,32	5,53	20,97	60,3	32,83	43,8	51,9	37,06	56,7	30,0

Continuation *Table 3.*

2,3- butylene glycol racemate + meso	528,6	573,3	674,9	557,7	746,9	746,9	640,7	681,3	567,4	559,7	464,8	705,5	634,1	452,5
1,2 propylene	50,0	22,75	33,97	40,32	35,84	35,97	34,99	44,26	26,9	27,35	29,45	51,36	31,04	41,03
1,3 propylene	–	–	–	–	19,4	–	–	18,19	–	22,32	5,13	–	–	–
The amount of polyhydri c alcohol	587,6	601,4	708,9	598,0	802,1	782,9	675,7	743,7	594,3	609,4	499,4	756,9	665,1	493,5
Methyl etilformiat	46,7	17,77	11,19	–	41,8	–	–	–	4,50	7,62	4,19	9,29	40,14	30,23
ethylacetat e	32,21	39,93	66,32	65,95	67,32	32,2	19,26	62,55	26,16	66,63	39,48	–	36,36	–
etilbutano at	–	–	–	–	–	–	–	–	–	5,99	2,87	37,48	–	–
izobutilace tat	–	–	1,66	–	–	–	–	–	–	–	–	–	–	–
etilvaleria nat t	0,25	0,24	–	–	–	1,03	–	–	–	0,77	0,41	–	0,83	–
izoamilace tat	–	0,42	–	–	–	–	–	–	–	–	–	–	–	–
etilcaprilat (etiloctano at)	–	1,75	–	22,45	–	37	3,83	4,79	–	–	1,21	–	–	–
Etilcapri- nat (etil- decanoat)	–	17,23	7,45	10,61	36,92	64	–	33,16	31,5	8,86	9,14	18,76	–	13,35

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Continuation **Table 3**

metilcapri nat (metideca noat)	–	4,96	–	22,35	4,34	20,30	–	3,36	–	12,26	4,00	–	2,08	13,48
etilactat (etilundec anoat)	–	–	–	–	–	–	–	4,38	–	7,43	–	–	26,33	–
etillaurat (etildodec anoat)	–	–	–	3,25	–	–	–	–	–	9,48	–	–	–	–
Amount esters	79,2	82,31	86,63	147,4	152,1	97,30	27,47	108,2	62,16	119,0	57,34	71,51	135,6	96,54
Ethyl	4,58	1,24	2,64	2,1	5,86	–	–	–	2,64	1,06	1,46	0,923	5,98	2,91
Methanol	40,84	45,26	34,0	37,48	37,2	32,1	30,1	38,49	69,4	49,62	54,97	34,35	49,76	38,76
2- propanol	6,28	4,61	–	–	5,71	–	–	1,88	3,52	7,34	15,18	–	8,06	5,48
1- propanol	5,07	8,24	11,32	6,88	10,5	9,53	7,37	10,55	15,7	10,59	20,0	12,31	20,0	15,97
isobutanol	35,66	37,68	70,37	85,1	47,31	41,1	54,1	93,71	36,2	57,98	33,12	53,28	89,22	62,47
1-butanol	3,14	0,38	–	–	–	–	357	–	–	–	–	–	–	–
isoamyl alcohol	313,1	330,6	467,9	414,5	380,2	339,0	7	341,5	525,2	408,0	481,4	341,7	357,9	406,6
1-amilol	–	3,85	–	–	–	–	–	–	–	–	2,34	–	–	–
1-hexanol	7,43	3,67	6,47	–	5,57	22,64	–	4,06	7,38	5,0	7,78	–	8,06	3,28
The amount of higher alcohol	370,7	389,0	556,1	506,5	449,3	412,3	419,2	441,7	588,0	488,9	559,8	407,3	483,2	492,8
propionic acid	15,04	15,16	12,85	25,37	–	–	11,01	9,14	4,74	21,38	6,2	–	15,78	10,98
isobutyric acid	19,88	2,73	–	2,35	–	–	1,98	0,49	–	2,84	1,53	–	4,62	3,76

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Continuation <i>Table 3.</i>														
butyric acid	1,15	–	4,98	–	2,38		2,92	1,32	7,35	2,80	3,58	–	4,14	–
isopentanoate acid	3,31	1,85	–	4,41	–	4,60	10,62	2,17	10,52	1,77	1,64	7,41	10,45	2,04
pentanoic acid		4,91	–	–	7,81	4,41	–	–	–	–	–	–	–	–
hexanoic acid	–	7,88	–	–	–	11,47	4,79	15,41	10,54	10,94	0,77	–	4,38	4,14
The amount of volatile organic acids	39,39	32,53	18,57	36,35	10,20	20,48	31,32	28,54	33,14	39,73	13,54	7,41	39,36	20,91
Phenylethyl acetate	–	2,9	7,52	–	–	24,90	8,87	–	14,77	–	3,60	12,56	2,87	26,92
2-phenylethyl acetate	–	3,11	3,97	8,14	–		2,30	3,74	5,29	2,80	–	4,71	51,91	–
2-phenylethanol	72,89	67,77	78,5	84,9	64,66	78,7	79,95	67,4	82,7	72,1	64,7	69,4	75,49	65,84
ionone	–	2,23	–		–	–		1,57	–	7,21	–	16,59	–	–
The amount of aromatic compounds	72,89	70,00	78,5	84,9	64,66	78,7	79,95	68,9	82,7	79,3	104,7	85,9	75,49	65,84

Tryptophan is a heterocyclic amino acid and is one of the most easily digestible amino acids by yeasts and bacteria, which is why it is found in lower concentrations in wines. In our experimental wines it is in the range of 1 to 6.27 mg/dm³.

α -alanine and glycine are monocarboxylic acids. Glycine or glycocholic acid (has a sweet taste) was determined at low concentrations up to 2.65 mg/dm³ in control wine. Alanine concentrations were determined up to 6.46 mg/dm³ for strain collection no. 89 (sample no. 11).

It is known that [2, 3, 4], from histidine by the mechanism of decarboxylation histamine is obtained – that indicates on the load with biogenic amines index of wines. It has been detected in experimental wines no. 1 (endogenous microflora) and 3 (strain no. 11) at low concentrations. This indicates on its possible decarboxylation and conversion to histamine.

As a result, in Chardonnay experimental wines, by gas-liquid chromatography aromatic method, the analysis of 14 samples of the experimental wine was performed.

The results obtained are shown in Table 3.

Analyzing the data in Table 3 we find that the experimental wine fermented on spontaneous microflora the acetaldehyde concentration reaches 37.11 mg/dm³. Concentrations of up to 20 mg/dm³ were determined in the experimental wine fermented with strain no. 11, 29 and 47 (12.3; 16.6 and 5.53 mg/dm³) and its concentration in the rest of experimental wine varies from 20.3 to 37.6 mg/dm³, which shows a lack of sulphitation of experimental wines (see table 5).

Butylene glycol – a compound responsible for wine's extractivity and softness. Naturally its concentration is 0.5–0.7 g/dm³, a value that is proportional to the square of the concentration of alcohol, higher values indicate that oxygen penetrates the wine [3].

The concentration of this compound in experimental wines analyzed, the information in Table 7 varies in the range from 332.0 to 746.9 mg/dm³, which shows that these wines are extractive.

The main oenological role of esters is the participation to the formation of olfactory-taste characteristics of the wine, which is why this group of products can be found in the highest number of compounds in wine. The formation of the esters takes place in two ways – biochemical and chemical.

The most representative neutral ester is ethylacetate, which usually is obtained by biochemical method. From the data in Table 3, it was found that in the experimental wines ethylacetate concentration varies from 19.26 mg/dm³ (strain no. 55) to 67.32 mg/dm³ (strain 30). However, the fermentation wines stems from dry active imported yeast Oenoferm and IOC 18–2007 ethylacetate was not identified.

After the total amount of esters highlighted strain collection no. 30 with 152.1 mg/dm³, and the poorer is the strain collection no. 55 with only 27.47 mg/dm³.

Another group of compounds of aromatic volatile complex are higher alcohols.

The most significant is the isoamyl alcohol (3-methyl-1-butyl) obtained by the fermentation of leucine. It is known that it causes alcohol intoxication more obvious than ethyl alcohol. Thus the data in Table 3 show that the highest concentration of isoamyl alcohol is synthesized by collection strain no. 81 with 525.2 mg/dm³ that has a negative impact on wine's organoleptic properties and corresponds with weak points to organoleptic appreciation of wines (Table 1) and the lowest in spontaneous microflora fermented wine – 313.1 mg/dm³.

Another representative is methyl alcohol or methanol, also known as the toxic effects even at low doses, although the concentration is regulated to max. 350 mg/dm³ in EU, in the National technical regulations it was divided for white and rosé wines – 250 mg/dm³ and tomatoes – 400 mg/dm³. At lower concentrations has no organoleptic and adverse effect because of the presence of the antidote – ethyl alcohol. In our experimental wines its concentration ranging from 27.48 mg/dm³ (strain 29) up to 69.4 mg/dm³ (collection strain no. 81). It was found that enzymes contribute to an increase in methanol concentration for both strain collection and for strains of dry active imported yeast.

Other compounds present in wine in this group are 1-propanol and isobutanol, concentration of isobutanol in the experimental wine was determined in the range of from 33.12 till 90.76 mg/dm³.

Summary, the highest concentration of higher alcohols was determined in the wine fermented with strain no. 81 with 588.0 mg/dm³.

One of the higher alcohols with aromatic character is 2-phenylethanol being the characteristic aroma of rose, while his volatile ester – phenylethyl acetate gives wine shades of honey. The data in Table 3, 2-phenylethanol was determined in the range of 64.6 to 84.9 mg/dm³.

Another group of compounds are aliphatic fatty acids, obtained as a by-product of the alcoholic fermentation, also called the volatile acid, and represent the volatile acidity of wine. According to the data in Table 3 the highest concentration of volatile acids was determined in spontaneous microflora fermented wine – 39.39 mg/dm³.

Thus, we find rich composition of experimental wines in aromatic compounds, allowing to identify about 38 aromatic components, each character is dependent on the strain of yeast used in alcoholic fermentation, and the variation of its concentration determines the specific organoleptic-taste of each experimental wine.

Generalizing the data presented in the study results that of the 10 collection strains taken in research to study their influence on the physico-chemical and biochemical indexes were appreciated as perspective the strain no. 29, 30 and 55, and of the dry active yeast the strain Oenoferm F3.

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