

Using the microflora of grapes for the production of young sparkling wines

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Abstract. In order to meet the growing demand for ecological products, it is necessary to conduct research on the impact of technological methods of organic winemaking on the quality of finished products. The possibility of preparing high-quality young sparkling wines using wild microflora of Cabernet Sauvignon grapes grown in the conditions of the southern coast of the Crimea without the use of pesticides was studied. The analysis of physical and chemical parameters of sparkling wines was carried out with the help of generally accepted in enochemistry and modified methods of analysis. The use of wild microflora contributes to a greater accumulation of glycerol (by 12-19%), amine nitrogen (by 18%), polyphenols (by 14-17%), the formation of combined forms of carbon dioxide (by 1.2%), better foaming (by 4-8%) and sparkling properties (by 2-3 times) in finished sparkling wines than in control samples. However, fermentation on wild microflora may do not go to the end, which leads to the appearance of undesirable sauerkraut tones. Control samples prepared using pure yeast culture "Odessa black SD13" had a pure varietal aroma and harmonious taste. To improve the bouquet and taste of young sparkling wines produced using wild microflora, it is necessary to select promising strains of wild yeast *Saccharomyces cerevisiae*, suitable for champagnization. The technology under study can be applied in small enterprises without the use of complex technological equipment. The introduction of this technology will help to increase the total output of sparkling wines.

1 Introduction

The current trends in the global wine market include an increase in the popularity of ecological products. That is, obtained without the use of synthetic fertilizers and pesticides in the cultivation of grapes, with the maximum use of manual labor, with minimal doses of sulfur dioxide and fermentation on the wild microflora of grapes. Such wines have the appropriate certificate and mark on the label and, as a rule, are more expensive than their usual counterparts. The demand for such products is associated not only with the absence of residual amounts of pesticides in it, but also with its uniqueness, since in this case the influence of the terroir is very significant, which includes not only natural and climatic conditions, but also local yeast living on grapes [1]. It is the combination of these factors

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that makes such wines unique, often made from widely distributed European grape varieties. In particular, when studying the fermentation process of Chardonnay grape must using the "Pide de Cuve" method, it was found that up to 150 strains of *Saccharomyces uvarum* yeast were contained in the wild microflora of grapes [2]. Moreover, at the first stage, must fermentation can be carried out, including yeast that does not belong to the genus *Saccharomyces* [3-8], which also has a significant effect on the organoleptic properties of the finished product. Some strains of wild yeast *Saccharomyces* have a good fermentation ability, including at low temperatures, and wines with a lower ethanol content and a higher glycerol content are obtained [9]. However, at the last stages of fermentation, as a rule, the yeast *Saccharomyces cerevisiae* dominates [10,11], since this type of yeast can produce antimicrobial peptides that can inhibit the growth of bacteria and yeast of other species [12,13]. However, to suppress the development of undesirable microflora at the initial stage of fermentation, is sometimes added wine to the fermented grape must to a total alcohol concentration of 1.5-3.0% by volume [14], or sulfur dioxide (up to 30-40 mg/dm³). At the same time, some strains of *Saccharomyces cerevisiae* are able to dominate at the fermentation without adding alcohol and SO₂ additives and completely ferment sugars, while maintaining grape polyphenols and high antioxidant activity [15]. But, what is very important for obtaining high-quality products, including for young sparkling wines, there are cold-resistant races of *Saccharomyces cerevisiae*, resistant to high concentrations of alcohol and CO₂, which do not form hydrogen sulfide [16-18]. However, in some cases, an addition of ammonium phosphate and thiamine is used to better ferment the must with wild yeast [19]. In recent years, there has also been great interest in non-saccharomycete yeast, which is suitable for use in the production of sparkling wines [20]. Such yeast races allow you to get finished products with a low alcohol content, which is important for regions with a hot climate, and also allows you to get original products for small enterprises that produce sparkling wines.

The purpose of this work was to study the possibility of obtaining high-quality young sparkling wines in the conditions of the southern coast of the Crimea by fermentation of must on the microflora of ecologically grown Cabernet Sauvignon grapes.

2 Materials and methods

The objects of research were young sparkling wines produced from Cabernet Sauvignon grapes grown on the experimental site of the southern coast of Crimea without the use of pesticides, using wild microflora of grapes (experience) and pure yeast culture (PYC) "Odessa black SD13" (control). The race "Odessa black SD13" from the Collection of microorganisms of winemaking "Magarach" belongs to *Saccharomyces cerevisiae* (Kreger-van Rij N. J. W., 1984), S-sensitive, promotes the formation of aliphatic alcohols, esters and lactones, synthesizes β -phenylethanol, enhances spicy shades in the aroma. This race is recommended for the preparation of red wine materials and sparkling wines [21]. A week before the mass harvest, five batches of grapes (10 kg) were hand-picked. The most ripe undamaged berries were separated from the ridges and the grape must was obtained using a basket press, which was placed in a clean container for spontaneous fermentation with the participation of wild microflora of grapes. The state of the resulting a fermenting mixture of wild microflora (FMWM) was evaluated by direct microscopy. From five batches of FMWM at the stage of active fermentation, an actively fermenting one was selected, which contained more than 80% of *Saccharomyces* yeast cells. The generic identity of yeast was determined by cell morphology and by the nature of colonies on dense media. A week later, the main grape harvest was carried out, which took place in the early hours at a mass concentration of sugars of 202 g/dm³, titratable acids – 7.8 g/dm³ and pH – 3.2. The technological reserve of phenolic substances was 2842 mg/dm³. When processing grapes by

the white method, the grape must was obtained by crushing the grapes on a roll crusher with grape comb separation → separation of the must-gravity on the drain and pressing on a basket press (the total yield of the grape must is not more than 65%). The resulting pink grape must was divided into two parts:

Scheme I included sulfitation of the must (30 mg/dm³) → clarification of the must by settling at a temperature of 14-16°C for 16-18 hours → removal the sediment → introduction of FMWM in an amount of 2% → fermentation of the clarified grape must at a temperature not exceeding 18°C → removal the yeast sediment at a sugar concentration of 22-24 g/dm³.

Scheme II included the sulfitation of the grape must (75 mg /dm³) → clarification of the must by settling at a temperature of 14-16°C for 16-18 hours → removal the sediment → introduction of PYC "Odessa black SD13" in an amount of 2% → fermentation of the clarified must at a temperature not exceeding 18°C → removal the yeast sediment at a sugar concentration of 22-24 g/dm³.

When processing grapes by the red method, the pulp was obtained by crushing the grapes on a roll crusher with comb separation.

Scheme III included sulfitation of the pulp (30 mg/dm³) → introduction of FMWM in an amount of 2% → fermentation of the pulp with constant stirring at a temperature not exceeding 18°C to 1/3 of the residual sugars → pressing of the fermenting pulp → fermentation of the red must in the fermentation tank → removal the yeast sediment at a sugar concentration of 22-24 g/dm³.

Scheme IV included sulfitation of the pulp (75 mg/dm³) → introduction of PYC "Odessa black SD13" in an amount of 2% → fermentation of the pulp with constant stirring at a temperature not exceeding 18°C to 1/3 of the residual sugars → pressing of the fermenting pulp → fermentation of the red must in the fermentation tank → removal the yeast sediment at a sugar concentration of 22-24 g/dm³.

Then, from each version of the must, a tirage mixture was prepared using the available live yeast cells of primary fermentation (at least 1 million cells/cm³) and bentonite (0.2 g/dm³) and the tirage mixture was poured into a champagne bottle → capping → stacking → fermentation at a temperature of 12-14°C → remuage → cooling to a temperature of minus 3-4°C → freezing the sediment in the bottle neck →degorging →topping up with the same wine→ capping.

In the obtained sparkling wines, the analysis of physical and chemical parameters was carried out according to [22]. The parameters of the foaming properties (the maximum volume of foam and the time of foam destruction) were determined by bubbling by air in a measuring cylinder (with a capacity of 1 dm³) of a degassed wine sample using a portable compressor and a sprayer lowered to the bottom of the cylinder. The volume of the formed foam was determined visually by means of cylinder calibration, the time of foam destruction - by means of a stopwatch. The content of organic acids, residual sugars, glycerol, and ethyl alcohol was determined by HPLC on a Shimadzu LC 20AD chromatograph (Japan) with a spectrophotometric detector. The total content of carbon dioxide in sparkling wines was determined according to the developed method, according to which the CO₂ released from the wine under the action of ultrasound displaced the gate liquid from the graduated container. The volume of the displaced gate liquid corresponded to the volume of carbon dioxide contained in a bottle of sparkling wine [23]. The content of combined forms of carbon dioxide was calculated from the difference between the measured CO₂ content and the solubility of CO₂ at a certain pressure and concentration of ethanol [22]. The sparkling properties were determined according to the developed method by measuring the rate of desorption of CO₂ from a wine sample at pressure relief to atmospheric pressure [24].

Organoleptic evaluation of sparkling wines was carried out in accordance with GOST 32051-2013 " Wine products. Methods of organoleptic analysis". And ISO 5492:2008 Sensory analysis-Vocabulary. And ISO 11035:1994 Sensory analysis — Identification and selection of descriptors for establishing a sensory profile by a multidimensional approach. The organoleptic evaluation was carried out according to a 10-point system (the minimum acceptable score is 8.0 points). And also by the quantitative expression of the contribution of individual descriptors to the formation of color, taste, and aroma of wines. The descriptors were selected in accordance with ISO 5492, ISO 11035, and [25].

The obtained data were processed by mathematical statistics methods using the Microsoft Excel software package.

3 Research results

At the first stage of the work, the dynamics of fermentation of must with use FMWM and PYC were compared (Fig. 1 and 2).

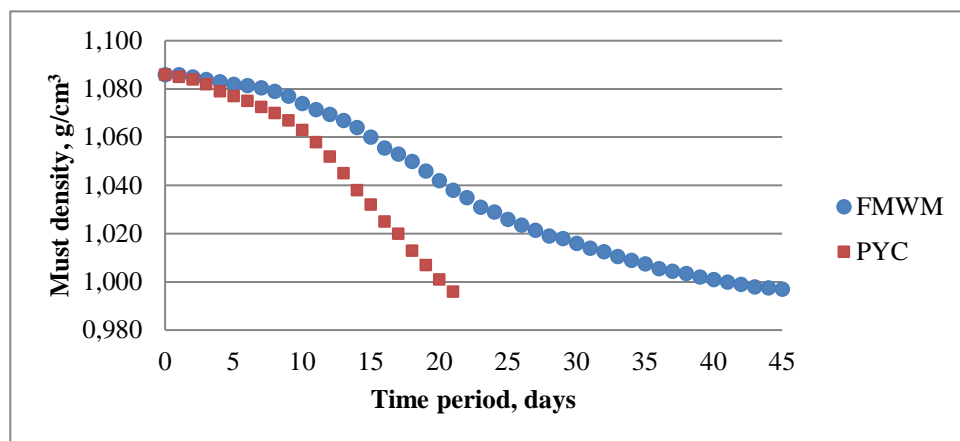


Fig. 1. Dynamics of fermentation of rose must.

Fermentation of rose must (Fig.1) with use "Odessa Black SD13" PYC took place for 21 days, and on the wild microflora of grapes-1.5 months.

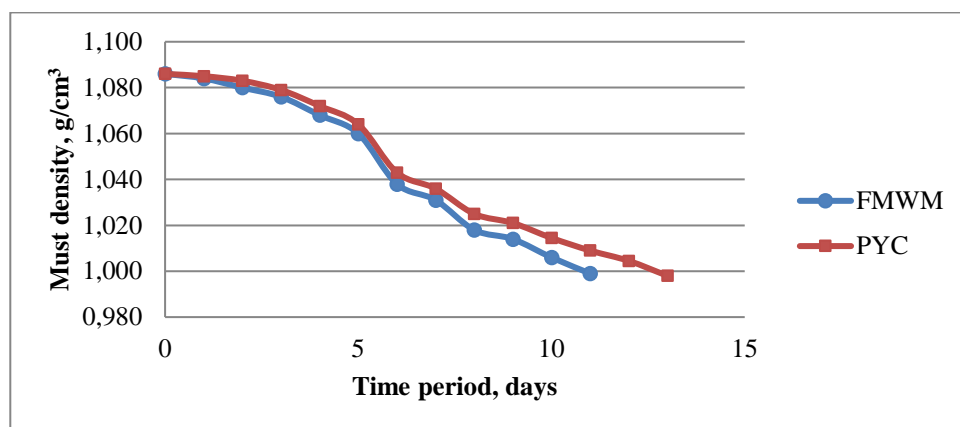


Fig. 2. Dynamics of red pulp fermentation.

The fermentation of the pulp (Fig.2) on the wild microflora of grapes proceeded a little faster than on the PYC and ended 2 days earlier. In this case, this may be due to the fact that on the skin of crushed grapes, despite the sulfitation (30 mg/dm^3), a certain amount of active initial microflora remained, which, together with the added FMWM, accelerated the fermentation process. At the same time, PYC had to compete with the wild yeast remaining after sulfitation, which could slow down the fermentation process. A slight curvature of the fermentation schedule in the area of the density of 1.030 g/cm^3 is associated with the process of pressing the pulp. In the young sparkling wines obtained after the end of the champagnization process (45 days after placing the tirage), the physical and chemical parameters were determined (Tables 1-5).

Table 1. Physical and chemical parameters of experimental sparkling wines

Title of the sample	Volume concentration of ethanol, vol. %	Mass concentration							Value	
		g/dm ³						mg/dm ³		
		sum of titratable acids	volatile acids	sugars	glucose	fructose	glycerol	amine nitrogen	pH	Eh, mv
Rose sparkling wine, FMWM	11.20	6.45	0.46	7.1	2.04	5.07	7.00	140	3.25	204
Rose sparkling wine, PYC	12.04	7.58	0.53	3.2	1.75	0.91	5.66	116	2.94	220
Red sparkling wine, FMWM	11.93	6.98	0.40	2.7	2.08	0.60	6.08	196	3.49	191
Red sparkling wine, PYC	12.20	7.13	0.26	2.1	1.56	0.34	5.35	161	3.38	197

Where: Eh – value of redox potential

Table 2. Physical and chemical parameters and foaming properties of experimental sparkling wines

Title of the sample	Mass concentration, mg/dm ³				Color intensity (D ₄₂₀ + D ₅₂₀)	Value of dynamic viscosity, mm ² /s	Foaming properties	
	TPh	MPh	PPh	C			V _{max} , cm ³	t _{br} , s
Rose sparkling wine, FMWM	492	199	293	20	0.289	1.684	500	26
Rose sparkling wine, PYC	440	187	253	8	0.107	1.549	460	17
Red sparkling wine, FMWM	2166	639	1526	257	1.248	1.566	1150	65
Red sparkling wine, PYC	1836	569	1268	220	1.129	1.536	1100	61

Where: TPh – total content of phenolic substances, MPh – content of monomeric fraction of phenolic substances, PPh – content of polymeric fraction of phenolic substances, C – content of coloring agents, I – value of color intensity (D₄₂₀ + D₅₂₀), V – value of dynamic viscosity, V_{max} – max foam volume, t_{br} – time of foam break.

Table 3. Mass concentration of organic acids in experimental sparkling wines.

Title of the sample	Mass concentration of acids, g/dm ³						Ratio of tartaric and malic acids
	citric	tartaric	malic	succinic	lactic	acetic	
Rose sparkling wine, FMWM	0.10	2.84	2.09	1.20	0.55	0.20	1.359
Rose sparkling wine, PYC	0.15	3.24	3.22	0.93	0.10	0.10	1.006
Red sparkling wine, FMWM	0.10	2.65	2.84	1.20	0.26	0.10	0.933
Red sparkling wine, PYC	0.15	2.75	3.02	1.09	0.21	0.10	0.910

According to the data obtained, in rose wines obtained with the use of PYC, alcoholic fermentation was more complete. While, during fermentation on the wild microflora residual sugars remained. Moreover, in rose sparkling wine, residual sugars were determined to be 7.1 g/dm³, of which 2/3 were fructose. In the samples obtained using wild microflora, more glycerol was detected, which may indicate the passage of side reactions of glycolysis along the glycerol-pyruvate pathway, and to a greater extent than in the control samples. This is also evidenced by the greater accumulation of succinic acid in the experimental samples. In addition, a greater accumulation of glycerol may be associated with the vital activity of non-saccharomycete yeast [26] at the initial stage of fermentation. It should be noted that the samples obtained using wild microflora contained more amine nitrogen, their redox potential and mass concentration of titratable acids were lower, and the pH index was higher than in the control. Moreover, the decrease in acidity was due to a decrease in the content of tartaric, malic and citric acids, while the content of lactic and succinic acids was higher than in the control samples. It should be noted that the ratio of tartaric and malic acids in rose wines was more than 1, and in red wines-less than 1. The content of various forms of phenolic substances and anthocyanins and, accordingly, the color intensity in the experimental samples was greater than in the control samples. If in the case of red wines, this can be explained by the better extraction of phenolic substances by must during fermentation on wild yeast due to the work of their enzyme systems (76% of the technological stock of phenolic substances in the experimental sample and 64% in the control sample were extracted), then in the case of rose must, this is explained by the better adsorption of phenolic substances (mainly polymer forms) by cultured yeast and products of their beginning autolysis. The dynamic viscosity index was higher in the experimental samples, and if in red wine this was apparently due to a higher concentration of glycerol, then in rose wine with a higher content of glycerol and residual sugars. The values of the foaming properties were higher in the samples obtained using wild microflora, which correlates with the mass concentration of amine nitrogen (k=0.893) and polyphenols (k=0.994). This is due to the fact that substances of the polymer structure (proteins, polypeptides, polyphenols, protein-phenolic complexes) stabilize the foam [27,28].

At the next stage of the work, the content of various forms of carbon dioxide and indicators of sparkling properties were determined (the results are presented in Table. 4 and in Fig. 3,4).

Table 4. Typical properties of experimental sparkling wines

Title of the sample	Equilibrium pressure of CO ₂ , kPa	CO ₂ content per bottle (0,75 dm ³), g				Weight ratio of the combined CO ₂ , %	V ₁₋₃₀₀ , mg/min	Angle of deflection of CO ₂ desorption curve, °	Coefficient of sparkling properties, min · 10 ⁻³
		total in the bottle	dissolved	gasiform	combined				
Rose sparkling wine, FMWM	520	7.318	6.172	0.187	0.958	13.09	2.813	0.1612	2.60
Rose sparkling wine, PYC	500	6.952	5.957	0.170	0.825	11.87	4.886	0.2799	1.42
Red sparkling wine, FMWM	620	8.233	7.036	0.211	0.985	11.97	1.860	0.1066	4.43
Red sparkling wine, PYC	500	6.861	5.993	0.130	0.737	10.74	4.742	0.2717	1.45

Where, V₁₋₃₀₀ - average desorption speed of CO₂ on the timespan 1-300 min.

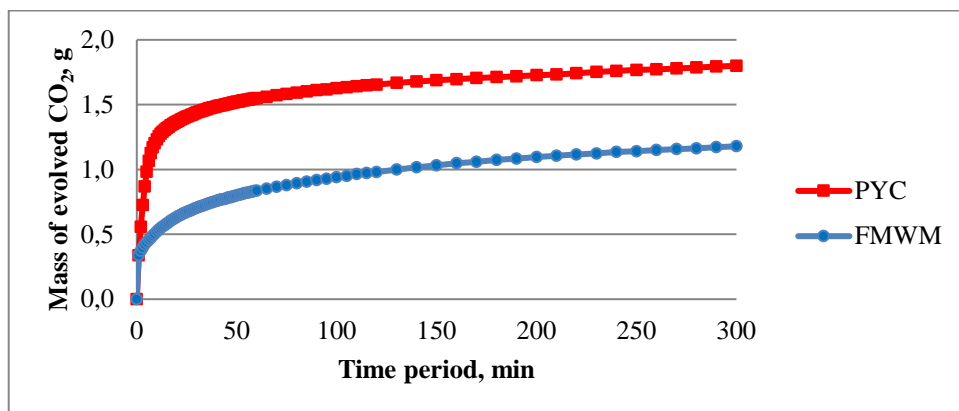


Fig. 3. Dynamics of carbon dioxide desorption from rose sparkling wines

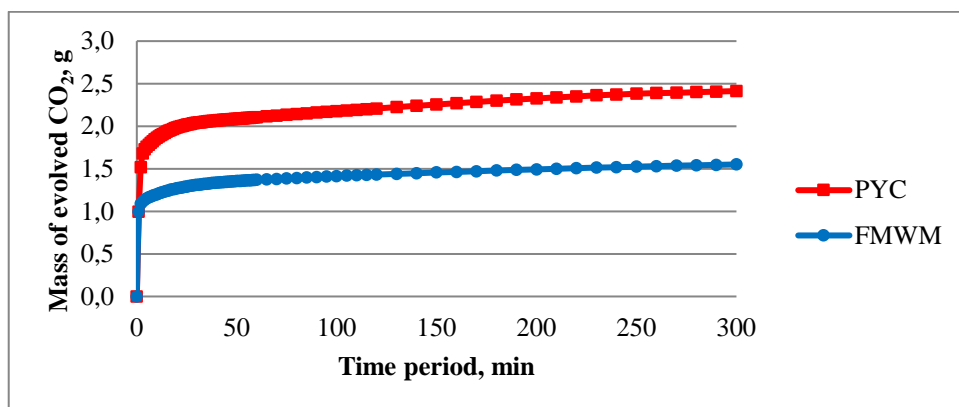


Fig. 4. Dynamics of carbon dioxide desorption from red sparkling wines

According to the obtained data in the experimental samples of sparkling wines, the excess pressure, the total content of carbon dioxide and his combined forms were higher, which in this case depended on the initial concentration of CO_2 and sugars in the tirage mixture at the time of laying the tirage and the degree of their fermentation. In addition, the slower fermentation of the rose must on the wild microflora contributed to a greater accumulation of combined forms of CO_2 in it. In turn, the higher content of combined forms of CO_2 contributed to a decrease in the rate of desorption of carbon dioxide ($k=-0.566$) and an increase in the coefficient of sparkling properties. Also, a decrease in the rate of desorption of carbon dioxide was facilitated by a higher content of glycerol ($k=-0.659$), which increased the viscosity of the wine, and amine nitrogen ($k=-0.683$).

The results of the tasting evaluation of young sparkling wines are presented in Table. 5 and in Fig. 5-6.

Table 5. Organoleptic characteristics of the taste of experimental samples of young sparkling wines

Title of the sample	Taste characteristics	Tasting assessment, points
Rose sparkling wine, FMWM	Not harmonious, with residual sugars, sauerkraut tones, with good CO ₂ saturation	7,93
Rose sparkling wine, PYC	Full, refreshing, with berry shades, with a "piquant" bitterness, with good CO ₂ saturation	8,38
Red sparkling wine, FMWM	Full, velvety, with berry shades, with light extraneous shades, with good CO ₂ saturation	8,12
Red sparkling wine, PYC	Full, harmonious, velvety, refreshing, with varietal shades, with good CO ₂ saturation	8,44

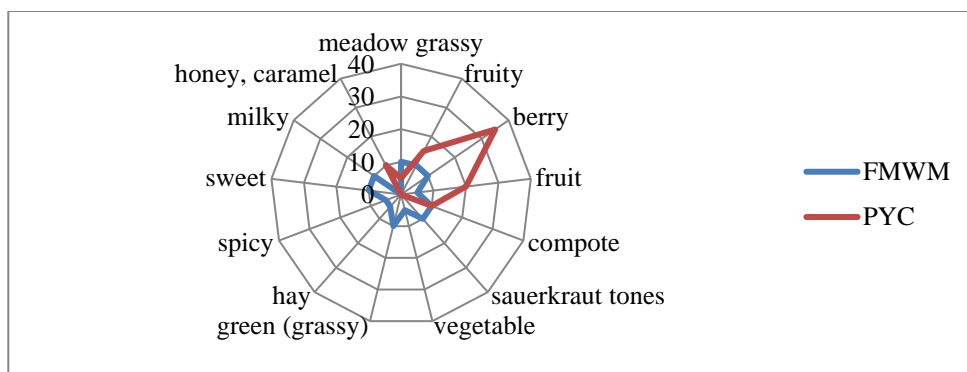


Fig. 5. Aromatic profiles of young sparkling rose wines

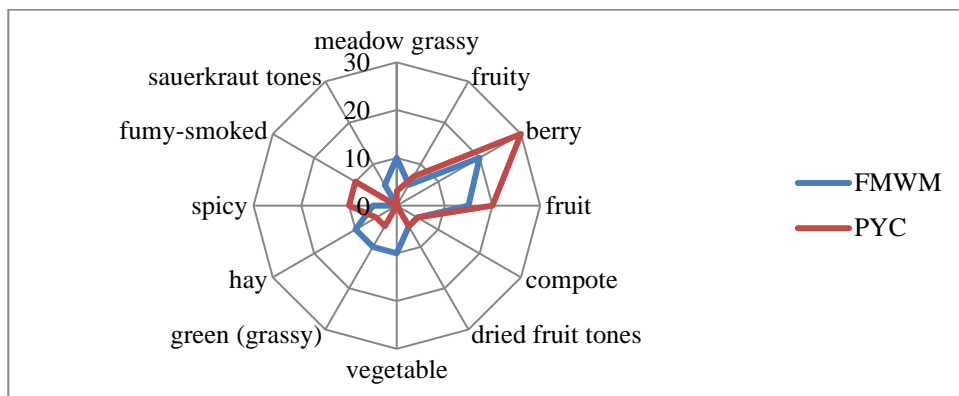


Fig. 6. Aromatic profiles of young sparkling red wines

Visual evaluation showed that a sample of rose sparkling wine prepared on wild microflora was with opal. The rest of the samples were transparent. The appearance of sauerkraut tones in the experimental sample of rose wine was the result of the vital activity of lactic acid bacteria, which activated the processing of residual sugars into lactic acid after the alcoholic fermentation was stopped [25]. A sample of red sparkling wine prepared on wild microflora was more pure in bouquet and taste, although it also had light extraneous shades. All control samples received higher tasting ratings for a clean, bright varietal aromatic complex with berry and fruit shades and a harmonious taste. In this

connection, to obtain higher-quality young sparkling wines using the microflora of grapes, it is promising to carry out the selection of yeast with the specified properties.

4 Conclusions

For the preparation of young sparkling wines, you can use the fermenting mixture of wild microflora, it contributes to the formation of combined forms of carbon dioxide, better foaming and sparkling properties and greater accumulation of glycerin. However, to improve the bouquet and taste of young sparkling wines, it is necessary to select perspective strains of wild yeast *Saccharomyces cerevisiae*, suitable for champagnization. The technology under study can be applied in small enterprises without the use of complex technological equipment. The introduction of this technology will help to increase the total output of sparkling wines. Research in this area is planned to continue.

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