

# Potential of production of orange type wines with increased bioactive substance content from new selection grape cultivars from the Republic of Moldova

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## ABSTRACT

Dry white wines with an increased content of bioactive substances, known as "Orange wines," have become a captivating subject in the wine industry, rooted in the millennia-old tradition of the South Caucasus and gaining global popularity. In the Republic of Moldova, producers such as "Crama Domească", "Gogu Winery", and "Vinăria Novac" have explored the "Orange wine" method with indigenous cultivars, bringing innovations to the wine landscape. The study, conducted at the Laboratory of "Biotechnologies and Microbiology of Wine," focused on four promising local cultivars: 'Viorica', 'Florica', 'Riton', and 'Legenda'. To optimize the content of bioactive substances, the process involved selecting grapes at technological maturity, crushing-destemming with sulfiting, and fermentation with active yeasts. The maceration-fermentation process, ranging from 30 to 90 days, proved to be a crucial factor for the maximum extraction of phenolic substances. Physico-chemical analyses revealed slight changes in titratable acidity and pH, remaining within appropriate limits, with significant concentrations of antioxidant phenolic substances. In this context, "Orange" wines from Moldavian cultivars have demonstrated the potential to provide an innovative alternative in the wine market. However, in light of the results, further studies are recommended to precisely determine the influence of technological procedures on the composition of "Orange" wines and identify other cultivars with increased potential. This approach would strengthen the position of the Republic of Moldova in the production of wines with high bioactive substance content, solidifying its reputation in the global wine landscape.

**Keywords:** new selection grapes cultivars, proanthocyanidins, must, maceration-fermentation, bioactive substances (BAS)

## INTRODUCTION

Dry white wines with elevated levels of bioactive substances have become a prominent focus of research and innovation in the wine industry, known as "Orange wine," a term coined by British importer David Harvey in 2004. Originating from the ancient winemaking traditions of the South Caucasus, particularly in Georgia for over 8,000 years (Makhviladze and Kvartskhava, 2022) this method has generated increasing interest in countries such as Italy, Slovenia, and Georgia, presenting an innovative alternative less embraced in France, Austria, Croatia, and the USA.

The "Orange" wines gained high popularity in 2017, earning the title of the "drink of the

year" by "The Independent" newspaper. In the Republic of Moldova, these wines are still relatively undiscovered, but local producers, including 'Crama Domească', 'Gogu Winery', 'Castel Mimi', 'Carlevana Wineri', 'Vinăria Novac', 'Vinăria Purcari', 'Basavin Wineri', 'Crama Mercești', and 'Kvint' ([\\*https://diez.md/2022/08/27/lista-vinariilor-din-moldova-care-produc-vinuri-orange-cu-arome-intense/](https://diez.md/2022/08/27/lista-vinariilor-din-moldova-care-produc-vinuri-orange-cu-arome-intense/)), have started to gain significant attention by adopting the "Orange wine" method, introducing innovation to the Moldavian wine industry.

Despite extensive research on the production of red wines rich in bioactive substances, there are few reports regarding the influence of technological processes on the physicochemical composition of dry white wines, especially concerning antioxidant phenolic substances. Market trends increasingly emphasize the production of wines from new or indigenous grape cultivars, fully leveraging the terroir effect of the viticultural area and creating original wines specific to each country.

In this context, the technology for producing dry orange wines from indigenous grape cultivars in the Republic of Moldova represents a relatively high-profile area, more of an experiment in limited quantities than a planned and stable process due to the lack of extensive research in this domain. The study focuses on four promising grape cultivars from the Republic of Moldova: 'Viorica', 'Florica', 'Riton', and 'Legenda' evaluating their high potential to produce (Perstnirov *et al.*, 2020; Makhviladze and Kvartskhava, 2022) wines with an increased content of bioactive substances and analyzing the high impact of various technological processes on the physicochemical, microbiological, and organoleptic characteristics of the obtained "Orange" (<https://diez.md/2022/08/27/lista-vinariilor-din-moldova-care-produc-vinuri-orange-cu-arome-intense/>).

## **MATERIALS AND METHODS**

The research was conducted at the Laboratory of Biotechnologies and Microbiology of Wine at the Scientific Practical Institute of Horticulture and Food Technologies (SPIHFT) in the Republic of Moldova. White dry wines obtained from newly selected grape cultivars 'Viorica', 'Florica', 'Riton', and 'Legenda', (Taran, 2022) cultivated in the mother vineyards of SPIHFT, were used for the research. To produce white wines with an increased content of bioactive substances and to avoid SBA oxidation, grapes reaching technological maturity with a minimum sugar content of 220 g/dm<sup>3</sup> were used. The grapes underwent the process of crushing-destemming with the addition of sulfur dioxide to the must at a rate of 70 mg/kg SO<sub>2</sub>. The obtained must was transferred to fermentation vessels and inoculated with dry active yeasts Oenoferm Freddo (ERBSLOH) at the recommended dose of 2-3 g/hL and a complex fermentation activator, Phosphates titres (IOC) 2.5 g/hL, and vitamin B1 Actibiol (LAMOTHE-ABIET) 2-3 g/hL. Fermentation took place at temperatures ranging from 14 to 18°C. After the fermentation process was completed, the vessels were filled and sulfited with a dose of 30 mg/dm<sup>3</sup>. Additionally, the must underwent a maceration process for 30, 60, and 90 days, which is a mandatory condition for the maximal extraction of phenolic substances and proanthocyanidins predominantly located in the grape seeds.

Physico-chemical indices were determined according to standardized methods and OIV methods outlined in the Technical Regulation "Methods for the Analysis in the Field of Wine Production" (Government Decision of the Republic of Moldova No. 708 dated September 20, 2011). The total content of phenolic substances was determined using the spectrophotometric method with the Folin-Ciocalteu reagent, where gallic acid served as the reference substance (Gherjicova *et al.*, 2022).

Gallic acid and ascorbic acid were determined using the gas-liquid chromatograph HP 4890D with a flame ionization detector (Scorbanov *et al.*, 2012) The total content of

proanthocyanidins was determined using the spectrophotometric method with DAMAC reagent (Tardea, 2007).

## RESULTS AND DISCUSSION

In the context of the Republic of Moldova, the technology of producing dry orange wines from native grape cultivars represents a relatively new field, characterized more as an experiment than a planned and widespread process. For this reason, we selected four promising new selection cultivars from the Republic of Moldova, 'Viorica', 'Florica', 'Riton', and 'Legenda' to assess their technological potential in producing dry white wines with an increased content of bioactive substances.

One of the essential factors in the technological process is the use of grapes rich in phenolic compounds, and their optimal ripening influences the content of proanthocyanidins in seeds and skins, contributing to achieving a superior aromatic and antioxidant profile in the resulting wines. The evaluation of the technological potential of these grape cultivars included determining the optimal contact period between the liquid and solid phases, with detailed results presented in Table 1, which encompasses the physico-chemical data of wines from the 'Riton', 'Florica', 'Viorica', and 'Legenda' cultivars, harvested in 2022 and subjected to maceration for up to 90 days.

**Table 1.** Physico-chemical indices of dry white wines with advanced content of bioactive substances produced from new selection grape cultivars with varying maceration periods (harvested in 2022).

№	Grape cultivar	Maceration time	Ethyl alcohol, % vol.	Mass concentration of				pH
				sugars, g/dm <sup>3</sup>	titratable acids, g/dm <sup>3</sup>	volatile acids, g/dm <sup>3</sup>	SO <sub>2</sub> free / total g/dm <sup>3</sup>	
1.	'Riton'	blank	13,2	0,9	6,68	0,33	30/100	3,15
2.		1 month	12,8	1,5	6,60	0,33	13/85	3,44
3.		2 months	12,7	1,5	6,53	0,46	15/85	3,44
4.		3 months	12,7	1,5	6,38	0,40	15/85	3,46
5.	'Florica'	blank	15,4	2,6	7,05	0,36	15/107	3,13
6.		1 month	15,5	2,7	6,98	0,40	10/80	3,28
7.		2 months	15,4	2,8	6,90	0,40	12/66	3,28
8.		3 months	15,4	2,7	6,80	0,43	25/62	3,29
9.	'Vioroca'	blank	15,8	3,0	6,45	0,33	38/93	3,28
10.		1 month	15,7	2,1	6,38	0,40	16/74	3,48
11.		2 months	15,5	1,9	6,30	0,46	16/74	3,50
12.		3 months	15,4	1,4	6,23	0,50	10/71	3,50
13.	'Legenda'	blank	15,6	2,8	5,93	0,40	23/105	3,18
14.		1 month	15,6	2,6	5,85	0,46	26/80	3,28
15.		2 months	15,5	2,2	5,78	0,46	21/75	3,31
16.		3 months	15,5	2,2	5,70	0,50	20/69	3,33

From the results presented in Table 1, it can be observed that the lowest ethyl alcohol content in dry white wines was determined in the 'Riton' dry white wine with maceration-fermentation periods of 2 and 3 months, having an alcohol content of 12.7% vol., while the 'Riton' (control) wine has an alcohol content of 13.2% vol. The dry white wine from the 'Florica' (control) has an alcohol content of 15.4% vol., and the variants with maceration-fermentation periods of 1, 2, and 3 months all have an alcohol content of 15.5% vol. The

dry white wine from the 'Viorica' cv. (control) has the highest alcohol content of 15.8% vol., and the variants with maceration-fermentation periods of 1, 2, and 3 months have alcohol concentrations of 15.7%, 15.5%, and 15.4% vol., respectively. The difference between alcohol content values based on the maceration process ranges from 0.07% to 0.13% alc., which is approximately 1% of the respective parameter value. Therefore, we can conclude that the influence of the maceration-fermentation process is not significant on the alcohol content.

Regarding the mass concentration of titratable acids in dry white wines, it decreased after alcoholic fermentation, with values ranging from 5.70 to 7.05 g/dm<sup>3</sup>. Wines obtained from the 'Florica' cultivar have a higher concentration of titratable acids (6.98 and 7.05 g/dm<sup>3</sup>), but the values fall within the limits for dry white wines. The 'Riton' dry white wine has a titratable acid concentration ranging from 6.38 to 6.68 g/dm<sup>3</sup>, and from the 'Viorica' grape cultivar, a concentration of 6.23 and 6.45 g/dm<sup>3</sup> is observed.

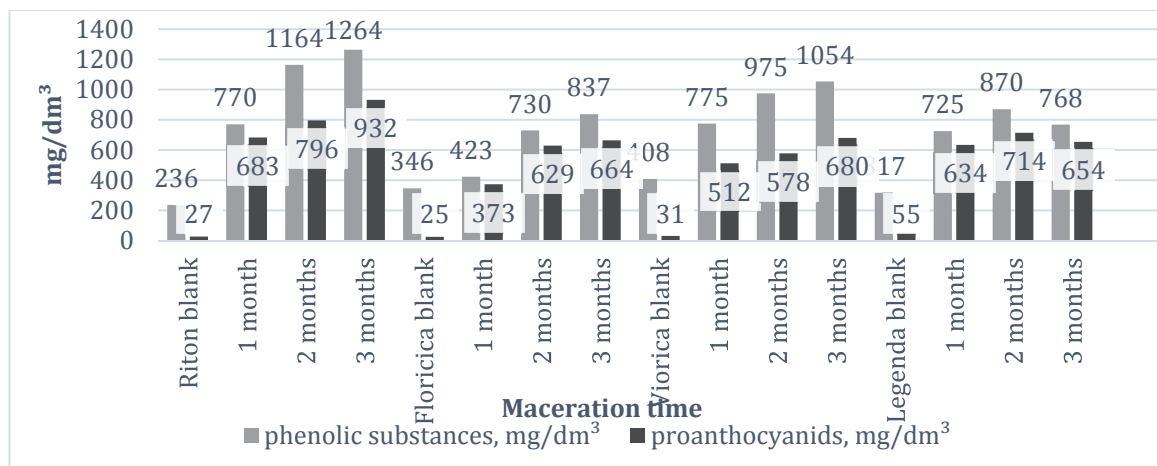
Regarding the influence of the maceration-fermentation process on the mass concentration of titratable acids, a decrease of about 3% can be observed in all wines obtained with maceration-fermentation.

The studied dry white wines present low values of volatile acidity within the permissible limits for young wines, without exceeding the limit of 0.46 g/dm<sup>3</sup>.

pH index values vary between 3.13 and 3.50 in the studied white wines, with the lowest value recorded for the 'Florica' dry white wine and the highest for the Viorica dry white wine. In the case of the 'Legenda' cultivar, the pH index value varies between 3.18 and 3.33. During the maceration-fermentation process, an increase in the pH index was observed in all studied wines, with the most significant increase recorded in the 'Riton' wine, where an increase of 0.29 units was determined.

In this way, the maceration-fermentation process lasting 30-90 days does not negatively influence the basic physico-chemical indicators of dry white wines. The mass concentration of titratable acids registered a slight decrease after maceration, and the pH values showed a slight increase, but they remained within the appropriate limits for dry white wines. Additionally, the maceration process had a minor impact on the alcohol content.

The content of phenolic substances and proanthocyanidins was determined in the dry white wines. The results are presented in Figure 1.



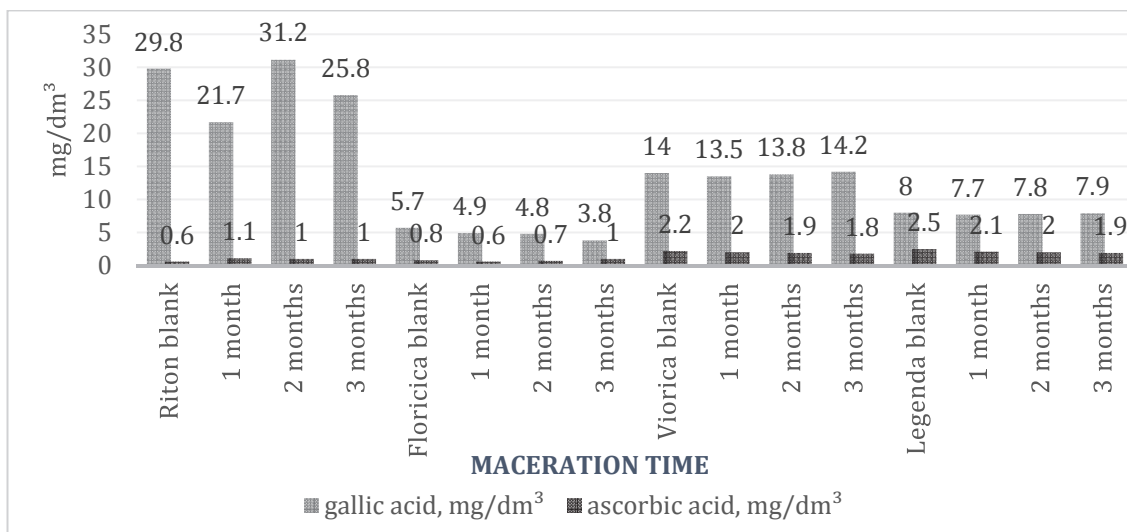
**Figure 1.** The content of phenolic substances and proanthocyanidins in dry white wines with different maceration time.

Analyzing the data in Figure 1, it can be observed that extending the maceration duration for the white wines obtained from the 'Riton' cv. leads to an increase in the concentration of phenolic substances, especially in the first 60 days. The concentration of

proanthocyanidins accumulates intensively in the first 30 days, increasing on average by 125 mg/dm<sup>3</sup> per month and reaching a value of 932 mg/dm<sup>3</sup> at 90 days of maceration. Initially, dry wines produced from the 'Florica', 'Viorica', and 'Legenda' cvs. have a higher content of phenolic substances. However, during the maceration process, the extraction is more intense in the first 60 days. Prolonging the maceration duration to 90 days is characterized by a reduced accumulation of phenolic substances, and in the case of wine produced from the 'Legenda' cv., there is a decrease in these compounds. The accumulation process of proanthocyanidins follows the same dynamic as phenolic substances, but the total accumulated amount is lower compared to the 'Riton' cv.

Furthermore, the impact of the maceration duration on the production of Orange wines from the studied new selection cultivars was evaluated, considering the content of gallic and ascorbic acid, two compounds important for the antioxidant capacity of wine. The results of these analyses are presented in Figure 2.

From the obtained results described in Figure 2, it can be observed that dry white wines from the 'Riton' new selection cultivar record the highest gallic acid content, reaching 31.2 mg/dm<sup>3</sup>, with a constant extraction throughout the entire maceration process. These are followed by dry white wines produced from the 'Viorica' cultivar. Dry white wines from the 'Legenda' cv. have a medium gallic acid content, highlighting efficient extraction up to 60 days of maceration. In the case of dry white wines produced from the 'Florica' cv., the lowest gallic acid content is recorded, reaching its maximum at 3 months of maceration, with a value of 7.9 mg/dm<sup>3</sup>.



**Figure 2.** The content of gallic acid and ascorbic acid in dry white wines with different maceration time.

The positive role of ascorbic acid as an antioxidant is well-known, which is why its content has been evaluated in the studied wines to analyze its contribution to the total biologically active substances in wines derived from the 'Riton', 'Florica', 'Legenda' and 'Viorica' grape cultivars. The highest content of ascorbic acid is identified in 'Legenda' wine, reaching 2.5 mg/dm<sup>3</sup>. Dry white wines from the 'Riton' and 'Florica' cvs. record the lowest values of ascorbic acid, ranging from 0.6 to 1.1 mg/dm<sup>3</sup>. In all wine samples, the highest values are recorded in the control samples, and the maceration-fermentation process leads to a reduction in the concentration of this antioxidant.

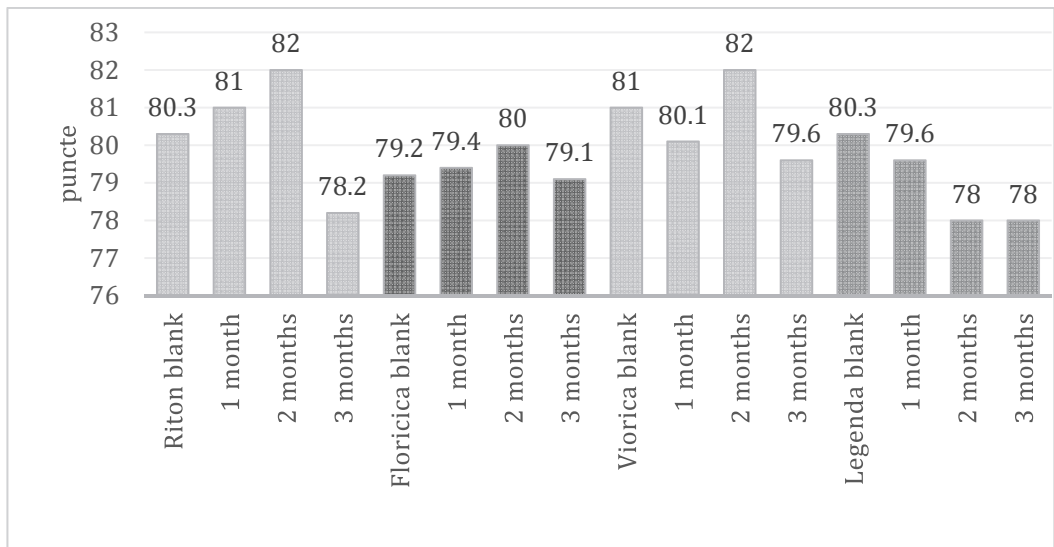
In the samples of dry white wines, the content of rutin, quercetin, resveratrol, and the redox potential were measured, because these are also components of the biologically active substances. The results are presented in Table 2.

From Table 3, it can be observed that the concentration of resveratrol in all samples of dry white wines is below the method's detection threshold. Maceration-fermentation up to 30 days increases the content of rutin and quercetin, especially in the dry white wines produced from the 'Florica' and 'Viorica' cultivars. However, these values are lower compared to red wines. The extension of the maceration period leads to a decrease in these compounds in all examined samples.

**Table 2.** Rutin, quercetin, resveratrol concentrations, and redox potential in dry white wines obtained from newly selected grapes at different maceration periods (h.y. 2022)

Nº	Grape cultivar	Maceration time	Routine, mg/dm <sup>3</sup>	Quercetin, mg/dm <sup>3</sup>	Resveratrol, mg/dm <sup>3</sup>	Redox potential, mV
1.	'Riton'	Martor !!!	0,3	0,2	≤ 0,1	216
2.		blank	0,2	0,2	≤ 0,1	219
3.		1 month	0,3	0,1	≤ 0,1	220
4.		2 months	0,3	0,2	≤ 0,1	220
5.	'Florica'	3 months	4,6	2,2	≤ 0,1	213
6.		blank	4,4	2,3	≤ 0,1	228
7.		1 month	4,0	1,9	≤ 0,1	228
8.		2 months	3,2	2,0	≤ 0,1	229
9.	'Vioroca'	3 months	3,4	1,8	≤ 0,1	208
10.		blank	5,2	1,1	≤ 0,1	216
11.		1 month	4,1	1,3	≤ 0,1	216
12.		2 months	3,8	1,9	≤ 0,1	221
13.	'Legenda'	3 months	5,0	0,9	≤ 0,1	212
14.		blank	4,8	0,6	≤ 0,1	232
15.		1 month	3,9	0,7	≤ 0,1	233
16.		2 months	3,2	1,0	≤ 0,1	231

The redox potential in all analyzed dry white wines increases due to the maceration process, but extending the process beyond 30 days practically does not modify this index. Dry white wines with an increased content of bioactive substances underwent organoleptic evaluation by the Sensory Analysis Commission of ISPHTA, and the results obtained are presented in Figure 3.



**Figure 3.** The organoleptic evaluation of dry white wines with different maceration time.

The organoleptic analysis from Figure 3 of the dry white wines produced from the new selection cultivars 'Riton', 'Florica', 'Viorica', and 'Legenda', with different maceration periods, reveals significant variations in organoleptic characteristics.

'Riton' Orange wines (1-month maceration) receive an average score of 81.0 points, characterized by a light straw color, a clean aroma with fruit nuances, and a fresh, full taste with slight tannins and extract. Extending the maceration period to 2 months improves the qualities, reaching 82.0 points, but prolonging it to 3 months results in a decrease in quality.

'Florica' and 'Viorica' wines show variability depending on the maceration duration. 'Florica' with 1 month of maceration scores 79.4 points, while the 2 and 3-month variants highlight oxidation and alcoholization nuances, affecting quality. 'Viorica' with month scores 80.1 points, and those with 2 and 3months exhibit orange and tannin nuances, with average scores of 82.0 and 79.6 points, respectively.

The 'Legenda', with 1 month of maceration, scores 79.6 points, and those with 2 and 3 months show oxidation nuances, with a decrease in quality and average scores of 78.0 points. In conclusion, the analysis shows that the maceration duration significantly influences the qualities of the wines, with notable variations depending on the cultivar and processing period.

Analyzing the presented data highlights that the new selection cultivars with the highest potential for producing wines with an increased content of bioactive substances are 'Riton' and 'Viorica'. These cultivars have achieved notable results regarding the concentration of phenolic substances, especially proanthocyanidins, depending on the maceration duration. They have also demonstrated superior organoleptic qualities compared to the other cultivars, showcasing clean, complex and full aromas, fresh taste.

'Riton', in particular, achieved the highest content of phenolic substances, and the 2-month maceration variant highlighted the best organoleptic characteristics. On the other hand, 'Viorica' presented a complex and intense aroma with specific cultivar notes and received notable ratings, especially for the 2-month maceration variant.

In conclusion, 'Riton' and 'Viorica' represent cultivars with the highest potential for producing dry white wines with an increased content of bioactive substances, offering a balanced combination of chemical and organoleptic aspects.

## CONCLUSIONS

The results of maceration on dry white wines from the cultivars 'Riton', 'Florica', 'Viorica', and 'Legenda' indicate that the maceration-fermentation process does not negatively affect their basic physico-chemical properties. Titratable acids and pH remain within appropriate limits, with slight changes in concentration and pH.

For obtaining an optimal concentration of bioactive substances (BAS), the maceration-fermentation process of dry white wines from 'Riton' cultivar demonstrates remarkable efficiency in extracting these substances, with significant values throughout up to 90 days of maceration.

'Riton' and 'Viorica' cultivars stand out as having the highest potential in producing wines with an increased content of bioactive substances.

In light of the obtained results, it is evident that the 'Riton' and 'Viorica' cultivars show significant potential for producing "orange" wines with an increased content of bioactive substances. It is crucial to continue studies to confirm and strengthen these conclusions and determine the optimal maceration-fermentation periods, ensuring the success and stability of the technological process.

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