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Differences in muscat wine aroma composition depending maceration and fermentation processes

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Abstract

The present paper represents a study upon the influence of the prefermentative operations and of certain biotechnological factors on the flavored complex and the main quality chemical parameters of the wines obtained from the grapes variety Muscat Ottonel, from the Dragasani vineyard, Romania. As a result of the studies carried out by means of the gas-chromatography, it has been noticed that the different types of maceration enrich the must in free flavors and influence the contents in hexanol, hexanal and the PH of the obtained wines. The association of the maceration with the addition of enzymes in the must present favorable consequences of oenological type.

Keywords: maceration, enzymatic preparations, free volatile terpenes, terpenes bound in precursors, phenol compounds.

1. Introduction

The flavored wines, obtained from certain varieties of grapes which have the ability to biosynthetisised and accumulate in the peel of the grapes flavored substances of terpenic type (linalool, geraniol, terpineol), which confer them the character of „muscat", require a specific technology because the flavored substances from the grapes are stored in the superficial or more profound layers of the peel (STOICA FELICIA, [26]).

Over the last century, the origins of wine aroma have been a big interest, developing in this way modern analytical techniques (POLÁŠKOVÁ & al. [20], S.E. EBELER & J.H. THORNGATE [10], A. L. ROBINSON & al., [21]).

Due to the organoleptic and composition qualities of the flavored wines, these ones are more and more required by the competent consumer.

The flavored wines have witnessed a clear commercial progress. The researchers P. TORRES, A. SEGUIN, A. BRUGIRARD, [31], taking into account both the consumers demands and their own observations, sustain that this increase is not the result of the hazard and the obtaining of the „modern Muscat" represents a sustained scientific effort of experiments and researches that has lasted for 15 years.

Aromatic volatile compounds are crucial to determine consumer approval of wines. The simultaneous presence aromatic constituents like terpenes associated with grapes and winemaking defines a high quality and unique wine (A. SCACO & al., [25]).

Researchers from all over the world, passionate and interested in both, the raw material used – varieties of flavored grapes- and the extraction, intensification and maintenance of the

flavor in the finished product, turned their attention to the most important technological link: maceration, fermentation-maceration (by using different enzymatic preparations and selected yeasts), the control and suspend of the alcoholic fermentation, the capture of the flavors (M. ROSARIO SALINAS & al., [22]; J. BAKKER, [2]; M.M. LOSADA & al., [17]) .

CORDONNIER R. & al. [8] point out that at certain varieties, the flavored potential of the grapes consists of a „pool" of free, flavored substances, so odorants represented especially by terpenols and a hidden "pool", un-odorant represented by the precursors of the terpenols formed from polyols and complex terpenil-glycosides. The flavored fraction under the form of precursors is superior to the free fraction and can reach important contents of some mg/l. Thus, in the grapes there is a capital of flavors insufficiently capitalized.

CANAL – LLAUBERES [6] proposes the use of the enzymatic pectolytic preparations for the hydrolysis of the flavor precursors, after he proved in 1992 the efficacy of the Novoferm 12 G device upon certain flavored varieties such as Muscat, Gewurztraminer. Same results obtain KELEBEK M. & al., [16].

GÜNATA Y.Z. & al. [14], studying the influence of exogenous glycosidase of fungal origin (α-arabinosidase, α-ramnosidase, β-apiosidase, β-glucosidase) in the obtaining the sweet, natural wines and of dry wines of Muscat de Frontignan variety, noticed that the use of the enzymes in the case of the dry wine ended with the increase of the free fraction of the flavor, through the increase of certain terpenes (nerol, geraniol, citronellol, α-terpineol, hidroxilinalol), on one hand and on the other hand, through the appearance of nonisoprene compounds. At the use of the enzymes in the elaboration of the natural sweet wines, the hydrolysis of the glycosidase precursors of the flavor was interrupted in the monoglucosides phase because of the inhibition of the β-glucosidase in the presence of the glucose.

Also, D. TATE and A. G. REYNOLDS, [30] notice that activity of β-glucosidase in presence of yeasts starins and comercial enzyme preparation increasing wine aroma intensity.

CELLOTI E., & al., [7] also sustain the previous statements concluding that by means of these combined methods, the flavored quality of the product is intensified and maintained at a high level.

So, the capitalization possibility of the wines flavored potential in flavored hydrolyzed precursors has been the research subject for many authors. Thus, BOIDRON J.N., TORRES P., [5]; RAZUNGLES A. & al., [23]; GUNATA Y.Z & al., [14]; STOICA FELICIA, [28] studied the action of the grape glycosidase; DARRIET P. & al., [9]; GUERZONI M. & al., [13], ARAGON P. & al., [1] studied the action of the addition of yeasts upon the flavoured potential, MARENGHI M. [18] pointed out the importance of the maceration temperature and BERTRAND A., & al., [4]; HEROIU ELENA, [15]; NININO M.E., [19]; SALLINAS M. R. & al., [24]; STOICA FELICIA & al., [27] showed the benefic influence of the use of enzymatic preparation on the extraction and quality of the flavors through the precursors hydrolysis.

2. Material and methods

Sampling and Processing

The study was preformed on the variety of Muscat Ottonel, flavored grapes, cultivated in Drăgăşani (Vâlcea) vineyard, the harvest of 2012 and 2013.

Analyzes were performed on grapes at harvest: the contents in glucides, acidity, polyphenols and free volatile and bond terpenes.

At the involvement of maceration, pre-fermentative (before fermentation) technique, which implies a controlled and limited contact between the two phases of the mixture resulting after pressing, from flavored grapes, there have been experimented the following aspects:

The influence of duration of maceration on the terpenic flavored nature of the Muscat Ottonel wines. Have used these contact durations between phases: 0, 6, 12, 24, 36 hours. The effects of the maceration length of time have been compared to the free and bound in precursors flavors content, from the grapes.

Having as an objective the establishment of the most favorable length of maceration, there have been set the following experimental variants: V_1 – vinification in white, V_2- classical maceration, at 25^0C for 36 hours; V_3- maceration in ROTO at 20^0 C for 12 hours; V_4- the treatment of the must (mixture resulting after pressing) with thermic shock at 4 ^{0}C and maceration with pectolytic enzymes for 24 hours.

To specify coupling effects maceration with pectolytic enzymes on aromatic constituents of wines Muscat Ottonel, it has been set an experience which comprises the following variants: V- control sample, vinified in white; V_1- maceration of 12 hours, V_2- maceration of 24 hours; V_3- maceration of 12 hours +Ep- Suprazym; V_4- maceration of 12 hours +Ep+ LSA.

The maceration temperature was of 18 ^{0}C and the suphitation dose was the same at all the variants of 3 g SO_2/100 kg. The enzymatic preparation (Ep) that was used was Suprazym in a dose of 1,5 g/100kg of grapes and the selected yeasts (LSA) Enolevure A.R.

The influence of the different variants of maceration on the terpenic complex of the Muscat Ottonel wines.

The two „coordinates" of the maceration process have a decisive influence on the extraction of the flavors from the quantity but especially from the quality point of view, being considered both independent from each other and in interaction with each other. The set of experimental variants was the following one: V- vinification in white, V_1- maceration of 8 hours at 22^0 C; V_2- maceration of 12 hours at 22^0 C; V_3- maceration of 24 hours at 22^0 C; V_4- maceration of 12 hours at 8^0 C; V_5- maceration of 24 hours at 8^0 C.

At all the experiments that have been carried out, it was assured a minimum dose of antioxidant protection, with the application of 3g SO_2/l, in the conditions of the use of certain raw materials found in a perfect maturation and phytosanitary state.

Analitycal Methods

The content in glucids, PH, superior alcohol has been determined in accordance with the official methods of the European Union (European Union, [11]).

Extraction of the musts/wines

The wine sample is treated with sodium chloride or ammonium sulphate and is introduced in a mixed extractor. It comes into contact with the solvent kept at boiling through a thermostatic electrical heating. The extracted substances which are less volatile than the solvent gather up and can be kept at temperatures of -4 degrees Celsius in a balloon with a cork. The extract can be concentrated through distillation in a Vigreux column and stored at -4 degrees Celsius.

Chromatographic Method

The samples have been analyzed with a Perkin Elmer Model 900 chromatograph. The device includes a system of the carrying gas which provides a constant flow at two test injectors, a thermostat for the exact control of the column temperature and a manifold, which allows the use of the FID detectors and a catharometre which makes possible the collecting on fractions. The separation columns that have been used are: SCOTT column of 15 meters length and 0,5 mm inner diameter, filled with Carbpwax 1540, SCOTT column of 15 meters length and 0,5 mm inner diameter, filled with Carbpwax 20M, stain column of 3,6 mm length and 2 mm inner diameter filled with Diglicerol, stain column of 3,6 mm length and 2 mm inner diameter filled with a mixture of Eritriol, sorbitol and diglycerol, stain column of 3,6

mm length filled with Reoplex 400, stain column of 3,6 mm length filled with FFAP, stain column of 3,6 mm length filled with DEGS(succinatglicol diethyl) and a column of 1 m length filled with Porapak Q. The work conditions for each column have been established through testing, by using the groups of the compounds to be analyzed and the maximum temperatures for each stationary phase. The calculation of the tops surface has been done with an electronic integrator. In order to confirm the presence of certain compounds, signaled in the literature, there have been determined the restraint times on several types of columns and they have been compared to the restraint times of the pure compounds. The comparison has been made through successive chromatography of the control sample and extract.

3. Results and discussion

Maceration – fermentation-maceration – is a complex process of biochemical and biophysical nature, but also a technological link with essential implications in the obtaining typical flavored wines. It implies keeping in contact, for a certain period of time, the two phases of the mixture resulting after pressing in order to extract the flavored substances from the peel.

Taking into account the information contained in Table 1 regarding the influence of maceration upon the content in terpenes and upon other main features of the must, it is emphasized the fact that maceration determines the enriching of the must in terpenic compounds from all the categories.

Of the first three variants, the V3 one clearly detaches, being macerated in rotative recipients but also in the absence of alcoholic fermentation which ensures the accomplishment and maintenance of the highest contents in free terpenic compounds and especially in: linalool, geraniol, α -terpineol.

Table 1. The influence of the maceration process upon the content in terpenes compounds and upon other main features of the Muscat Ottonel must

Variants	Characteristics									
	Glucides g/l	pH	Hexanal µg/l	Hexanol µg/l	2hexenol µg/l	TVF µg/l	TBP µg/l	Linalol µg/l	α terpineol µg/l	Geraniol µg/l
V_1	226	3,21	1499	216	146	985	2240	246	159	74
V_2	160	3,26	2201	339	42	1925	2900	299	289	244
V_3	226	3,25	2072	729	46	2130	3000	299	289	244
V_4	226	3,31	3284	687	59	2190	3070	381	320	185

TVF- free volatile terpens, TBP-bound in precursors terpens

Applying a thermical shock before maceration (V4) was followed by the increase of the content in terpenes bound in precursors, on one hand, and of linalool and a-terpineol, on the other hand, geraniol recording a slight decrease.

The combined effect of cooling-maceration determines essential increases in terpenes concentration, situation which can be attributed to the additional enriching of the free forms.

The greater quantity of terpenols obtained at the V4 variant confirms the fact that these compounds are produced either through chemical synthesis in the fraction juice + pulp or through acid hydrolysis of the glycosides terpenols.

The maceration at low temperatures presents advantages and disadvantages according MARENGHI M [18] in 2004. The advantage is represented by a better extraction of the flavored compounds and lower quantities of polyphenols and the disadvantage is represented by the existence of a higher duration of contact between the phases of the mixture resulting after pressing.

Adopting the classical procedure of maceration fermentation, at the elaboration of the Muscat Ottonel wine, leads to the obtaining of more reduced contents of both free, volatile terpenes and terpenes bound in precursors and especially linalool. The more reduced level of these ones is a consequence of the fact that, beside extraction, there are important losses through fermentation (Table 2).

Applying a thermic shock before maceration was followed by the increase of bound terpenes, of linalool and a-terpineol.

The extraction that is achieved in the maceration phase or the maceration-fermentation until the phases separation is not of the same intensity for all the terpenic compounds. The geraniol records the maximum rhythm of accumulation being followed in a decreasing order by the free, volatile terpenes, a-terpineol, linalool and finally the terpenes bound in precursors.

Table 2. The evolution of the content in terpenes throughout the elaboration of the Muscat wine

Variants	Glucides g/l	pH	Alcohol vol%	Terpenes µg/l		Linalol µg/l	α terpi-neol µg/l	Geraniol µg/l
				TVF	TBP			
Must initially	226	3,21	-	985	2240	247	159	74
The solid fraction separation								
V_2	160	3,26	3,5	1927	2925	298	289	240
V_3	226	3,25	-	2130	3023	375	258	209
V_4	226	3,31	-	2195	3072	379	310	182
At the end of alcoholic fermentation								
V_1	36,0	3,13	11,2	1021	1922	208	159	120
V_2	36,0	3,32	11,2	1610	2609	270	248	255
V_3	32,5	3,38	11,4	1860	2515	323	225	274
V_4	35,0	3,38	11,4	1810	2712	336	239	323
At 15 days after alcoholic fermentation								
V_1	36,0	3,01	11,2	1100	1908	209	160	120
V_2	36,0	3,14	11,2	1905	2506	275	252	261
V_3	32,0	3,15	11,4	1851	2410	333	230	270
V_4	35,0	3,15	11,4	1873	2602	346	250	322

TVF- free volatile terpens, TBP- terpens bound in precursors

During the alcoholic fermentation, the content of terpenic compounds diminishes with the exception of geraniol.

This behavior becomes the balance point of the double process extraction, on the one hand, and of transforming + loss, on the other hand. Under the conditions of the main sources disappearance, the solid phase of he must, the prevalence of the consume takes place at the decrease of the content in terpenes compounds. The adopted maceration proceeding determines

differences regarding the decrease rhythm of the terpenes compounds during the fermentation. Thus, the contents in linalool and terpenes bound in precursors lower quickly in the must obtained through maceration in rotative tanks according with the results presented by HEROIU ELENA, [15].

The procedure of maceration in ROTO rotative tanks, characterized through a process going on in a closed system and featured by an obvious diminished fermentative activity, makes easier the gathering of volatile terpenes.

Obvious differences regarding the decreasing speed of the content in terpenes during the fermentation are revealed in accordance with the examined compound. The bound terpenes have a superior rhythm of decreasing unlike the volatile ones, α - terpineol decreases quickly than the linalool and the geraniol keeps increasing the content even during the alcoholic fermentation.

Vinification in white offers interesting data regarding the particular behavior of each type of terpenes compound.

The free volatile terpenes present increases in content. This indicates, on one hand, that the fractions of pulp and peels remained in the must constitute a source of terpenes and on the other hand, that their release from the bound forms is quite important. According to BAYONOVE [3] the increase of the free volatile terpenes is ascribed to the activity of the glucosides produced by leavens upon the release of the terpenes or that the synthesis and metabolism of the terpenes is the result of the activity belonging to a yeast strain.

The terpenes bound in precursors decrease with approximately the same rate at all the variants. The decrease of the linalool content is the most stressed of all. The content of α - terpienol and especially of geraniol obviously increase throughout the fermentation of Muscat Ottonel must, vinified in white. After the end of the alcoholic fermentation, the content of free volatile terpenes increases, especially at the variants which are macerated in a classical manner and with thermic shock. It increases slowly at the vinification in white and remains constant at the maceration wine in rotative tanks. The content in terpenes bound in precursors decreases with approximately the same rate at all the variants. The content in linalool increases at all the variants and even more at the ones macerated in ROTO with or without thermic shock. In accordance with the applied technology, the content of geraniol modifies in one way or another, in very tight limits. The content in α - terpineol behaves likewise, the only difference being the existence of a more obvious increase in variant V4 (thermic shock + classical maceration with enzymatic preparations) according with the results presented by M. MARENGHI [18].

From the data in Table 3, under these conditions of experimentation, the maceration leads to the increase in the must of the terpenic constituents, both free and bound in precursors.

Table 3. The influence of the different types of maceration upon the terpenes complex of the Muscat Ottonel wine

Variants	Terpenes µg/l		Linalol µg/l	α terpinol µg/l	Geraniol µg/l	Flavonoids µg/l	Un-flavonoids µg/l
	TVF	TBP					
V	2350	4480	780	166	320	140	36,2
V_1	3200	5680	940	300	580	481	67,08
V_2	3500	5900	980	360	550	504	70,3
V_3	3400	6975	985	325	575	565	59,6
V_4	3500	6300	1010	380	550	577	71,7

TVF- free volatile terpens, TBP- terpens bound in precursors

Also, the must enriches in polyphenols that can become oxidation support of the must and can imprint astringency and bitterness. It is interesting to notice the fact that an extended maceration length (Variant V2), does not bring significant pluses in the content of terpenic compounds unlike the variant which was steeped throughout a shorter period of time (V1). On the other hand, the involvement of the pectolytic enzymes of extraction accompanied by the maceration leads to an essential increase of the terpenes compounds bound in precursors (V3) but has a negligible effect upon the free volatile compounds. The same thing is also noticed at the V4 variant, steeped in similar conditions, with the specification that for fermentation there have been added the selected Enolevure A R yeasts.

Taking into account the obtained data, it can be noticed that of all the free volatile terpenes, the α-terpineol and geraniol displayed significant increases in the steeped variants, especially in the V3 and V4 ones, when maceration takes place in the presence of the enzymatic preparation of extraction.

In what concerns the influence of the temperature and maceration length of time, the data of Table 4 are eloquent.

The influence of the duration and of maceration temperature emphasize the fact that maceration determines an enriching of the must in both terpenes compounds and total polyphenols at all the 5 variants registered at witness according to certain previous researches at LOSADA M & al., [17]. The most important values are recorded at the variants that have been steeped at low temperatures (V4 and V5).

Table 4. The influence of the temperature and maceration length of time upon the terpenes and polyphenol complex of the Muscat Ottonel wines

Variants	Terpens µg/l		Total polyphenols mg/l
	TVL	TLP	
V	910	2210	256
V_1	1420	2760	415
V_2	1630	3280	440
V_3	1730	3700	465
V_4	1890	3780	401
V_5	1810	4030	352

TVL- free volatile terpens, TLP- terpens bound in precursors

Of the variants steeped at a higher temperature (22 ^{0}C), the V3 variant stands out in bold relief as it has the longest maceration length of time. But, the content in polyphenols also has the highest value, which can imprint the wine an astringent, unpleasant taste.

At the variants with maceration at low temperature, there are emphasized higher contents in terpenes compounds (V4) and a lower content in polyphenols (V5) which confirm the fact that the enzymatic activity at low temperature is minimal.

4. Conclusions

The high level in the flavor constituents from the peel as well as the presence of a bound fraction of the flavor, much greater than the free, volatile one, which form a flavored potential slightly valorized in the present but which remains a natural source in reserve, impose the choice of proper technological actions for the exploitation of this flavor source.

Maceration brings important increases in the flavored potential of the wine both through the increase of the free volatile terpenes and the ones bound in precursors.

Through maceration at low temperatures, the extraction processes of the flavor compounds are slowly, being necessary the extension of the maceration period. The application of a thermic shock followed by maceration brings an increase in the content of both free volatile terpenes and the ones bound in precursors.

In the conditions of maceration in rotative thermostat metal tanks, the extraction phenomena of the terpenic compounds from the solid fraction are more intense compared to the procedure of classical maceration. The wine content in volatile compounds of the flavor is influenced by the clarification of the must.

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