INFLUENCE OF *NON-SACCHAROMYCES* YEASTS ON WHITE DRY WINES

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Abstract. *It was demonstrated a positive action ofthenon-Saccharomyces yeasts on the organoleptic properties of wines. Also, their participation in fermentation process did not involve an excessive accumulation of volatile acidity or other taste and aroma defects. The involvement of the non-Saccharomyces yeasts in practical oenology that keeps on recent achievements in oenological biotechnologies allow an increase of aromatic intensity (floral, fruitful etc.) in varietal wines and preserve the varietal identity of obtained wines.*

Keywords: yeasts, *non-Saccharomyces, Saccharomyces cerevisiae,* alcoholic fermentation, kinetics of alcoholic fermentation, white dry wines.

Introduction

Yeasts are microscopic fungi that transform naturally the sugar from grapes into ethylic alcohol. These microorganisms have an extremely simplified anatomy. They are the basic agent in wine production because they are responsible of alcoholic fermentation mentioned above. These yeasts are retained by berries skin by the pruine. Their dispersion across plantation is realized by insects, named drosophila, also by the wind etc.

Nowadays, the world market offers to winemakers a wide spectrum of products that ensure a good alcoholic fermentation. The enzymes of selected *Saccharomyces* strains prevalent the fermentation environment and thereby provide a rapid and reliable fermentation, ensuring wines with a constant quality. On the other part, the wines that are produced by mono-seeding are recognized as being less complexes and too "standardized". However, once in the alcoholic fermentation process the *non-Saccharomyces* yeasts are involved, it can be obtained a positive influence on the organoleptic characteristics of wines. At the same time natural microflora fermentations risk to stop because of the sensibility of these yeasts to the environmental conditions (alcohol, pH and others. From these reasons it is proposed to use a multi-starter culture that contains both strains *Saccharomyces* and *non-Saccharomyces*. This seeding technique

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allowsmimicking a natural fermentation at the beginning to increase wine complexity and at the same time to avoid the risk of stagnation of fermentation. During the alcoholic fermentation with different yeasts pairs different interactions between these are noticeable [1].The last years the *non-Saccharomyces* yeasts have become increasingly studied dues to those technological proprieties. Many works refer to those benefic influences on the white dry wines [2, 3, 4, 5, 7].
In view of the above, several *non-Saccharomyces* strains were proposed for co-fermentation process in association with *Saccharomyces cerevisiae.* French Institute of Vine and Wine (Nantes) last ten years were studied the fermentation process, its kinetics and also organoleptic and physic-chemical characteristics of produced wines from Melon B and Sauvignon varieties.

Materials and methods

T he research objective supposes the association of non-*Saccharomyces* yeasts with *Saccharomyces cerevisiae* in sequential seeding to prove the positive changes involved by these strains.
Non-*Saccharomyces*strains targeted in this study are:
- ✓ *Candida pyralidae*,
- ✓ *Metschnikowiapulcherrima*,
- ✓ *Torulasporadelbrueckii*.

Candida pyralidae is a selected strain that was studied earlier at the French Institute of Vine and Wine (Nantes). It has an oenological interest in enriching wine with aroma. The other two tested strains (*Metschnikowiapulcherrima* and *Torulasporadelbrueckii*) are already recommended to be produced and marketed. During this study were used three Lots of different varieties and geographical provenience:
- ✓ LOT 1 : Melon de Bourgogne
- ✓ LOT 2 : Sauvignon de Poitou
- ✓ LOT 3 : Sauvignon de Touraine

Given the trends of last years of substitution of manual harvest with mechanical, in all three Lots the harvest was perform using the combine and received as marc (Melon B) and must (Sauvignon).

The success of implantation of the strains was verified by performing an implantation control when the density of musts was ranged between 1,020 and 1,030 g/dm^3. Biomass analysis was realized by amplification Polymerase Chain Reaction (PCR). The genetic profile of biomass recovered from must, compared with referential strain allow the validation of successful yeast implantation. In order to determine the basic physical and chemical indexes were used recommended a standardized methods proposed by OIV [6].

Results and discussions

The analytical composition of every lots is different and especially in assimilable nitrogen. In order to ensure a reliability of alcoholic fermentation, in this study it was proceed to an increase of nitrogen content by using acloholic fermentation activators „Go Ferm„ and „Fermaid E„ in two halves. Table 1 presents an analytical composition of 3 lots of must.

Table 1. Analytical composition of musts

Indexes	Melon B	Sauvignon de Poitou	Sauvignon de Touraine
Total acidity, g/l H_2SO_4	4.0 (82 me/l)	6.42 (131 me/l)	5.0 (102 me/l)
pH	3.20	3.08	3.17
Assimilated nitrogen, mg/l	66	190	55
Tartric acid, g/l	2.9 (38 me/l)	3.9 (52 me/l)	4.1 (54 me/l)
L-malic acid, g/l	5.7 (85 me/l)	8.8 (131 me/l)	7.0 (104 me/l)
Turbidity,NTU	100	50	110
Potential alcohol concentration, % vol	10.0	10.0	12.0
Carbohydrates concentration, g/l	166	166	195

Table 2.Comparative characteristics of fermentative activity of yeasts

Yeasts Strains	Melon B			Sauvignon de Poitou			Sauvignon de Tourraine		
	Alcoholic Fermention, days		Residual sugar before sulfitage, g/l	Alcoholic Fermention, days		Residual sugar before sulfitage, g/l	Alcoholic Fermention, days		Residual sugar before sulfitage, g/l
	Latency	Duration		Latency	Duration		Latency	Duration	
1	2	3	4	5	6	7	8	9	10
Saccharomyces cerevisiae	1	20	1.8	2	16	2.0	3	10	1.9
Torulaspora delbrueckii	2	28	1.9	2	25	2.5	4	35	2.0
Candida pyralidae 2%	3	20	1.9	2	23	1.9	3	24	1.9
Candida pyralidae 3%	3	20	1.8	2	23	2.0	3	24	1.9
Candida pyralidae 5%	3	20	1.9	2	25	2.5	3	10	2.1
Metschnikowia pulcherrima	3	22	1.9	2	16	1.5	3	10	1.8
Metschnikowia pulcherrima IFV	4	20	1.8	2	11	1.25	3	10	1.5

Analysis results show a fundamental difference in assimilable nitrogen concentration, ranging from 55 to 66 g/l for Touraine Sauvignon and Melon B but up to 190 g/l for Poitou Sauvignon.

Also, the total acidity range from 4,0 g/l in Melon B must up to 6,42 g/l in those obtained from Poitou Sauvignon.

Chemical composition of must, as well as the interactions that occur between pairs of strains involved in every fermentative process influence first of all the duration of the fermentation (Table 2).

Table 2 results shows a difference in duration (days) of alcoholic fermentation carried out with *Saccharomyces cerevisiae* strain in 24 hours, while, under the same experimental conditions, this characteristic for *Torulasporadelbrueckii*and *Candida pyralidae* strains was respectively 48 and 72 hours. In terms of the duration of fermentation of must, it is observed only small deviations for experimented varieties, being higher in Melon B and quasi identical in the two lots of Sauvignon.

Also, the interactions between strains and the fermentation kinetics will alter (Fig. 1, 2, 3).

The curves of each series had a similar shape to that of the reference sample (seeded with *Saccharomyces cerevisiae*), but the using of *non-Sachharomyces* yeasts increase the latency period because of the concurrence between the strains. *Torulasporadelbrueckii* provides a quick beginning of alcoholic fermentation but towards the end the sugar consumption decrease and the fermentation slows compared to the previous steps.

This can be seen in all launched lots. *Metschnikowiapulcherrima,* didn't involve fermentation difficulties in any sample, also its competition with *Saccharomyces* is less noticeable.

Regarding to *Candida pyralidae* strain, the graphs are quasi identic, showing that the initial number of microorganisms doesn't involve changes in duration and speed of alcoholic fermentation of the musts.

The alcoholic fermentation of Lot No. 2 (Poitou Sauvignon) took place in a higher speed than the other two lots but the curves of lot No.3 (Touraine Sauvignon) have greater slopes at the beginning, showing that the fermentation speed decrease with the decrease of the content of sugar and increase of the content of alcohol.

Legend:
LT1-TD-*Torulaspora delbrueckii*
LT1-RnMO2-*Candida pyralidae 2%*
LT1-RnMO3-*Candida pyralidae 3%*
LT1-RnMO5-*Candida pyralidae 5%*
LT1-SC-*Saccharomyces cerevisiae*
LT1-MP- *Metschnikowia pulcherrima*
LT1-MPL8- *Metschnikowia pulcherrima*
selected from IFV

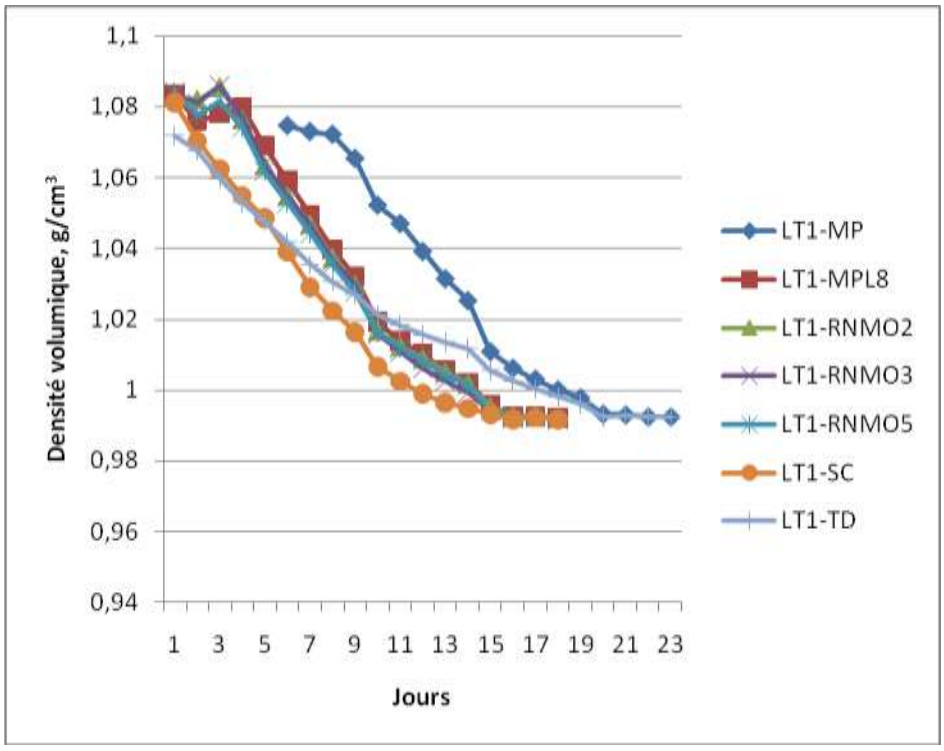

Fig. 1. Alcoholic fermentation kinetics of Lot Nr1 (variety Melon B)

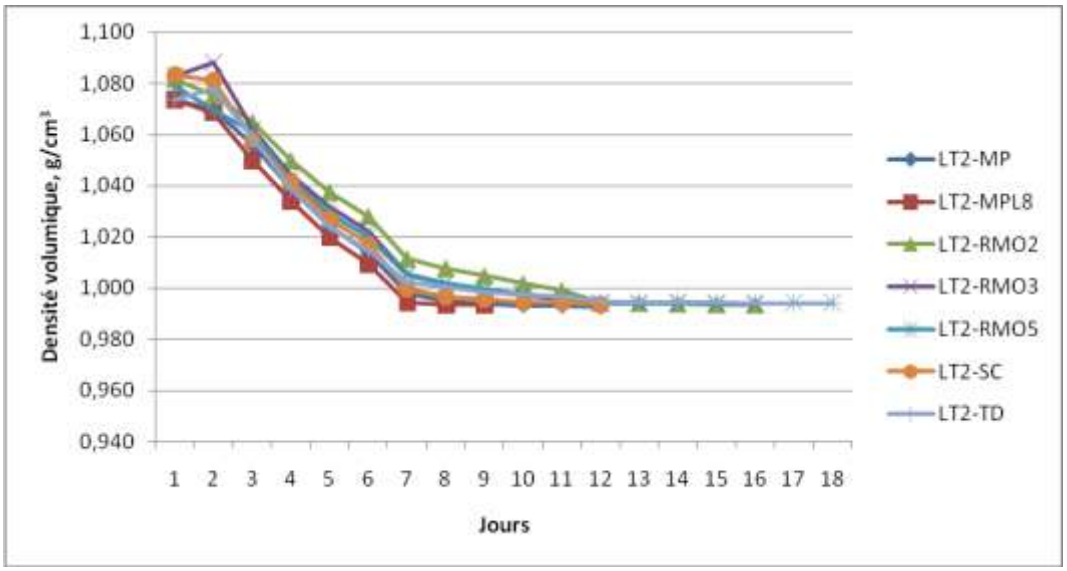

Fig. 2. Alcoholic fermentation kinetics of Lot Nr2 (variety Poitou Sauvignon)

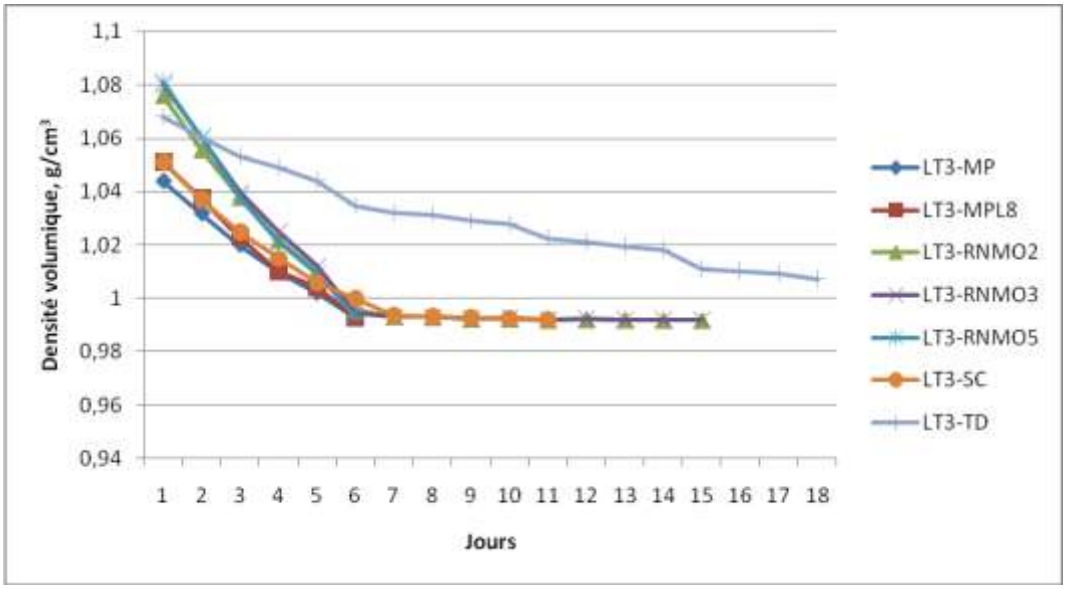

Fig. 3. Alcoholic fermentation kinetics of Lot Nr3 (varietyTouraine Sauvignon)

Tables 3, 4 and 5 summarize the analytical composition of produced wines

Table 3. Analytical composition of obtained wine from variety Melon B (Lot 1)

Indexes	Melon B						
	Saccharomyces cerevisiae	Torulaspora delbrueckii	Candida pyralidae 2%	Candida pyralidae 3%	Candida pyralidae 5%	Metschnikowia pulcherrima	Metschnikowia pulcherrima IFV
Alcohol concen-tration, % vol	12.48	12.37	12.43	12.43	12.32	12.79	12.35
Glucose +Fructose, g/l	1.3	0.6	1.2	0.9	1.3	<0.4	1.2
Total acidity (in H_2SO_4), g/l	4.19	4.15	4.02	4.11	4.01	4.10	4.14
Volatile acidity corrected (in H_2SO_4), g/l	0.21	0.51	0.24	0.23	0.23	0.33	0.27
pH IRTF	3.23	3.28	3.25	3.23	3.24	3.24	3.22
L-malic Acid, g/l	5.0	4.1	4.4	4.3	4.5	4.6	4.7
Tartric Acid, g/l	1.4	1.6	1.3	1.4	1.4	1.4	1.5
Total sulfure dioxide (total SO_2), mg/l	89	24	113	83	107	14	100

According to Table 3, the alcoholic fermentation was finished in all samples (residual sugar < 2g/l).

Alcoholic concentration of Lot No. 2 samples is very close to the first Lot, except for two (*Torulasporadelbrueckii*-TD and *Candida pyralidae*5%-RnMO5), that are 12,29 and 12,23 respectively.

The volatile acidity of samples is a little bit high but has no gap between samples of the same Lot.

The total acidity is less high comparing to the first Lot but the samples are homogeneous by this parameter, except samples TD and RnMO5(about 4,8g/l). Because these two samples had finished fermenting later than the others, their chemical analysis was carried out before sulfitage (total SO_2 54 and 50 mg/l respectively), also before treatement with cold that explain a higher concentration in tartric acid.

Tartric and malic acid consumption by yeasts is higher comparing to the precedent Lot (about 2 g/l of malic acid and 3 g/l of tartric acid with respect to their initial concentration in must).

Table 4. Analitical composition of wine obtained from Sauvignon de Poitou variety *(Lot 2)*

Parameters	Sauvignon de Poitou						
	Saccharomyces cerevisiae	Torulasporadelbrueckii	Candida pyralidae 2%	Candida pyralidae 3%	Candida pyralidae 5%	Metschni-kowia pulcherrima	Metschnikowia pulcherrima IFV
Alcohol concentration , % vol	12.69	12.29	12.58	12.42	12.23	12.42	12.44
Glucose+Fructose, g/l	1.3	2.1	1.2	1.4	1.5	0.5	0.8
Total acidity (in H_2SO_4), g/l	4.44	4.8	4.53	4.5	4.79	4.44	4.24
Volatile acidity corrected (in H_2SO_4), g/l	0.33	0.41	0.42	0.36	0.34	0.42	0.33
pH IRTF	3.33	3.44	3.37	3.37	3.38	3.35	3.09
L-malic Acid, g/l	6.6	6.4	6.6	6.4	6.7	6.7	6.9
Tartric Acid, g/l	1.0	2.1	1.3	1.4	2.1	1.0	1.0
Total sulfure dioxide (total SO_2), mg/l	119	54	117	116	51	115	115

Table 5. Analytical composition of wine obtained from variety Touraine Sauvignon (Lot 3)

Parameters	Sauvignon de Touraine					
	Saccharomyces cerevisiae	Candida pyralidae 2%	Candida pyralidae 3%	Candida pyralidae 5%	Metschnikowia pulcherrima	Metschnikowia pulcherrima IFV
Alcohol concentration , % vol	13.25	13.05	13.17	13.20	13.27	13.21
Glucose +Fructose /l	1.2	<0.4	1.0	1.0	0.4	<0.4
Total acidity (in H_2SO_4), g/l	4.00	4.32	4.2	4.12	3.84	3.92
Volatile acidity corrected (in H_2SO_4), g/l	0.34	0.26	0.24	0.24	0.32	0.24
pH IRTF	3.19	3.17	3.17	3.15	3.18	3.15
L-malic Acid, g/l	3.8	4.1	4.2	4.2	4.0	3.9
Tartric Acid, g/l	2.0	2.1	2.0	1.8	1.8	1.8
Total sulfure dioxide (total SO_2), mg/l	96	101	91	92	74	83

The alcoholic fermentation of Lot Nr 3 showed the fastest kinetics for several samples and from the amount of residual sugars - the most advanced. The obtained ethylic alcohol concentration (about 13,2 % vol) highlights the rich potential of must. The sample inoculated with *Torulasporadelbrueckii* strain allows the slowest fermentation of all analyzed samples. Regarding the total acidity, the gap between samples is not significant (about 0,2 g/l). Despite the chemical de-acidification of Lot, the consumption of tartric and malic acids is the most significant between all three researched groups (decrease with 3 g/l of malic acid and 2 g/l of tartric acid compared to the initial concentration of acids in must). The volatile acidity of samples of this Lot is the smallest and shows a quasi linear homogeneity.

The sensorial analysis of wines at this stage can not give the definitive results on the quality of products but provides an objective opinion on the ulterior development of wine during maturation. The samples do not show important organoleptic defects. The intensity of aroma and taste of the products was determined both by yeasts activity and aromatic varietal potential of grapes. Overall, the most appreciate was the Lot 2 (Poitou Sauvignon) because of more pronounced secondary aroma and a balanced taste.

The results of organoleptic analysis are listed in figures 4, 5 and 6.

Legend:
TD-*Torulaspora delbrueckii*
RnMO2-*Candida pyralidae 2%*
RnMO3-*Candida pyralidae 3%*
RnMO5-*Candida pyralidae 5%*
SC-*Saccharomyces cerevisiae*
MP- *Metschnikowia pulcherrima*
MPL8- *Metschnikowia pulcherrima selecteted from* IFV

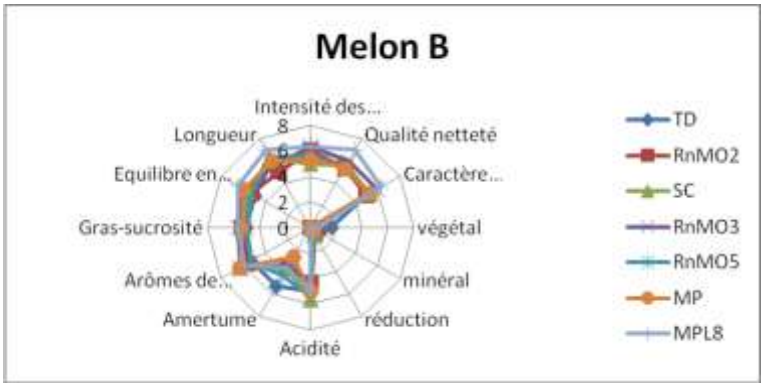

Fig.4. Sensorial analysis of wines (Lot 1)

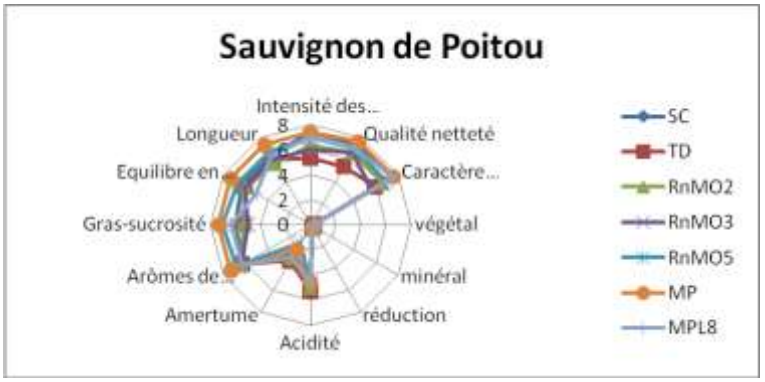

Fig.5. Sensorial analysis of wines (Lot No.2)

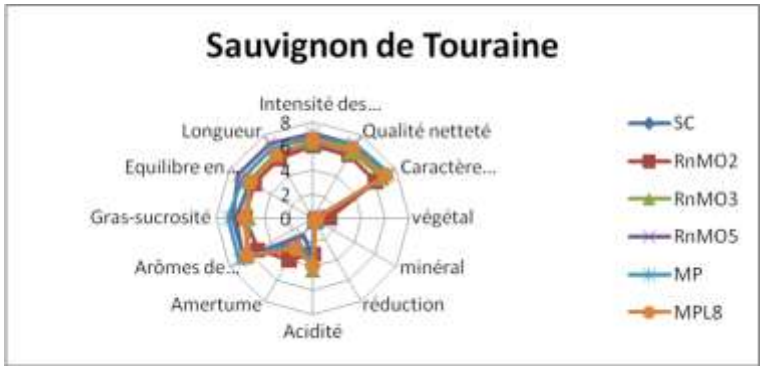

Fig. 6. Sensorial analysis of wines (Lot No.3)

Conclusions

(1) *Non-Saccharomyces* and *Saccharomyces* strains inoculated in musts from Melon B and Sauvignon varieties shows different kinetics behaviors and alcoholic fermentation was finished in almost all samples.

(2) An exception was found in a sample of Lot No.3 seeded with *Torulasporadelbrueckii*which has a slow kinetics of carbohydrates degradation. It was established that the fermentation kinetics is more a strain function than a function of used variety of grapes. The shape of curves of kinetics of fermentation was similarly to the curve of the referential sample.

(3) It was demonstrated the positive action of non-*Saccharomyces* yeasts on the organoleptic characteristics of wines.

(4) At the same time, their involvement in fermentation process doesn't achieve an excessive volatile acidity and other defects of aroma and taste.

(5) The involvement of *non-Saccharomyces* strains in practical oenology that keeps recent achievements in oenological biotechnologies allows an increase of aroma intensity (floral, fruitful etc.) in varietal wines with preservation of varietal aromas and taste in natural dry wines.

References

[1] Flanzy Claude, « Oenologie –fondementsscientifique et technologiques», Lavoisier TEC&DOC, pp.165-219, 1998;
[2] Application à l'oenologie des progrèsrécents en microbiologie et en fermentation », Office International de la Vigneet du Vin. ENSAA Dijon, ENSA Montpellier, pp.171-202, 1998;
[3] Ribereau-Gayon, J., Peynaud, E., Traitéd'oenologie. Vol I, Paris et Liège, 1960;
[4] Maîtrise des fermentations spontanées et dirigées, ITV-Nantes, pp.26, 2008
[5] Huet Myriam, Lauzeral Valérie, Dictionnaire des vins et alcools, Edition Hervas, pp.440, 1999;
[6] Recueil des méthodesinternationalesd'analyse des vins et des moûts, OIV, volume 1, Paris, edition 2011;
[7] Poulard Alain, Pascari Xenia, Rapport de stage « Sélection d'une souche non-Saccharomyces pour la fermentation des vins blancs », Institut Francais de la Vigne et du Vin, 2013, 54 pages;
[8] Gherciu-Musteță Lidia, Poulard Alain, Gore Ecaterina, « Etude de la fermentation alcoolique en flores mixtessur Melon de Bourgogne », Universtite Technique de Moldova, 2009, 16 pages.