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Using Malolactic Fermentation with Acid- And Ethanol- Adapted Oenococcus Oeni Strain to Improve the Quality of Wine from Champs Bourcin Grape in Sapa - Lao Cai

Pham Thu Thuy, Nguyen Lan Huong, Chu Ky Son

***Abstract*—**Champs Bourcin black grape originated from Aquitaine, France and planted in Sapa, Lao cai provice, exhibited high total acidity (11.72 g/L). After 9 days of alcoholic fermentation at 25°C using *Saccharomyces cerevisiae* UP3OY5 strain, the ethanol concentration of wine was 11.5% v/v, however the sharp sour taste of wine has been found. The malolactic fermentation (MLF) was carried out by *Oenococcus oeni* ATCCBAA-1163 strain which had been pre-adapted to acid (pH 3-4) and ethanol (8-12%v/v) conditions. We obtained the highest vivability (83.2%) upon malolactic fermentation after 5 days at 22°C with early stationary phase *O. oeni* cells pre-adapted to pH 3.5 and 8% v/v ethanol in MRS medium. The malic acid content in wine was decreased from 5.82 g/L to 0.02 g/L after MLF (21 days at 22°C). The sensory quality of wine was significantly improved.

***Keywords*—**Champs Bourcin grape, malolactic fermentation, pre-adaptation, *Oenococcus oeni*

I. Introduction

THE history of wine production spans thousands of years and is closely intertwined with the history of agriculture, cuisine, civilization and humanity itself. Wine is mainly produced by fermenting crushed grapes cultivated in the temperate zones of several European countries, such as Italy, France, Greece, etc. The quality of grape wine has been known to depend directly on grape varieties. This implies that the geographical regions and climates of grape cultivated areas may considerably affect on the quality of grape wine. In Vietnam, grapes are cultivated mainly in Ninh Thuan and Sapa, of which Sapa is considered as a suitable region for the grape cultivation since it has a temperate and/or subtropical climate. In recent years, some kinds of domestic grape wines have been available in the market, however their quality in sensory and flavor seem to be lower than those imported from other countries.

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Especially, it is easy to recognize that these have a sharp sour due to the high content of malic acid in raw materials. Consequently, technological problem related to the reduction of malic acid content in raw materials seems to play a key role in the improvement of grape wine quality. Previous studies found that grape wine quality could be remarkably improved under malolactic acid fermentation conditions, which allow reducing the malic acid content in crushed grapes [1]-[3]. To date, most studies have focused on enhancing the efficiency of malolactic acid fermentation, especially the adaptation of selected bacteria with young wine under specific conditions, such as low pH value, high alcohol concentration and acidity, have been increasingly gained more attention [4]-[6]. The objective of this study was using malolactic fermentation with acid- and ethanol-adapted *O. oeni* strain to improve the quality of wine from Champs Bourcin grape in Sapa-Lao cai.

II. Materials and Methos

A. Materials.

1) Grapes: Champs Bourcin black grapes, originated from Aquitaine, France and planted in Sapa, Lao cai provice, were harvested on June, 2010.

2) Microorganisms: *Saccharomyces cerevisiae* UP3OY5 yeast strain was isolated at the University of Burgundy, France. Stock cultures (kept frozen at -80°C) were grown until the stationary phase then inoculated in YPD (2% glucose, 1% yeast extract, 2% peptone) medium. *Oenococcus oeni* ATCC BAA-1163 strain was kindly provided by the Institut Universitaire de la Vigne et du Vin, University of Burgundy, France. *Oenococcus oeni* ATCC BAA-1163 (formerly *Oenococcus oeni* IOB 8413) was cultured at 30°C in modified FT80 (mFT80) medium at pH 5.3.

3) Elaborate conditions: The grape juice in the presence of grape skins and seeds was adjusted to sugar concentration of 210 g/l and pH 3.2. The juice was sulphited by adding Na_2SO_3 (40 – 50 mg/l). Alcoholic fermentation was carried out in 5 L jar with 3.5 L of the juice by inoculating the *S. cerevisiae* UP3OY5 strain (0.25 – 0.3 g/l) at 25°C for 9 days. *Oenococcus oeni* ATCCBAA-1163 strain was grown on MRS medium with pH 6.2 at 25°C for 24 hours, and then was pre-adapted at different pH and ethanol concentration. The pre-adapted cell inoculum (10^8 CFU/ml) was tranffered into the wine as

the starter in MLF. The MLF was conducted at 22◦C for 21 days.

B. Analytical methods

1) Chemical compositions of grape must were determined: The total soluble solid was observed by the hand refractometer. The reducing sugars content was measured using DNS methods. The total acid was determined using titration with 0.1 N NaOH and bromothymol blue as indicator, and expressed as H_2SO_4. Total yeast and bacterial cells were counted by the counting chamber and plating on agar plate, respectively.
2) Ethanol concentration was measured by using distillation method.
3) Malic acid was determined by thin layer chromatography (TLC) and Malic acid kit (Biosentec, France).

III. Results And Discussions

A. The main compositions of the grape must

Champs Bourcin black grapes, originated from Aquitaine, France and planted in Sapa, Lao cai provice, were harvested on June, 2010. The chemical compositions of the grape must were presented in the Table I.According to the compositions of Champs Bourcin grape must in Table I, it seems that the grape was a sour type. The ratio of reducing sugar content to total acidity of that was 6.35. The reducing sugar content in the juice was low, while the total acidity and the malic acid content were high for alcohol fermentation process. It is necessary to adjust sugar so that the initial sugar content of the juice for fermentation was above 210 g/l and acidification by $CaCO_3$ (1 g/l) to reach pH≥3.2.

TABLE I
THE CHEMICAL COMPOSITIONS OF THE CHAMPS BOURCIN BLACK GRAPE MUST

Composition	Unit	Value
pH		2.83
Degree Brix	°Bx	11.0
Reducing sugar	g/l	74.4
Total acidity (as H_2SO_4)	g/l	11.72
Malic acid	g/l	8.0

B. Alcoholic fermentation

Alcoholic fermentation was conducted by following traditional procedures. The wines were elaborated from Champs Bourcin grapes in the presence of grape skins and pips, with the *S. cerevisiae* UP3OY5 strain (0.25 – 0.3 g/l), and after sulphiting by adding Na_2SO_3 (40 – 50 mg/l) to 3.5 l of wine. The temperature was maintained around 25°C. The kinetic of alcoholic fermentation was shown in Table II.The results showed that the sugar content was reduced quickly within 6 days, then went slowly down after 8 and 9 days of incubation. The amount of viable yeast cell was significantly increased after 2 days of fermentation and reached the maximum at 6th day then decreased slowly after 8 days. Beside that the total acidity was slightly increased, whereas the pH decreased weekly. It seems that the fermentation process took place very vigorously. After 9 days of alcoholic fermentation the ethanol concentration of wine was 11.5% v/v and the remained sugar content was 10.14 g/l. However the sharp sour taste of wine was found.

TABLE II
THE KINETICS OF ALCOHOLIC FERMENTATION

Time (day)	pH	Total soluble solid (°Bx)	Sugar content (g/l)	Total acidity (g/l)	Viable cells ($x10^6$/ml)
0	3.20	21.5	209.80	8.32	22
2	3.10	15.5	127.39	8.43	87
4	3.11	9.5	95.64	8.48	147
6	3.16	6.0	59.44	8.50	163
8	3.17	2.0	19.23	8.51	152
9	3.17	1.0	10.14	8.51	150

C. Acid and ethanol pre-adaptation of O. oeni strain

Oenococcus oeni is the major bacterial species found in wines during the malolactic fermentation, and is well adapted to the low pH and high ethanol concentration of wine [1], [7], [8]. Several authors have demonstrated the benefits of using well-adapted biomass of *O. oeni* that can be used as an effective starter to induce the MLP in wine [6]-[7]. The *O. oeni* ATCCBAA-1163 strain had been pre-adapted to acid (pH 3.5) and ethanol (8 %v/v) in MRS medium. The count of bacterium was presented in Fig. 1. The result showed that the count of bacterium began to rise slowly during incubation and reach maximum biomass after 5 days. The highest vivability was 83.2% at 5 days. The biomass was haverted at the early stationary phase *O. oeni* cells pre-adapted to pH 3.5 and 8% v/v ethanol in MRS medium, then transfered into the fresh MRS medium with the higher inhibitory conditions (pH 3-4, ethanol concentration 10-12% v/v). The results were shown in Fig. 1. As indicated in Fig.1, the ethanol concentration significantly influenced on the biomass. At the ethanol concentration of 10% v/v, the count of bacterium reached the maximum at 4th day in case of pH 4.0, while at 5th day in pH 5.0. With the higher ethanol concentration (12 % v/v), the maximum of biomass reached lower than that in the case of the ethanol concentration of 10 % v/v. The count of bacterium was also affected by pH. As the initial pH decreased from pH 4.0 to pH 3.0 at same the ethanol content (example 12 % v/v), the incubation time for the maximum of biomass dropped from 5 days to 7 days. To elucidate the effect of pH and the ethanol content on the count of bacterium, the *O. oeni* ATCCBAA-1163 strain had been pre-adapted at pH 3.5 and ethanol (8 %v/v) in MRS medium. This biomass was used as a starter strain for malolactic fermentation.

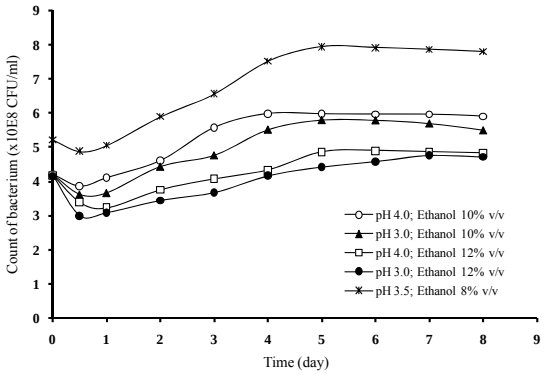

Fig. 1 The count of bacterium in MRS medium with pre-adapted to acid (pH3-4) and ethanol (8-12 % v/v) conditions versus time of fermentation

D. Malolactic fermentation

At the end of alcoholic fermentation, the wine was drawn off from the lees and placed homogeneously into fermentation jars for MLF. The temperature was obtained around 22°C. MLF was carried out in two samples: the control sample was not inoculated with *O. oeni* ATCCBAA-1163 strain and the other sample was inoculated with this strain. The initial count of bacterium for MLF was around 10^6 CFU/ml. The changes of pH, total acidity and malic acid content in the wine during 21 days of MLF were presented in Table III, Table IV, and Fig. 2. The results showed that the total acidity and malic acid content in the wine inoculated with *O. oeni* strain was

TABLE III
THE EVOLUTION OF PH, TOTAL ACIDITY AND MALIC ACID CONTENT IN THE WINE DURING MLF

	Before MLF	After the 21 days of MLF	
		Non-inoculated with *O. oeni* strain	Inoculated with *O. oeni* strain
pH	3.17	3.15	3.56
Acidity (g/l)	8.51	8.50	6.21
Malic acid (g./l)	5.82	5.96	0.02

TABLE IV
THE KINETICS OF ALCOHOLIC FERMENTATION

Time (in days	Malic acid content (g/l)	
	Non-inoculated with *O. oeni* strain	Inoculated with *O.oeni* strain
0	5.82	5.82
6	5.85	2.18
12	5.90	0.80
21	5.96	0.02

significantly decreased after 12 days incubation (Table III and IV). In the Fig. 2, the spots of malic acid in the wine inoculated with *O. oeni* strain appeared slightly, whereas the spots of L-lactic acid observed sharply during MLF. Interestingly in the wine without inoculation, the spots of malic acid in that kept constant.The malic acid contents in the wine inoculated with *O. oeni* strain were determined in Table IV. The malic acid content in wine decreased from 5.82 g/L to 0.02 g/L after 21 days.

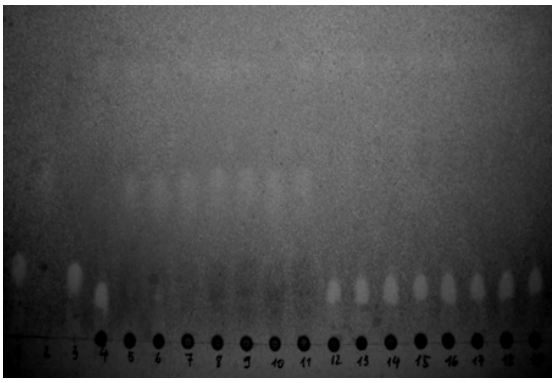

Fig. 2 Evolution of malic acid content in the wine during MLF. (1), (2), and (3) standard substrate; 6g/l of malic acid + 6g/l of latic acid; 6g/l of lactic acid; 6g/l of malic acid; respectively; (4, 5, 6, 7, 8, 9, 10, and 11) the wine inoculated with *O. oeni* strain at 0, 3, 6, 9, 12, 15, 18, 21 days; (12, 13, 14, 15, 16 , 17, 18, and 19) the wine non-inoculated with *O. oeni* strain at 0, 3, 6, 9, 12, 15, 18, 21 days.

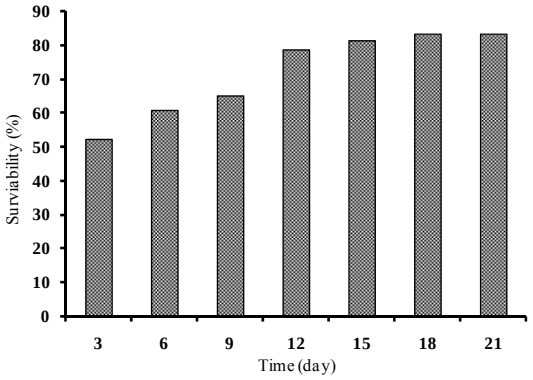

Fig.3 Survival of *O. oeni* in the wine during MLF

In this study, the implantation of *O. oeni* ATCCBAA-1163 strain was confirmed and advantage with respect to the non-inoculated jars was observed. MLF of wine inoculated with that lasted 21 days. In previous studies had shown that *O.oeni* strains IS-18 and IS-159 isolated from Roija red wine in MLF revealed as excellent candidates for selection as they succeeded 100% in a red wine with 13.5% v/v ethanol [4]. Lopez *et al.* [3] found that MLF of wines inoculated with *O. oeni* selected strains lasted 25 days in the stainless steal vasts, and 20-21 days in the barrels, whereas spontaneous MLFs lasted 38 and 36 days, respectively. Maicas *et al.* [9] detemined the *O. oeni* strain M42 was used for biological deacidification of wine, and the MLF was developed in red wines within 2-3 weeks [9].

IV. Conclusions

The main components of Champs Bourcin black grape must planted in Sapa, Lao cai provice showed that it was a sour grape for wine making. The initial sugar content and pH of juice in the alcoholic fermentation were 210 g/l and above pH 3.0. The MLF was carried out by *Oenococcus oeni* ATCCBAA-1163 strain which had been pre-adapted to pH3.5 and ethanol content 8 %v/v after 21 days at 22^{o}C. The obtained wine had alcohol content of 11% (data not shown), malic acid content of 0.02 g/l and pH 3.56.

References

[1] P. G. P. Versari. A, and Cattaneo. M., "Leuconostoc oenos and malolactic fermentation in wine: a review," J. Ind. Microbiol., vol. 23, pp. 447– 455, 1999.

[2] A. Lonvaud-Funel, "Lactic acid bacteria in the quality improvement and depreciation of wine," Antonie Van Leeuwenhoek, vol. 76, pp. 317-331, 1999.

[3] S. Q. Liu, " A review: malolactic fermentation in wine beyond deacidification," J Appl Microbiol., vol. 92, pp. 589-601, 2002.

[4] H. Teixeira, M. G. Goncalves, N. Rozes, A. Ramos, and M. V. San Romao, "Lactobacillic acid accumulation in the plasma membrane of Oenococcus oeni: a response to ethanol stress," Microb. Ecol., vol. 43, pp. 146-153, 2002.

[5] R. L. Lopez I., P. Santamaria, C. Torres, and F. Ruiz-Larrea, "Performance of malolactic fermentation by inoculation of selected Lactobacillus plantarum and Oenococcus oeni strains isolated from Rioja red wines," Vitis, vol. 47, pp. 123-129, 2008.

[6] C. T. Lopez I, M. Zarazaga, C. Torres, F. Ruiz-Larrea, "Evidence of mixed wild populations Oenococcus oeni strains of during wine spontaneous malolactic fermentations," Eur. Food Res. Technol., vol. 226, pp. 215-232, 2007.

[7] J. Guzzo, M. P. Jobin, F. Delmas, L. C. Fortier, D. Garmyn, R. Tourdot-Marechal, B. Lee, and C. Divies, "Regulation of stress response in Oenococcus oeni as a function of environmental changes and growth phase," Int. J. Food Microbiol. , vol. 55, pp. 27-31, 2000.

[8] A. Bastianini, L. Granchi, S. Guerrini, and M. Vincenzini. , "Fatty acid composition of malolactic Oenococcus oeni strains exposed to pH and ethanol stress," Ital. J. Food Sci. , vol. 12, pp. 333-342, 2000.

[9] P. G.-C. Maicas S., S. Ferrer, and I. Pardo, "Production of Oenococcus oeni biomass to induce malolactic fermentation in wine by control of pH and substrate addition," Biotechnology Letters, vol. 21, pp. 349-353, 1999.