PILOT SCALE VINIFICATIONS (100 l).

III : CONTROLLED FERMENTATIONS

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Introduction

The seasonal character of harvests limits winemaking considerably, especially oenological research. Thus, conserving sterile musts during the whole year to carry out experiments is certainly a very attractive concept. This can be done at a pilot scale level if specific equipment is available.

In fact, at the controlled fermentation facility of the INRA Experimental Unit in Pech Rouge, musts can be flash pasteurized for preservation, and then stored in sterile tanks under inert gas cover and at low temperatures.

This article describes these preservation procedures and their impact on must and wine characteristics. The consequences on fermentation kinetics is also described. This is very precisely monitored through in-line measurement of CO_2 production (Aguera et al, 2005)

Description of the must conservation and storage process:

Cellar:

Description of tanks

Musts are stored in stainless steel tanks. The total storage capacity is 210 hl, i.e. seven 10 hl tanks and seven 20 hl tanks. These tanks (Picture 1) are equipped with pressure valves and several ports allowing filling, cleaning and sterilization.

Cleaning and sterilization

Tanks are cleaned with caustic soda during the summer, after each preservation phase. They are then sterilized with steam from a steam generator (15 kW thermal power, Alfa Laval GEV 15 boiler).

First, steam is fed through the bottom and the top of the tank to be sterilized. After the heating phase, the temperature is maintained for 30 minutes. Condensing water is constantly removed through the bottom valve.

After steam injection, sterile filtered (Sartorius Sartofluor LG cartridge) inert gas (N_2/CO_2 mixture) is introduced into the tank. A pressure regulator (Air Liquide DCN 500 TBP) allows to the tank to be maintained at overpressure during the cooling stage.

After sterilization, the tanks are kept at overpressure (approximately 7.25 PSI) until filling to avoid contamination.

Picture 1: Storage tanks at the INRA controlled fermentation facility, Pech Rouge

Flash pasteurization

Principle - Application

The product is heated as fast as possible and kept at high temperature for a certain time before it is cooled down to room temperature again.

In oenology, flash pasteurization is used mainly for conditioning and stabilizing wines. Sometimes, it may also be used to remedy fermentation difficulties, i.e. to stop fermentations or volatile acidity development followed by re-inoculation with a starter culture. In contrast, this technique is rarely used with musts because of regulations prohibiting controlled fermentations, except for certain very specific products.

Application conditions

For white wines, keeping the temperature at 72°C for 20 seconds allows a sufficient level of sterility to be reached. These conditions correspond to 150 PU (Pasteurization units) with a z-value (temperature increase required for a one-log reduction of the D-value) of 4.5°C.

Must pasteurization is more difficult because of the absence of alcohol, and the high sugar concentrations increase the heat resistance of the microorganisms present in musts, thus requiring higher pasteurization temperatures.

The flash pasteurization system used is an electrical model with a capacity of 1000 l/h (Techniprocess thermabloc). Musts are heated for 30 seconds to temperatures between 82 and 85°C (i.e. between 40×10^3 and 180×10^3 PU).

In order to limit the contamination risk between the pasteurizer and the storage tank, the connecting line is sanitized with a peracetic acid and hydrogen peroxide based solution (Diversey Lever-Divosan plus).

Preservation

The tanks are located in a cold room kept at 4°C. Regular monitoring of the internal tank pressure allows the control of must sterility. This method offers several advantages:

(i) it is easy to implement
(ii) it is highly sensitive and allows the early detection of fermentation onset (before a significant change occurs in the composition of the easily assimilable nutrients, specifically vitamins and assimilable nitrogen)

(iii) it does not require samples to be taken, limiting contamination risks.

Once the must temperature has reached a constant value, a pressure increase should not exceed 0.1 bar, or another pasteurization would have to be performed.

Impact of must preservation and storage:

Pasteurization efficiency

Under the conditions of the pilot plant, must pasteurization does not lead to complete sterility (even though the process efficiency is generally sufficient to avoid measurable fermentation commencement during the 8-9 months of storage). Table 1 displays yeast counts (by viable cell counts on YEPD) of samples taken from different musts from the 2002 and 2003 harvests before and after pasteurization. It can be seen that pasteurization gave good (yeast count reduction always > 2×10^2) but also variable efficiency. Table 1 also demonstrates that it was not possible to establish a simple relationship between pasteurization efficiency and the main must properties, such as the initial sugar concentration, the acidity or the initial contamination level.

Must	Sugar concentration (g/l)	Total acidity (g/l)	pH	Yeast count before pasteurization / ml	Yeast count after pasteurization / ml	Yeast count reduction.
Chardonnay2002	201	6.55		6×10^4	10^2	6×10^2
Sauvignon2002	174	5.15	3.14	86×10^4	4.6×10^2	1.8×10^3
Clairette2002	187	3.50	3.41	34×10^4	< 1	$>10^5$
Viognier2003	175	3.50	3.70	10^5	<1	$>10^5$
Chardonnay2003	201	4.25	3.50	5×10^5	10	5×10^4
Sauvignon2003	192	3.40	3.50	7×10^2	<1	$>10^3$
Maccabeu2003	196	2.20	3.64	10^4	45	2×10^2
Maccabeu2003	215	2.50	3.70	6×10^5	10^2	6×10^3

Table 1. Pasteurization efficiency for different musts.

Impact of pasteurization and storage on must characteristics

Table 2 summarizes the effect of pasteurization and cold storage on the principal physiochemical parameters of musts. The main parameters modified by pasteurization were turbidity and colour. In fact, in most cases, a haze appeared after pasteurization, which was most likely caused by protein precipitation triggered by the temperature increase. In some cases, pasteurization also changed must colour: for example, the Maccabeu appeared to be less oxidized by visual inspection after pasteurization and this could be confirmed by the OD420 value, while the rosé colour of the Cinsault was less bright after pasteurization (transition from dark rosé to onion skin colour). The turbidity was also modified during storage because of cold settling. Cold storage of musts in tanks could also have an effect on the total acidity (as with Chardonnay) by precipitation of tartaric acid.

	Sugar g/l	TA g/l [tartrate]	pH	Turbidity	OD420	OD520	OD620
Maccabeu 1	215	4.4	3.59	320	0.976	0.459	0.229
Maccabeu 2	215	4.4	3.59	335	0.397	0.151	0.068
Cinsault 1	178	6.9	3.32	34	0.768	0.674	0.214
Cinsault 2	180	6.8	3.31	194	1.4	1.02	0.57
Viognier 1	174	5.4	3.70	11	0.24	-	-
Viognier 2	178	5.4	3.70	58	0.46	-	-

Viognier 3	175	5.3	3.63	8	-		
Viognier 4	176	5.4	3.61	2	-		
Chardonnay 1	201	6.4	3.50	36	0.86	0.53	-
Chardonnay 2	201	6.5	3.50	140	0.95	0.63	-
Chardonnay 4	200	5.5	3.50	83	0.54	0.24	

Table 2: Effect of pasteurization and cold storage on physiochemical characteristics of musts.
1: non-pasteurized must; 2: pasteurized must; 3: must pasteurized and kept 2 months at 4°C; 4: must pasteurized and kept 9 months at 4°C.

Effect of pasteurization and storage on must fermentability.

.Fermentation progress. X-axis: Time [h]; Y-axis: CO_2 production rate [g l^{-1} h^{-1}]

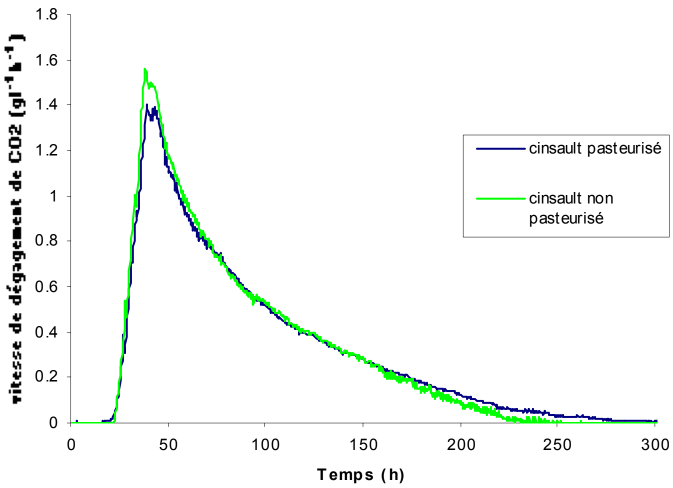

Fig 1: Comparison of fermentation progress of pasteurized (▬) and non-pasteurized (▬) Cinsault (A).

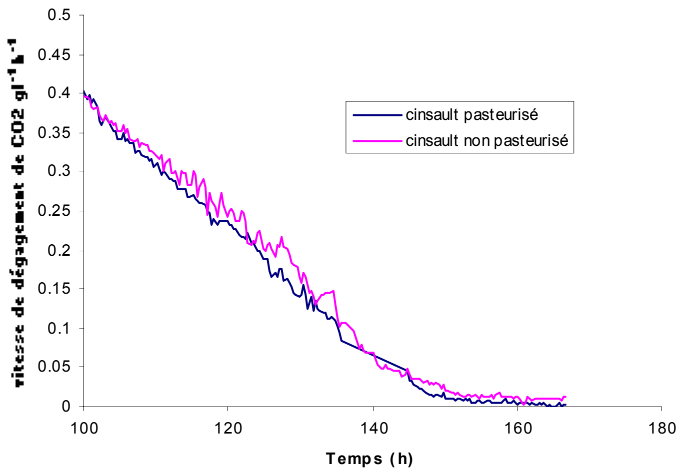

Fig 2: Comparison of final fermentation progress of pasteurized (▬) and non-pasteurized (▬) Cinsault (B).

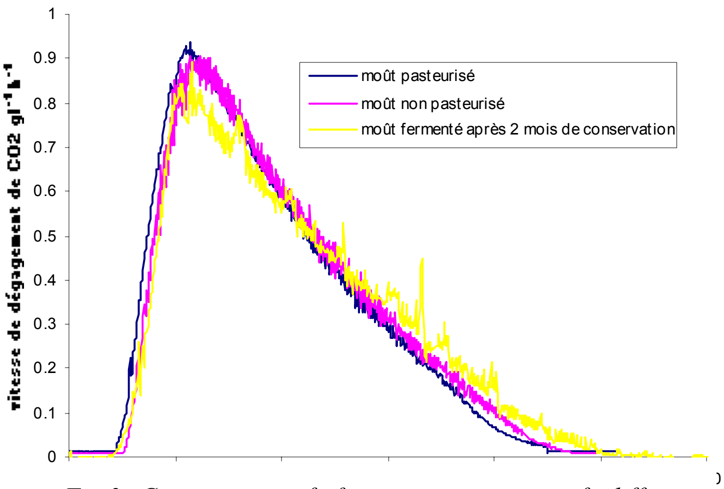

Fig 3: Comparison of fermentation rates of different Viognier musts: non-pasteurized (▬), pasteurized (▬), pasteurized and stored for 2 months (▬).

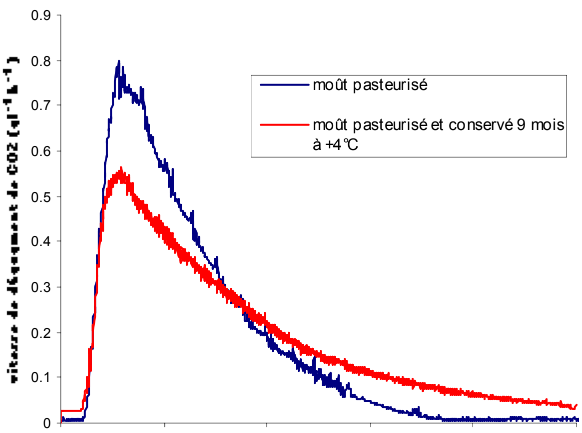

Fig 4: Effect of cold storage on fermentation rates of different Chardonnay musts : pasteurized must (▬), pasteurized and cold stored must (4°C, 9 month, ▬).

Pasteurization had no significant effect on must fermentability. In fact, for the 4 varieties tested (Figures 1 to 4), the fermentation kinetics obtained for pasteurized musts were congruent with the ones obtained for fresh musts.

However, in some cases (specifically with Chardonnay, Figure 4) cold storage of musts for several months led to a decrease in must fermentability with lower fermentation rates that approached those of a "sluggish" fermentation.

Improvement of the fermentability of stored musts

Pasteurization had no effect on the fermentability of musts. However, this was not the case for cold storage, which could lead to difficult fermentations.

Thus, we tried to improve the fermentability of these musts. The most likely hypothesis was that the negative impact of cold storage was due to clarification. Therefore, we studied (i) the effect of addition of sediments (stored frozen) to replace those which sedimented during storage, and (ii) oxygenation, which generally is very efficient in the case of highly clarified musts, since it compensates (at least partially) for the lipids lost in the sediments.

The effectiveness of sediment additions to highly clarified musts has been previously demonstrated by Aguera et al (2005).

Oxygenation was also very efficient in ensuring faster fermentations of stored musts (Figure 5). The oxygen addition was more effective if performed (i) towards the end of the growth phase, and (ii), if at least 10 mg/l were added.

Impact of pasteurization and storage on wine characteristics:

Wine analysis

The analysis of the wines obtained with 3 different varieties (Cinsault, Maccabeu and Viognier) indicates that the initial must pasteurization had very little effect on the "key factors" (Tables 3 and 4).

In the case of Viognier (Table 4), cold storage had an equally small effect, even after 9 month of storage (only the total SO_2 content changed significantly).

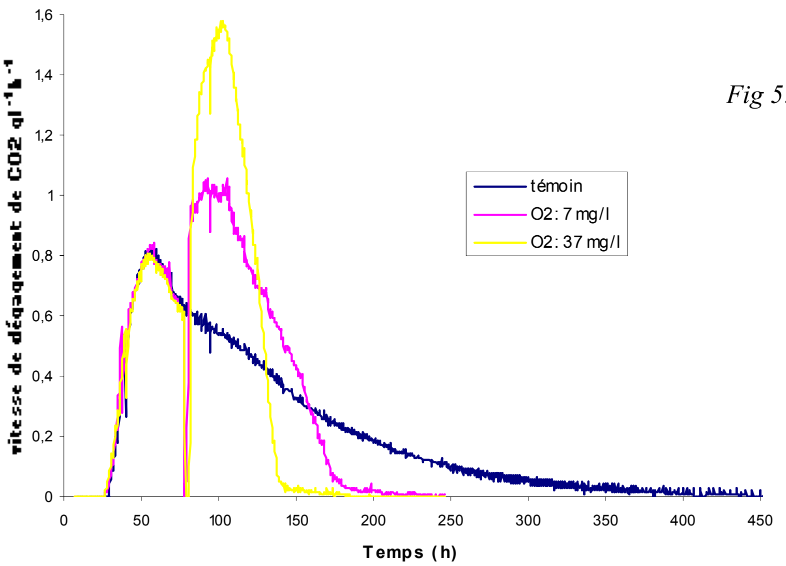

Fig 5: Effect of addition of various oxygen quantities on the fermentation rate of a Chardonnay must, which was pasteurized and cold stored for 3 months. Control (—); 7 mg l^{-1} oxygen (—); 37 mg l^{-1} oxygen (—).

	Pasteurized Cinsault	Non-pasteurized Cinsault	Non-pasteurized Maccabeu	Pasteurized Maccabeu
Volatile acidity (g/l acetic ac.)	0.25	0.27	0.16	0.26
Total acidity (g/l tartaric ac.)	4.4	4.4	5.8	5.3
pH	3.47	3.49	3.51	3.56
Alcohol (% $_{vol}$)	13.10	13.20	13.65	13.75
Density. (g/cm^3)	0.9884	0.9882	0.9886	0.9882
Free SO_2 (mg/l)	14	14	12	10
Total SO_2 (mg/l)	93	93	77	73
Sugars (g/l)	0.6	0.4	0,3	0.2
Glycerol (g/l)	6.4	6.5	8	7.4
OD 420	0.144	0.174	0.088	0.087
OD 520	0.074	0.096	0.020	0.016
OD420+520	0.218	0.27	0.103	0.108
OD 280	8.4	9	5.7	5.7

Table 3: Impact of pasteurization on wine characteristics.

	Fresh Viognier	Pasteurized Viognier	Viognier after 2 months storage	Viognier after 9 months storage
Volatile acidity (g/l acetic ac.)	0.05	0.04	0.05	0.11
Total acidity (g/l tartaric ac.)	4.90	5.05	5.20	4.90
pH	3.71	3.69	3.70	3.76
Alcohol (% $_{vol}$)		11.35	11.15	
Free SO_2 (mg/l)	23	16	16	10
Total SO_2 (mg/l)	128	115	115	77
Sugars (g/l)	<2	<2	<2	<2
OD 420	-	0.07	0.08	0.08

Table 4: Impact of pasteurization and storage on wine characteristics.

Sensory analysis
Triangular discrimination testing was performed with the Viognier must.
Results in Table 5 showed that:

- pasteurization significantly influenced wine characteristics.
- on the other hand, cold storage had no impact.

Certainly, it is impossible to make general conclusions from these results. However, other partial analyses are consistent with these results and seem to indicate that:

- storage (pasteurization and cold storage) can modify wine characteristics noticeably,
- these modifications are small enough to (i) avoid wine quality degradation, and (ii) allow comparative studies, which are generally recommended for oenological research.

	Correct answers	confidence interval (error margin)
Non pasteurized/ Pasteurized	19	<0.01
Pasteurized/cold stored for 2 months	12	12

Table 5: Impact of storage on organoleptic wine quality. Triangular testing with Viognier (panel of 26 tasters).

Conclusions

Flash pasteurization followed by cold storage in previously sterilized tanks, which are kept under inert gas cover, allows the conservation of musts for several months (up to 1 year). During this process, the contamination level is kept sufficiently low (below 10^2 – 10^3 cells /ml) to reduce the risk of must alteration, specifically with regards to the nutritional composition.

Under these conditions, fermentation rates are not affected, except after long storage of certain musts, which may lead to lower fermentability because of over-clarification (partial sedimentation). The negative effect of long storage periods can be compensated by adding back sediments or by oxygenation during fermentation.

Wines produced from treated musts can be significantly different from wines produced with fresh musts, but the differences are not large enough to question their utilization, including work where sensory analysis is desired.

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