Yeast species diversity and dynamic during cold maceration of grape must. Impact of temperature variations and commercial yeast inoculation

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Introduction:

Cold maceration (CM) of grape must is an oenological technique widely applied in the wine industry. It consists in the maceration of grapes and berries with their juice under cold temperature (undefined) for a certain time (undefined) before the beginning of alcoholic fermentation (AF) in order to improve wine quality. The dynamic and identity of the yeasts present during cold maceration and during AF is subject of this research.
Investigations were carried out using the same grape must of the 2006 vintage (winery A, located in the viticultural area of Bordeaux). The evolution of total yeast and non-*Saccharomyces* populations were followed during time of must cold maceration. The impact of inoculation with a selected *Saccharomyces cerevisiae* strain was studied and non-*Saccharomyces* strains where identified. Those real production size investigations were completed by a detailed laboratorial study on the influence of different temperatures on yeast development during CM.

The impact of different commercial yeast applications; yeast inoculation after CM; yeast inoculation before CM and instant yeast inoculation without CM on non-*Saccharomyces* population was studied by plate counting's on selective media (figure 1). The concentration of 1µg/ml cycloheximide was chosen for the NS medium due to the results of a preliminary study. 40 colonies were randomly isolated from the samples taken during CM and AF (NS-Media). Microscopic observation of the colonies was performed and non-*Saccharomyces* strains were identified by PCR-ITS analyses. Species identification was further confirmed by sequences analyses of the PCR-ITS amplified fragments (Esteve-Zarazoso *et al.*, 1999).

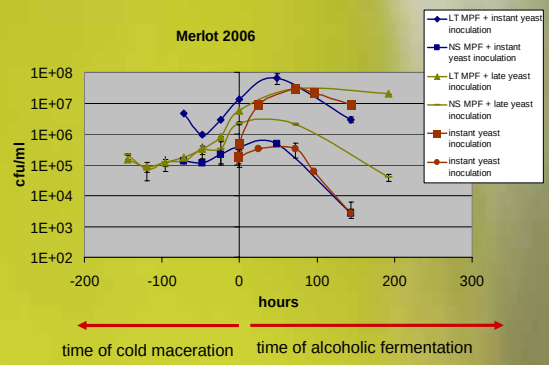

Figure 1: Development of non-Saccharomyces yeasts (NS) and total yeasts (LT) during CM and AF

Saccharomyces population increased at the end of maceration time (late yeast inoculation) but non-*Saccharomyces* yeast dominated the process reaching up to 10^6 cfu/ml at the end of cold maceration. During the early stage of fermentation their community were still growing then declining. Their population size at the end of alcoholic fermentation were strongly dependent on the yeast inoculation treatment.

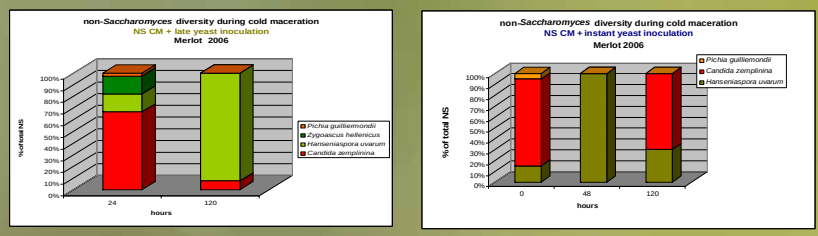

Figure 2: Identification of non-Saccharomyces yeasts present during cold maceration instant and late yeast inoculation

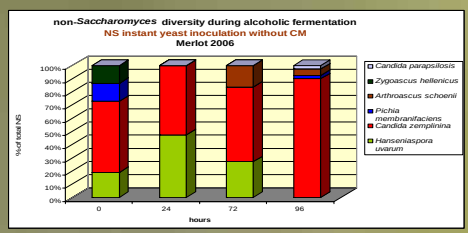

Figure 3: Identification of non-Saccharomyces yeasts present during alcoholic fermentation in non-macerated and instant fermented must

The results of the identification are showing a great diversity of non-*Saccharomyces* yeasts present during CM (figure 2) and AF (figure 3). However the major non-*Saccharomyces* yeasts present in 2006 were *Hanseniaspora uvarum* and *Candida zemplinina.* *Hanseniaspora uvarum* dies of after the first hours of fermentation where *Candida zemplinina* is gaining back importance at the end of alcoholic fermentation.

In order to **study the impact of temperature variation** on NS and LT evolution we performed CM under controlled conditions for 6 days. The grape must was afterwards inoculated with a commercial yeast strain (point 0) and kept at 25°C in order to allow alcoholic fermentation (figure 4). Population dynamics were studied by plate counting's on selective media (NS and LT).

Non-*Saccharomyces* yeasts are dominating the cold maceration process at the different temperatures tested. The initial yeast population of 10^5 cfu/ml is slowly declining and stable after three days of CM at 4°C and 10°C but increasing at the 15°C treatment after the fifth day.

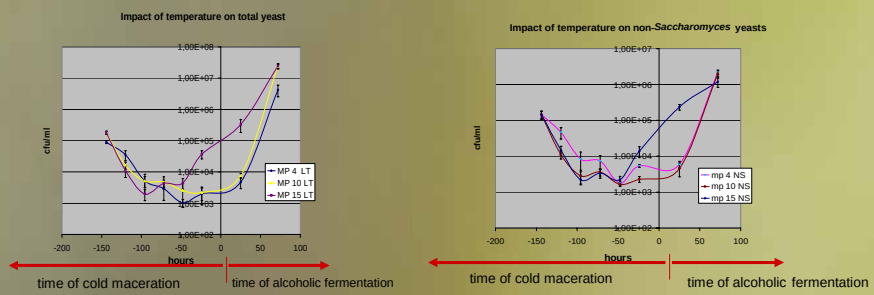

Figure 4: Impact of temperature on total yeast (LT) and non-Saccharomyces yeast (NS) at 4°C, 10°C and 15°C

Conclusion :

The cold maceration process is dominated by a wide diversity of non-*Saccharomyces* yeasts. *Hanseniaspora uvarum* and *Candida zemplinina* were very abundant in all 2006 samples independent of the instant or late yeast inoculation treatment (CM and FA). The population size of the non-*Saccharomyces* yeasts at the end of AF is strongly dependent of this treatment. Early yeast addition is leading to a lower non-*Saccharomyces* population at the end the alcoholic fermentation, representing between 1-10% of the total yeast population. Due to that observation, we are supposing an interaction between *Saccharomyces cerevisiae* and the non-*Saccharomyces* community. A temperature of 10°C makes it possible to control the yeast population during the whole CM process. It does not seem necessary to lower the temperature over this point.

References:
ESTEVE-ZARAZOSO, B., BELLOCH, C., URUBURU, F. et QUEROL, A. ; 1999 ; Int. J. Syst. Bacteriol. 49, 329-337