

## CHARACTERIZATION OF YEAST IN RELATION TO THE ABILITY TO UTILIZE NITROGEN - STUDIES OF AROMA COMPOUNDS.

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### SUMMARY

The aroma of wine is in part formed by yeasts during the fermentation of grape sugars. Volatile metabolites including esters, alcohols, fatty acids, carbonyls and sulfur compounds are formed from the metabolism of sugars, aminoacids, sulphate and other nutrients. Of the important nutrients of grape juice, the content of aminoacids varies widely and consequently affects yeast growth, fermentation and the formation of volatile metabolites.

Every Chapter of this thesis is organized with an index, an abstract, an introduction of what it is known, results and discussion of the experiments and a conclusion. There is a special Chapter with Materials and Methods, and some details of the methodology utilized are referred in each figure so as to facilitate the understanding of the results. There is a specific Chapter for the general conclusions of the work and the references section is organized with the bibliography in alphabetic order.

The first two Chapters are a general introduction to varietal aromas, winemaking, yeast metabolism and fermentation aroma. Chapter 3 is the characterization of growth and fermentation activity of the strains isolated and the advantage of WLN medium for colony differentiation in mixed strains fermentations. Differences were found between non-*Saccharomyces* strains with *Saccharomyces* in relation with nitrogen consumption profiles and percentage of viable cells in the process. The FAN content in Uruguayan musts of different regions and varieties is presented. It was measured during two consecutive years to understand the industrial conditions for vinification in our region. Chapter 4, studies the mixed cultures between non-*Saccharomyces* and *Saccharomyces* strains. Sluggish fermentations caused by competition for nutrients in mixed culture conditions are analyzed. A treatment with *Hanseniaspora* of a "Muscat Miel" must in comparison with pure inoculation with *Saccharomyces* alone and spontaneous fermentation. The effect on the profiles of varietal and fermentations aromas is discussed. The most interesting results are about an increase in varietal aromas like

terpenes, according to the analysis of free and bound compounds. Potential applications of these methods for winemaking are discussed.

In Chapter 5, *Saccharomyces* and its biotechnological characterization are the main focus in relation to aroma compounds produced in grape must. The use of a simple microbial medium like WLN was applied to industrial yeasts differentiation and the chemistry of aroma profiles is presented. Publications about characterization of strains by aroma profiles are discussed, and it is shown that the majority of these studies were done in high nitrogen musts or without information of the FAN of the musts used. The chemistry of aroma compounds obtained in certain conditions of low FAN made possible the definition of what it was called "**Yeast Administrative Capacity for Using Nitrogen**" (YACUN). By studying which are the compounds that vary in relation with the addition of ammonium phosphate in different musts and with different strains, it was possible to understand some of the aroma compounds that have negative or positive relations with the FAN levels in the medium. The effects of some phenomena that affect significantly the aroma profile are analyzed, like size of inoculum and the redox situation during the fermentation and how the profiles change. This chapter finished with the identification of the key compounds in relations with nitrogen metabolisms, but with the justification of why the use of an artificial medium is needed for obtaining more consistent results and repeatability.

In Chapter 6, an experimental design with an artificial medium permits a more precise look to the behavior of different fermentation compounds and the formation of terpenes and how the synthesis of these compounds is affected by the nitrogen content and the redox conditions of the cultures.

A strain defined as "good administrators of nitrogen" in Chapter 5, KU1 and a poor administrator M522, were used to ferment the chemically defined must to make a comparison between them in fermentations at different nitrogen levels of YAN: 50 mgN/L, 75 mg/L, 125, 180, 250 and 400 mgN/L. The aromas profiles are analyzed and the behavior of each family of compounds is characterized in relation to the nitrogen situation. Different behavior between KU1 and M522 are presented in relation to the alcohols, acids, esters and other compounds. This made possible the characterization of low and high nitrogen demand strains or YACUN in relation to the aroma compounds produced by them. An equation utilizing the aroma compounds profiles of commercial strains was tested at the end of this work where two possible groups may be

differentiated so as to categorize the YACUN classification of industrial yeast strains. The synthesis *de novo* of terpenes was demonstrated with Montrachet 522 in the artificial medium. Its relations with ammonium level in the medium and redox condition effects explain the possible way of synthesis utilized by *Saccharomyces cerevisiae*.