YEAST DIVERSITY RELATED WITH TOURIGA NACIONAL GRAPE VARIETY

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INTRODUCTION

Wine results from complex transformations of grapes that involve different groups of microorganisms. During alcoholic fermentation the non-*Saccharomyces* yeasts originating from grapes play an important role, influencing the structure, the complexity, the flavour and therefore the wine quality (Jolly *et al.* 2006; Ciani *et al.* 2010). Several factors can influence the ecology of non-*Saccharomyces* yeasts on grapes. The ripeness and the integrity of grape berries will largely determine the population numbers (Mortimer, Polsinelli, 1999; Fleet 2003). The use of pesticides also affects yeasts diversity on grapes, influencing both the number of different genera and the numbers within each genus (Guerra *et al.*, 1999; Cordero-Bueso *et al.*, 2011). The grape variety has been pointed out by several authors as affecting the yeast biota (Guerra *et al.*, 1999; Raspor *et al.*, 2006; Cordero-Bueso *et al.*, 2011).

MATERIAL AND METHODS

Yeasts isolation

Touriga Nacional grape samples were collected from two vineyards from Lisbon wine region (2005, 2006 and 2007), designated Dois Portos and Palhacana, one vineyard from Alentejo wine region (2006 and 2007), designated Montemor-o-Novo and one vineyard from Peninsula de Setubal wine region (2006), designated Azeitão. Replica samples of 2 Kg of grapes at the last stage of ripeness were aseptically collected at the same spots in different years, avoiding rotten grapes.

Samples were taken after 48 h and when must weight loss was 70 g/L, corresponding to the consumption of around two-thirds of the sugar content. Plate samples were incubated at 25 ºC for at least 48 h, afterwards 30 randomly chosen colonies were collected, isolated and kept at −80 ºC (glycerol 30 % v/v).

Identification of isolates by rDNA restriction profiles

Restriction analysis of a fragment from 26S rRNA gene with enzymes *Mse*I, *Hinf*I, *Apa*I, *Hae*III and *Cfo*I was performed according to Zanol *et al.* (2010). The identification of each isolate was achieved by using the restriction profiles library created on GelCompar II software.
Sequencing of the D1/D2 domains of the 26S rRNA gene was carried out to confirm identification and for profiles different from those existing in the library using the procedure described by Baleiras-Couto *et al.* (2005).
Hanseniaspora uvarum was differentiated from *H. guilliermondii* by testing growth at 37 ºC.

Statistical analysis

Classical ecology indexes were used to obtain the richness (S), the biodiversity (H′) and the dominance (D) of the species studied according to Cordero-Bueso *et al.* (2011).

RESULTS AND DISCUSSION

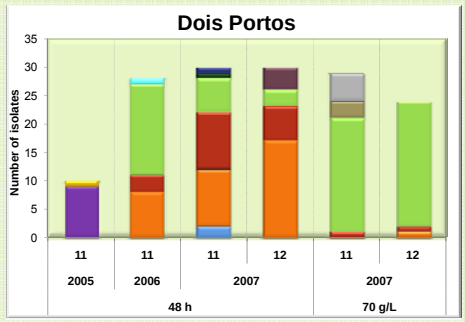
Five hundred and nine isolates from Touriga Nacional grape variety were characterised by restriction profiles analysis of 26S rRNA gene.

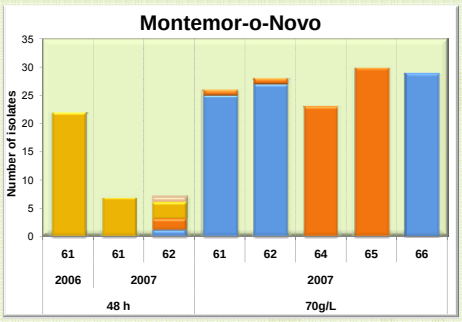
For Dois Portos vineyard a great diversity of species was found, with a total of 12 species detected. Among samples the species richness varied from two to six. The initial samples (48 h) presented higher richness and higher biodiversity when compared with the same samples after 70 g/L of weight loss. Higher dominance index was observed for sampling at two-thirds sugar consumption.

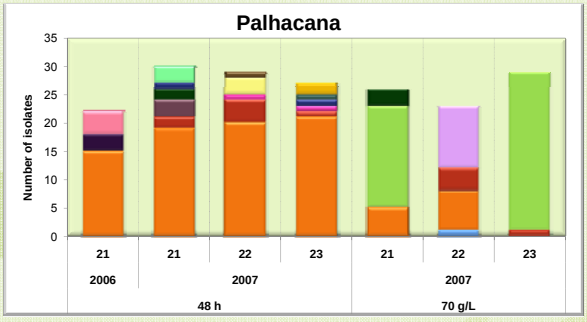
Palhacana musts presented even higher diversity, with a total of 16 yeast species detected. The species richness among samples varied from 2 to 6 and like observed for Dois Portos was lower for samples corresponding to weight loss of 70 g/L. Different values for the Biodiversity index were observed within each sampling time and was higher for one of the samples collected at 70 g/L weight loss, inversely to Dois Portos samples.

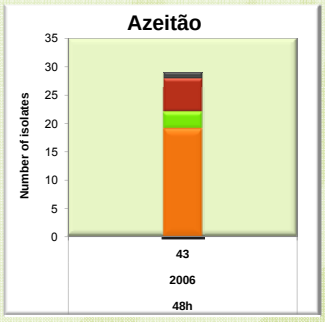
For Alentejo vineyard richness was very low, with only one yeast species detected for the majority of the samples. Low biodiversity and high dominance index were observed for this vineyard yeast biota. Its localisation far from any winery and from other vineyards may contribute to the low yeast diversity observed.

Higher species richness was found for Azeitão vineyard. *Issatchenkia hanoiensis* was only detected at this vineyard. This species has been scarcely isolated from grapes and musts, being detected at fermentations from Castelão and Catalanese grape varieties (Baleiras-Couto *et al.* 2005, Di Maro *et al.*, 2007).









H. guilliermondii · *M. pulcherrima* · *Crytococcus sp.* · *H. uvarum* · *Z. bisporus* · *C. californica*
C. zemplinina · *A. pullulans* · *P. kluyveri var kluyveri* · *C. oleophila* · *Z. hellenicus* · *Lachancea sp.*
I. terricola · *C. diversa* · *P. membranifaciens* · *C. fermentati* · *S.vini* · *C. railenensis*
I. orientalis · *Zygosacch. bailii* · *R. babjevae* · *I. hanoensis*

CONCLUSIONS

Variation of the yeast biota detected on different years and vineyards was observed. The year of 2007 presented higher yeast biodiversity. Higher yeast biodiversity was also found at the initial sampling time than at sampling after two-thirds sugar consumption. The most representative species was *Hanseniaspora uvarum*, which was detected in grape must from all the vineyards, followed by *Candida zemplinina*. Some species like *Pichia membranifaciens*, *P. kluyveri var kluyveri*, *C. railenensis*, *Saccharomycopsis vini*, *C. diversa* among others were only detected in grape must from one vineyard. *Saccharomyces cerevisiae* was not detected for any of the 22 samples analysed. At late fermentation the predominantly detected species was *H. guilliermondii*, also followed by *C. zemplinina*. Replicate samples were similar in relation to the most frequent species.

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