

Yeasts ecology and volatiles profile of spontaneously fermented Taggiasca black and green table olives

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Taggiasca table olives are produced with a spontaneous fermentation carried out by placing the raw drupes directly into a brine with a salt concentration of 8-12%. Such concentrations favor the growth of yeasts, and this process can last up to 8 months. During this period, yeasts are mainly involved in the debittering of the fruits and the production of volatile compounds. In this study, the microbial ecology of naturally fermented Taggiasca table olives was evaluated through culture-dependent and independent methods coupled with volatilome analysis via GC-MS. Two fermentation batches were considered and both brines and olives samples were analyzed. At the end of the process, yeasts populations reached 3,8 and 6 log₁₀ CFUcmL⁻¹ in olives and brines, respectively. Culture-dependent analysis revealed that the most abundant species overall were *Candida diddensae*, *Wickerhamomyces anomalus*, *Pichia membranifaciens* and *Aureobasidium pullulans*, with many differences in species distribution between batch 1 and 2. Culture-independent analysis confirmed the presence and dynamics of *W. anomalus* in batch 1 in brines and olives throughout the entire fermentation, whereas *Cyteromyces nyonsensis* and *Aureobasidium* spp. dominated the fermentation of brines and olives in batch 2, respectively. Volatilome results were analyzed and correlated to the microbiota data, confirming differences between the two batches. Such variations in microbiota and volatiles profile contributed to a successful fermentation of batch 1 and a poor fermentative process in batch 2, which did not proceed to the packaging step. This study will help improve the knowledge on the main microbial groups of the Taggiasca variety and their relationship with the quality of the final product. It will also guide the selection of potential autochthonous starters to better control the fermentative process while preserving the sensory qualities of the product.

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Introduction

Taggiasca table olives are produced with a spontaneous fermentation carried out by placing the raw drupes into a brine with a salt concentration of 8-12%. Such concentrations favor the growth of yeasts, in a process that can last up to 8 months. The aim of this study was to explore the yeast diversity of naturally fermented Taggiasca table olives and brines as an early step towards starter cultures selection. Culture-dependent strategies, amplicon-based sequencing (ABS) and the analysis of the volatile compounds was the approach used to understand the microbial diversity in our samples and to define the organoleptic profile of the final product. This study will help improve the knowledge on the main microbial groups of the Taggiasca variety and their relationship with the quality of the final product. It will also guide the selection of potential autochthonous starters to better control the fermentative process while preserving the sensory qualities of the product.

Culture-dependent: yeasts counts

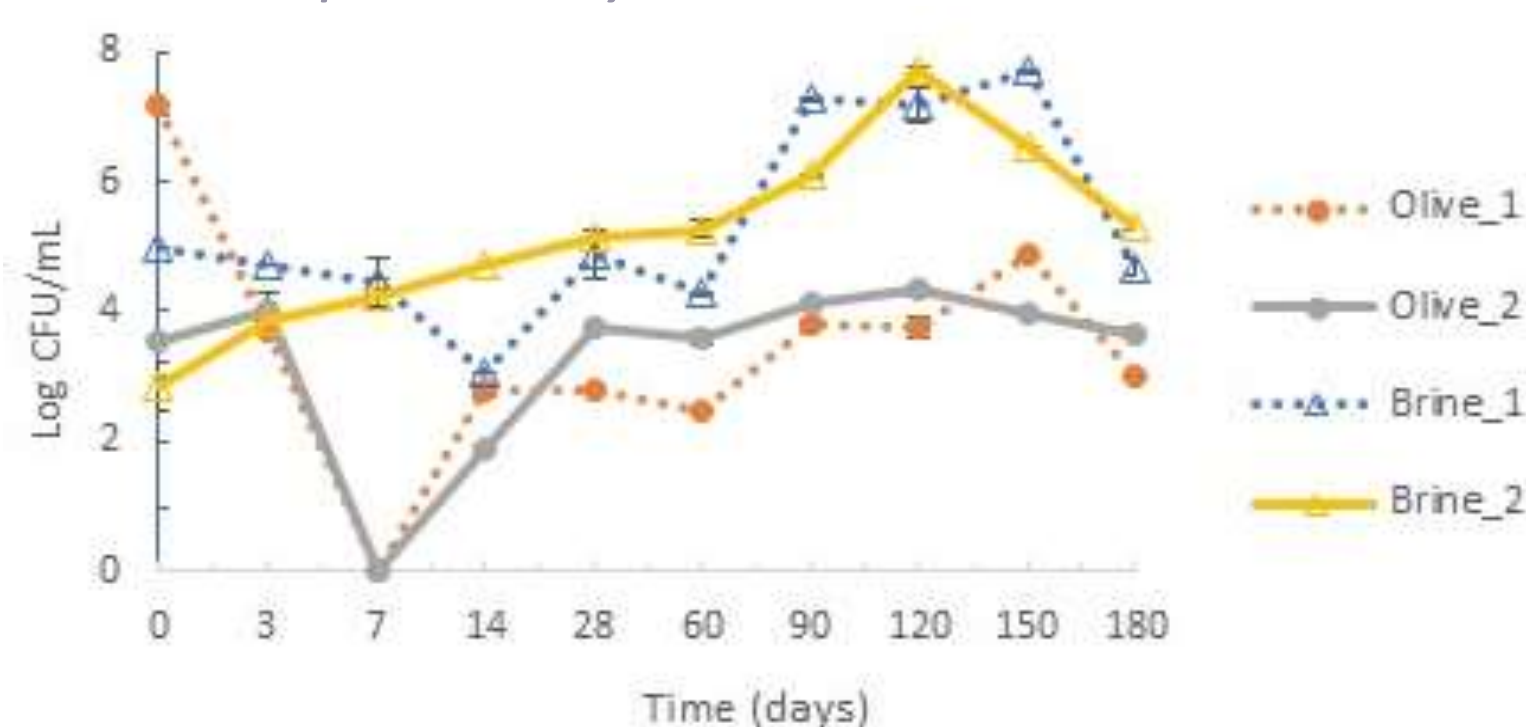


Fig. 1: Yeasts dynamics throughout the fermentation in olive and brine samples.

Culture-dependent: dereplication and clustering

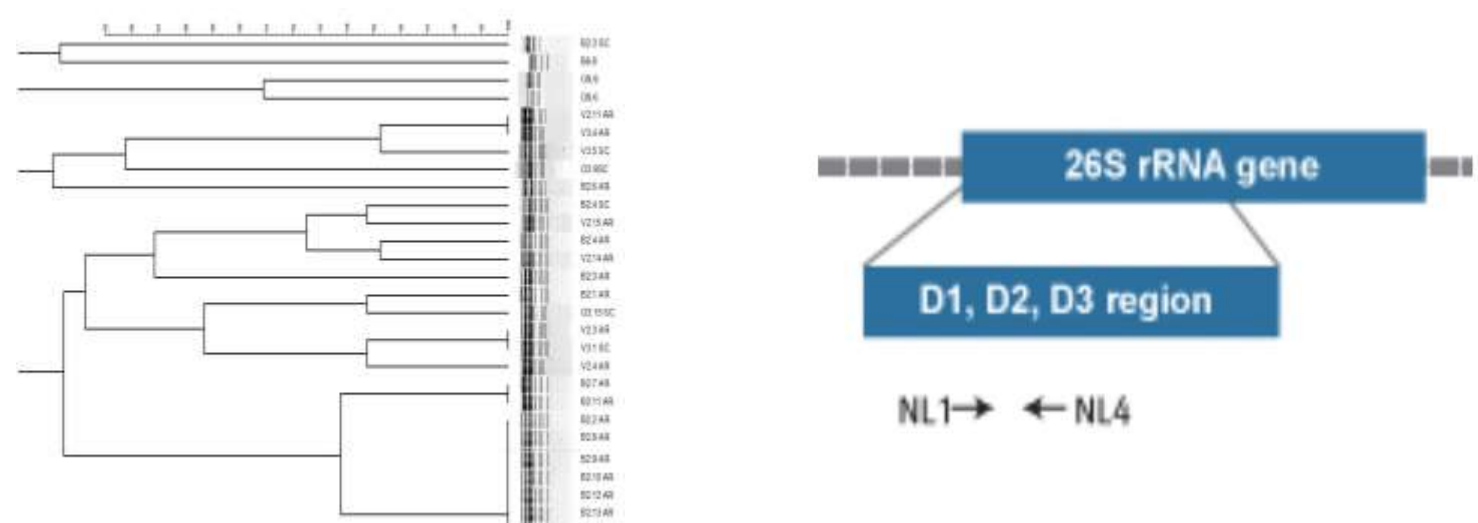


Fig. 2: Clusters generation with Bionumerics software and D1/D2 amplification of the 26S rRNA gene with primer pair NL1-NL4.

Culture-dependent: sequencing output

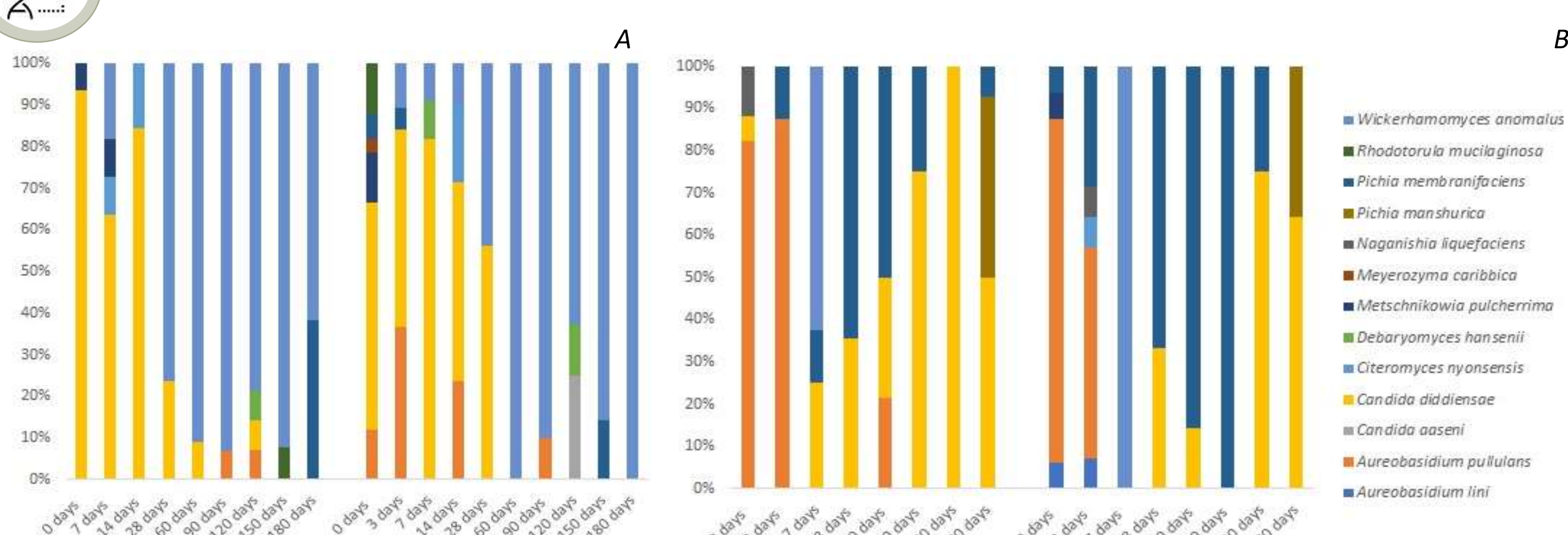
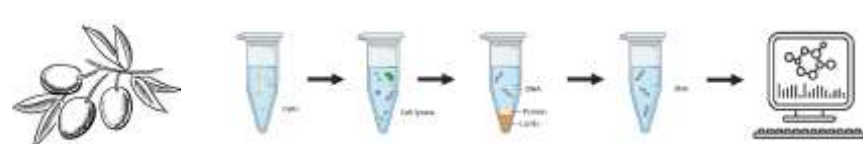


Fig. 3: Relative abundance (%) of yeasts species in brines and olives in batch 1 (A) and 2 (B).

The most dominant species in batch 1 throughout the entire process were *W. anomalus* (46,19%), *C. diddensae* (35,24%) and *A. pullulans* (6,67%), whereas in batch 2 the main ones were *C. diddensae* (33,74%), *A. pullulans* (26,38%) and *P. membranifaciens* (24,54%). These species were commonly isolated from both naturally fermented olives and Spanish-style fermented ones, and *W.anomalus* was also found in brines of fermented Taggiasca olives in a recent study [1]. The role of *W.anomalus* might be important for a potential application as starter culture, as it already showed β -glucosidase, anti-oxidant and strain-specific killer activities towards spoilage microorganisms [2].

Culture-independent: gDNA extraction



Total gDNA was extracted from brines and olives and was subjected to sequencing of the D1 domain of the 26s rRNA gene.

GC-MS: correlation with microbiota

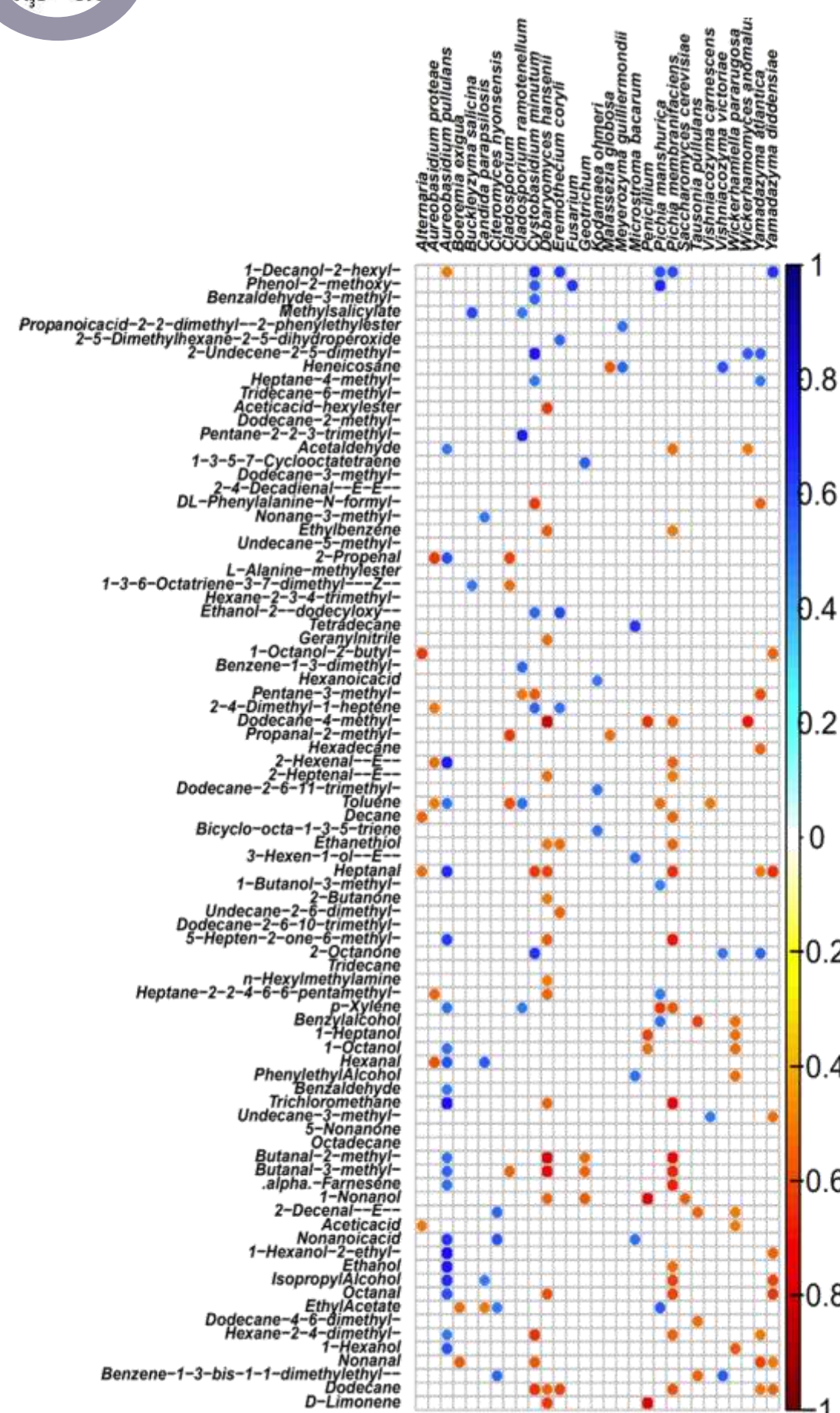


Fig. 4: Correlation between OTUs and volatiles. The intensity of the color stands for the degree of correlation (colored legend) according to Spearman's correlation. Blank squares show absence of significance ($P > 0.05$).

Culture-independent: sequencing output

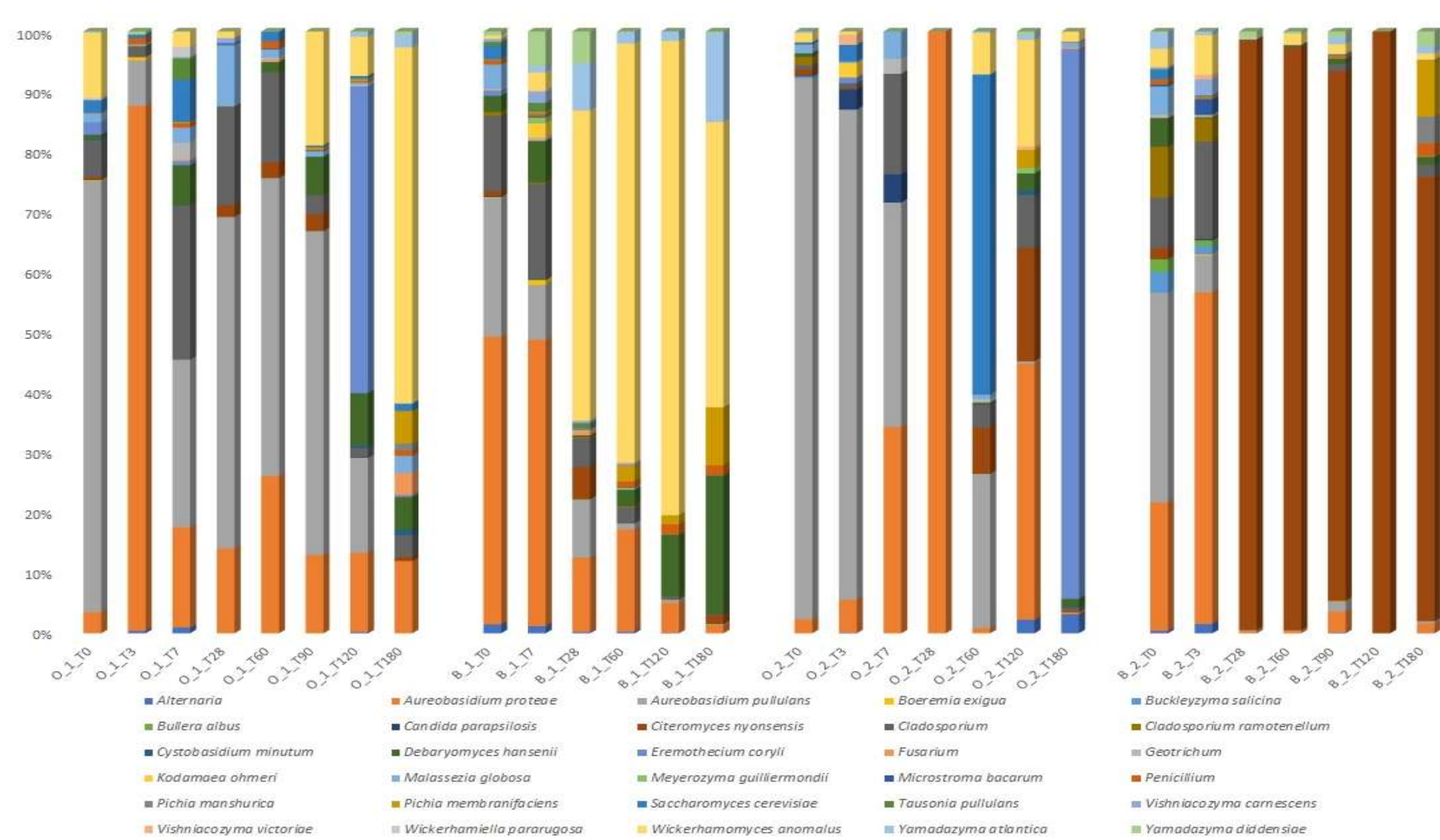


Fig. 3: Frequency of OTUs detected by 26s rRNA ABS in batch 1 (left) and batch 2 (right). Olive samples (O); Brine samples (B); first number (batch); Tnumber (sampling time expressed in days).

Predominant chemical classes:

- Hydrocarbons
 - Alcohols
 - Esters
- but chemical composition highly depends on the cultivar.

Conclusions

Yeasts dominated the entire fermentation, as common in spontaneous processes using high salt contents (>10% w/v). *W. anomalus* presence in brines was also confirmed by ABS and might be considered for further screenings as potential starter. The main ASV fraction, *A. pullulans* was positively correlated to 21 VOCs among which acetaldehyde and 2-hexenal-(E), commonly associated to desirable organoleptic characteristics [3]. In batch 2, presence of *C. nyonsensis* in the brine and *E. coryli* at the end of fermentation, might explain poor fermentative process.

[1] Cecchi, G., Di Piazza, S., Rosa, E., De Vecchis, F., Silvagno, M. S., Rombi, J. V., Tiso, M., & Zotti, M. (2023). Autochthonous Microbes to Produce Ligurian Taggiasca Olives (Imperia, Liguria, NW Italy) in Brine. *Fermentation*, 9(7), 1–14.; [2] Perpetuini G., et al. (2020). Table olives more than a fermented food, *Foods*, 9(2), pp.1–16; [3] Muzzalupo, I. et al. (2012) 'LOX gene transcript accumulation in olive (*Olea europaea* L.) fruits at different stages of maturation: Relationship between volatile compounds, environmental factors, and technological treatments for oil extraction', *The Scientific World Journal*, 2012.

