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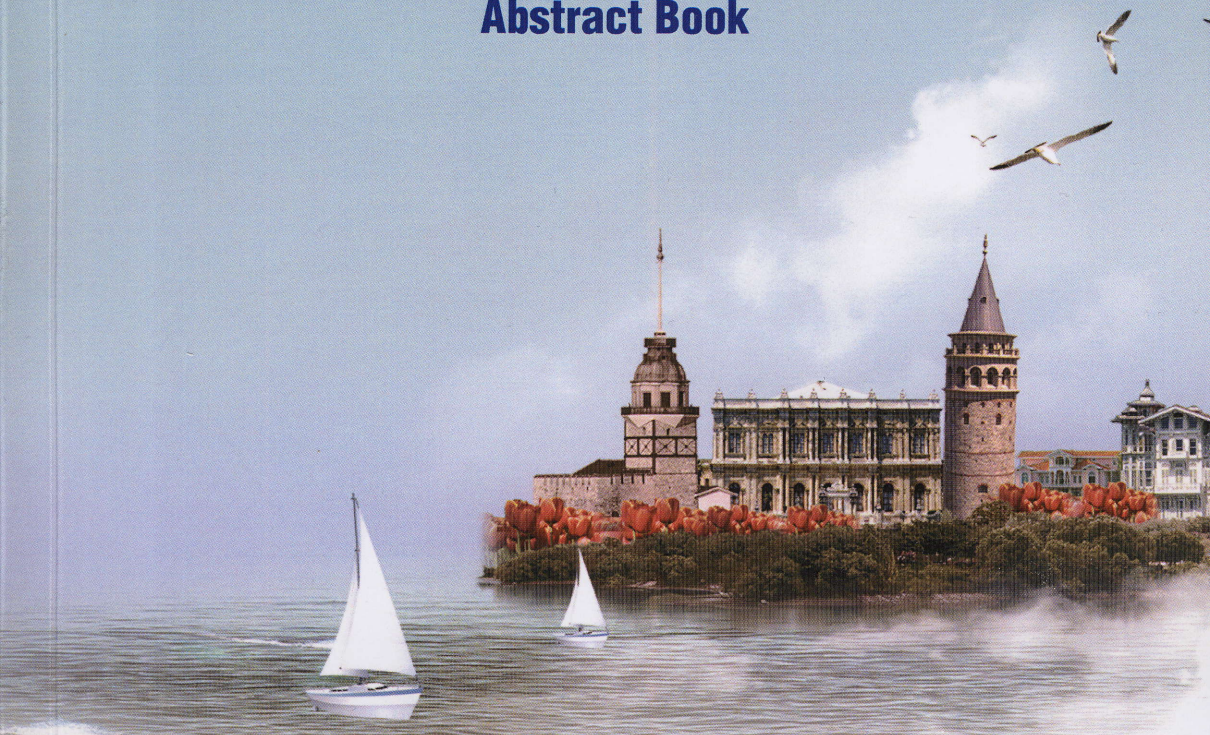

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Influence of certain technological variables on native fermenting microflora activity in biodynamic wine production

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The evolution of native yeasts during winemaking was followed in a traditional Italian winery which produces wines following the principles of biodynamic agriculture. Certain technological parameters were varied in the different trials to understand their impact on spontaneous yeasts. The experiments involved the addition of ammonium salts, thiamine, oxygen, and pied de cuve (a yeast inoculum made using grapes from the same winery). All observations were performed directly in the wine cellar during fermentation, the evolution of yeasts being followed by daily microscopic counts coupled with methylene blue staining to distinguish live and dead cells. During the winemaking process the samples were also analysed five times using plate counts with selective media to allow preliminary description of the microflora; the cellar equipment was also analysed. Following OIV methods, total yeasts, non-*Saccharomyces* yeasts, lactic and acetic bacteria were quantified in samples of grape, must, and wine. The identification of yeasts was performed using genotypic methods applied on pure cultures. A bulk cell DNA survey was carried out using denaturing gradient gel electrophoresis (DGGE)-PCR to confirm species identification. Several species of non-*Saccharomyces* yeast, mainly belonging to *Hanseniaspora* and *Candida* genera, were found at subdominant levels during must fermentation. The isolates identified as *S. cerevisiae* were subjected to molecular typing and a few strains were found to dominate all the fermentation trials. Considering the relative small size of the winery this result underlines the high biodiversity associated with biodynamic production. The trials involving the addition of both nitrogen and thiamine showed the highest fermentation rate and richness in terms of microbial composition, since lactic and acetic bacteria were counted at higher concentrations than in other trials. The trials with the addition of nitrogen showed a clear dominance of *S. cerevisiae* and were characterised by very low levels of bacteria. The internal surfaces of cellar equipment were characterised by a certain yeast biodiversity and hosted the species found during wine making; the wooden surfaces represented the primary source of inoculation of a strain of *S. cerevisiae* dominant in all wine making trials, independently of the technological regime applied.