Potential exploitation of lysozyme in the winemaking of Sicilian wines from "organic grapes"

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Lysozyme is a natural enzyme with muramidase activity which can work against a range of lactic acid bacteria (LAB), including *Oenococcus*, *Pediococcus* and *Lactobacillus* spp. that can affect wine stability (Cunningham *et al*., 1991). For such reason, over the last decade, there has been a growing interest in lysozyme as a supplement to sulphur dioxide for bacterial inhibition (Sonni *et al*., 2009). Since, lysozyme applications proved to be enough specific to require technical knowledge and expertise to be efficient, an experimental winemaking was performed to evaluate the efficiency of this enzyme application to produce a Sicilian wine from "organic grapes". Specifically, two experimental conditions were considered by using SO2 or lysozyme, alternatively, according to a defined industrial winemaking scheme, with periodically samplings in order to: i) control organic acid content, ii) control unwanted LAB and iii) prevent malolactic fermentation. Musts to be employed derived by grapes of "Catarratto", an ancient white variety, whose wine presented susceptibility to oxidation phenomena. The evaluation during time of lactic microflora revealed an appreciable microbial reduction following to lysozyme addition, even if this drop took a longer time (about 48 h) if compared to the production with SO2 (about 2 h). In addition, in wine produced with lysozyme the content of acetic acid was half of the value determined on the wine added with SO2 after 30 days by the end of alcoholic fermentation. Seventy-three LAB cultures isolated by different phases of both thesis were identified and characterized through molecular approach. Identification revealed the prevalence of *Lactobacillus plantarum* (about 60%) followed by the species *Lactobacillus mali*, *Lactobacillus hilgardii*, *Lactobacillus casei/paracasei/zeae* and *Leuconostoc mesenteroides* subsp. *mesenteroides*. By evidences emerged from minimal inhibition concentration (MIC) to lysozyme, lactobacilli resistance appeared to be strain's related; however, strains could be grouped into three classes according to their resistance behaviour. Representative strains of these three classes, (*Lactobacillus plantarum* B30, *Leuconostoc mesenteroides* subsp. *mesenteroides* B1 and *Lactobacillus mali* B4p) were employed in a winemaking trial by using synthetic must. In detail, each strain was inoculated in must added of lysozyme (20 mg ml^{-1}) singly or in combination with a commercial strain of *Saccharomyces cerevisiae*. Inoculated musts were used as controls. Lactic microflora was monitored for twenty days of fermentation, confirming evidences emerged by MIC determination. In all cases following to yeast addiction microbial loads appeared strongly reduced.
In conclusion, results obtained in this study confirmed the relative lack of antimicrobial activity of lysozyme on *Lactobacillus* strains already recorded during preceding surveys (Delfini *et al*., 2004). Nevertheless, in the chosen challenging experimental conditions, lysozyme activity proved to be a potential suitable tool to control lactic bacteria populations and to manage the start of the malolactic fermentation only if combined with a highly fermentative yeast, as already pointed out by several authors (Ribéreau-Gayon *et al*., 1998). Since lysozyme is a protein that partially remains in the white treated wine, further investigations are needed to highlight possible secondary effects and understand how to properly use it in this Sicilian wine production.

Keywords: lysozyme, white wine, *Lactobacillus*.

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