APPLICATION OF OZONE AT THE MICROBIAL CONTROL TROUGH THE OENOLOGICAL CHAIN

Raffaele Guzzon*, Sergio Moser, Tomas Roman, Loris Tonidandel, Tiziana Nardin and Roberto Larcher
Centro di Trasferimento Tecnologico. Fondazione Edmund Mach. Via Edmund Mach 1. 38010 San Michele all'Adige (TN), Italy. *raffaele.guzzon@fmach.it +39 0461 615184

ABSTRACT

In this work we present some applications of ozone in the prevention of microbial spoilage through the winemaking. The ozone was employed in the preservation of grapes during drying, necessary for the production of sweet wines, and as sanitizer agent of wood barrels. For each application we described the modification of microflora due to the action of ozone, combining plate count and NGS sequencing analysis. When the interaction between ozone and oenological matrices may have impact on the composition of materials and/or wines, the effects of ozone on the nature of most relevant components was evaluated by high-resolution analytical techniques (GC and UHPLC/MS). Results confirmed that ozone is a valuable alternative to chemical sanitizers, capable to obtain a complete sanitization in the technological interval of microbial contamination.
No alterations of the oenological matrices were observed, excluding interferences of ozone with the winemaking.

MATERIALS & METHODS

Ozone was produced by a cold plasma O_3 generator (Moving Fluid, I) having a nominal production of 80 g/h of O_3. Grapes treatment was performed in an hermetically closed chamber, reaching an O_3 concentration of 100 mg/L for 6 hours. In the barrels sanitization O_3 was employed in as gas or dissolved in water. In the first case, the generator was coupled to the barrels by a Teflon tube of 0.5 cm of diameter; the flow of gas was adjusted at 10 L/h. Aqueous O_3 treatment was performed coupling the O_3 generator to a water thank having a nominal volume of 100 L. To achieve a narrow ozone dispersion in water a valve having a porous membrane of glass (nominal porosity 2 µm) was inserted at the exit of O_3. After O_3 enrichment, water was employed to fill barrels, mantling the temperature at no more than 15 °C.
Microbiological analysis were performed according the OIV methods (OIV MA-AS4-01:2010). Pyrosequencing analysis of microbiota was based on the amplification of 525-nucleotide sequence of the V1-V3 region of the bacterial 16S rRNA gene and a ITS1-ITS4 region of the fungal Internal Transcribed Spacer (ITS).
Oenological parameters of wines were determined by FT-IR (Foss Winescan), the phenolic and volatile profiles of oenological matrices treated by O_3 were characterized respectively by an UHPLC equipped by a Hybrid Quadrupole-Orbitrap Mass Spectrometer and GC-MS. Major details about the analytical methods are reported in the in the papers listed below.

PREVENTION OF MICROBIAL SPOILAGE DURING GRAPE DRYING

Straw wine is an high-valuable oenological production, affected by relevant economical losses due to proliferation of spoilage microorganisms during drying of grapes, after the harvest. In this work ozone was employed to preserve grapes (*cv.* Sauvignon, Moscato, Riesling and Gewürztraminer) during drying, Results were evaluated in terms of qualitative and quantitative modifications induced in the epiphytic microflora of grapes. In addition, the alteration exerted by ozone on grape's chemical composition was investigated.

Botrytis cinera mycelium after O_3 treatment

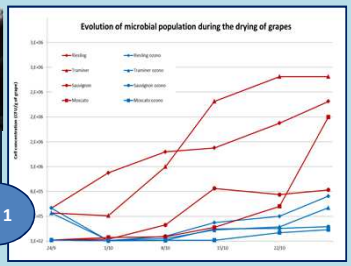
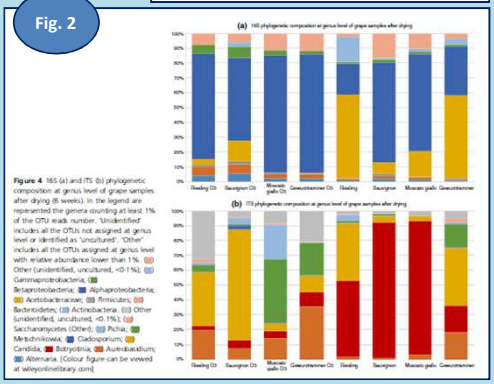


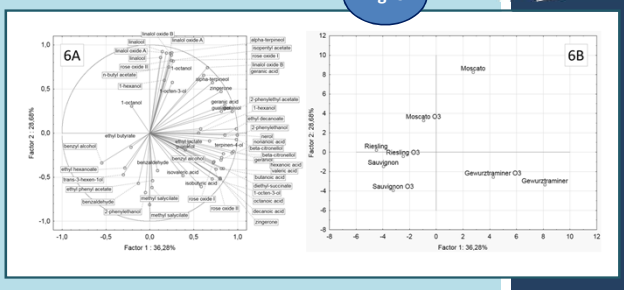
Fig. 1

Fig. 2



Grapes were treated with ozone during the drying period (6 weeks). The microflora was quantified weekly by plate counts and characterized by 454-pyrosequencing, in comparison with that of identical, untreated, grapes. Ozone reduced the microbial population up to 3 log units (Fig 1). From the qualitative point of view, ozone acts better against spoilage microorganisms, such as *Acetobacter* and *Botrytis cinerea* (Fig 2).
The statistical analysis (Fig 3) discriminates grape samples in function of the epiphytic microflora that residues at the end of drying and not for their chemical composition, excluding alteration due to O_3 treatments. These evidences confirmed that ozone controls spoilage microorganisms without altering the volatile profile of grapes. Chemical analysis revealed that untreated grapes are less suitable for winemaking owing to the deprivation of some valuable compounds during the microbial proliferation (APA, organic acids).

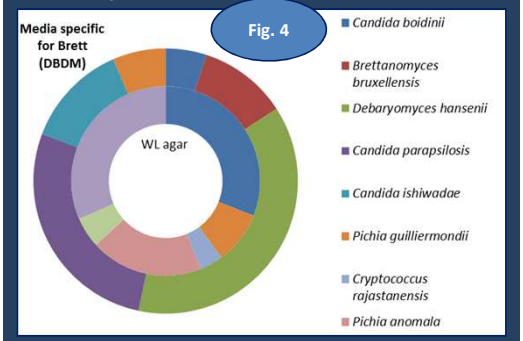
Fig. 3



MONITORING AND SANITIZATION OF OAK WINE BARRELS

The barrels play an important role in winemaking, giving to the wine specific flavours and assisting the ageing process. However, the porosity and inertness of wood encourage the proliferation of spoilage yeasts which can severely affect wine quality. The yeast microbiota that colonize barriques located in a traditional Tuscan winery was characterized (Fig 4). Yeasts belonged to the genera *Saccharomyces*, *Candida*, and *Pichia*, while *Brettanomyces/Dekkera* (the main wine spoilage yeast) represented a limited part of the population and was able to growth only onto DBDM medium. Bacteria were not detected in concertation having oenological relevance. 4 treatments (30 min of duration) were tested for their efficacy in eradicating yeast inside the barrels: aqueous steam, UV irradiation (36 W lamp), gaseous O_3 (40 mg/m³) and aqueous O_3 (2 mg/L). Steam and O_3 were the most effective treatments, eliminating about 70% of yeasts. UV appeared to be less effective, probably due to the porous nature of wood, which protects cells from direct irradiation (Fig 5).

Fig. 4



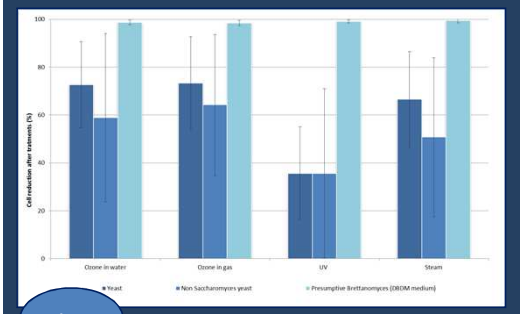


Fig. 5

Barrels sanitization by O_3 produced by a cold-plasma generator and biofilm formed by Brettanomyces on the internal surface of barrel

SANITIZATION OF BARRELS AND EVALUATION OF OZONE LONG-TERMS EFFECT ON WINE

In this work the efficacy of two sanitation treatments, carried out using ozone and sodium hydroxide, against barrel spoilage microflora. After sanitation, barrels were filled by a red wine (Lagrein) and the long-term (3 months) effects of the aging in barrels on the composition of the wines (simple phenol and main chemical parameters) were evaluated to understand if the different sanitizing treatments are able to interfere with the ordinary evolution of wine,
Fig 6 reports the results of microbiological survey inside barrels before and after the sanitization treatments. Data are referred to the microbial contamination of 50 liters of sterile water kept inside barrels for 24 hours under agitation (NT, not treated barrels; CT, barrels treated with a chemical sanitizer; OT, barrels treated with ozone. Number of barrels with the same treatment, n=5). Is evident that the ozone is the most active sanitizer especially against *Brettanomyces*.

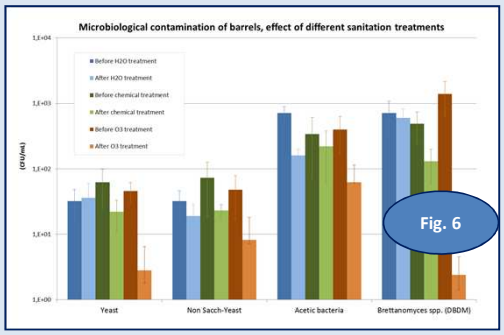
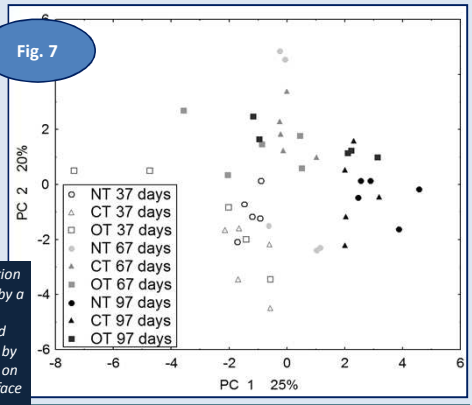


Fig. 6

The simple phenols of wine (38 compounds) were characterized using a UHPLC-MS approach during the first 3 months of wine ageing, to study possible alterations to the chemical exchange from wood to wine due to the sanitizing agents. The PCA analysis (Fig 7) of the entire set of data not reviled differences related to the sanitizer, but only due at the time of aging in barrels (1, 2 or 3 moths).

Fig. 7



REFERENCES

Guzzon, R. et al. (2018) ***Application of ozone during grape drying for the production of straw wine. Effects on the microbiota and compositive profile of grapes****. J. App. Microbiol. 125, 513-527.*

Guzzon, R. et al. (2017) ***Exploring the microbiota of the red-brown defect in smear-ripened cheese by 454-pyrosequencing and its prevention using different cleaning systems.*** *Food Microbiol. 62, 160-168.*

Guzzon, R, et al. (2017) ***The impact of different barrel sanitation approaches on the spoilage microflora and phenols composition of wine.*** *J Food Sci. Technol. 54, 810-821.*

Guzzon, R. et al. (2013) ***Antimicrobial activity of ozone. Effectiveness against the main wine spoilage microorganisms and evaluation of impact on simple phenols in wine.*** *Au. J. Grape Wine Res. 19, 180-188.*

Guzzon, R. et al. (2011). ***Survey of the yeast population inside wine barrels and the effects of certain techniques in preventing microbiological spoilage.*** *Eu. Food Res. Techno. 233, 285-291.*