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Assessing the Microbiological Quality of Innovative Sustainable Low- and No- Alcohol Wine Production

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INTRODUCTION & AIM

The wine industry is facing a growing demand for low- and no-alcohol wines (LNAWs), driven by shifting consumer preferences and the impact of climate change. The project "INNOWINE"—Cascading Grant Spoke 3 - Project OnFoods—applied an optimised production cycle to limit alcohol formation during fermentation, offering an innovative alternative to conventional post-fermentation dealcoholization. Among the strategies tested, we investigated X-ray irradiation, a non-thermal method proposed as a replacement for sulfur dioxide and to improve wine microbial stability. Microbiological quality was assessed in experimental low-alcohol wines (EW) and grape must permeate (P) compared to conventional wine (CW).

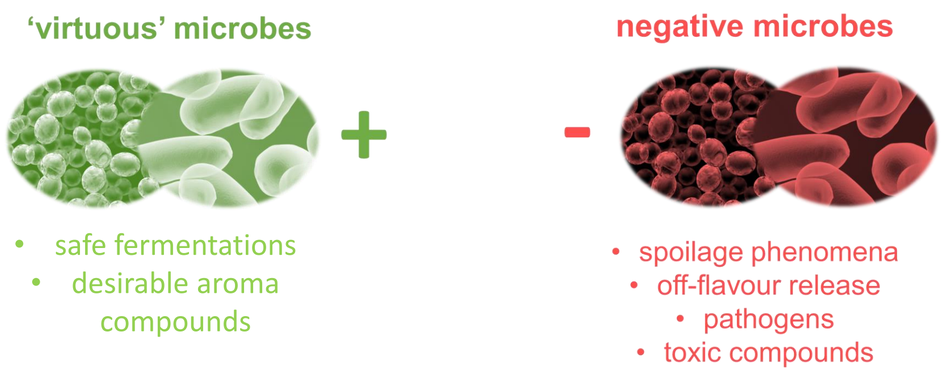

Figure 1. Virtuous vs negative microbes: assessing microbial impact is key in LNAWs production.

METHOD

Total mesophilic prokaryotes and yeasts were quantified using PCA and WLNM, respectively. Putative lactic acid bacteria (LAB), acetic acid bacteria (AAB), and *Brettanomyces* were enumerated on differential media (i.e. MRS, GYC, mWLNM), all supplemented with cycloheximide to suppress undesired yeasts and improve selectivity.

Culture Medium	Target Microorganisms	Selective Additives	References / Notes
Plate Count Agar (PCA)	Total mesophilic aerobic and facultative anaerobic bacteria	—	Total viable count of mesophilic microorganism
Wallerstein Laboratory Nutrient Medium (WLNM)	Total yeasts	Chloramphenicol 100 mg/L	Differential medium for yeasts in fermented products
De Man, Rogosa e Sharpe Agar (MRS Agar)	Lactic Acid Bacteria (LAB)	Cycloheximide 50 mg/L	*De Man, Rogosa & Sharpe, 1960* – Selective medium for LAB
Glucose-Yeast extract-Calcium carbonate Agar (GYC Agar)	Acetic Acid Bacteria (AAB)	Cycloheximide 50 mg/L	*Gullo et al., 2006* – Isolation of AAB from oenological matrices
Modified Wallerstein Laboratory Nutrient Medium (mWLNM)	*Brettanomyces* spp.	Cycloheximide 30 mg/L	*Di Toro et al., 2015* – Modified selective medium for *Brettanomyces* spp.

Table 1. Differential media used for selective enumeration of target microbial groups in EW, P, and CW with references for standard protocols.

Samples were analysed with and without X-ray treatment at 0.5, 1.0, and 2.0 kGy.

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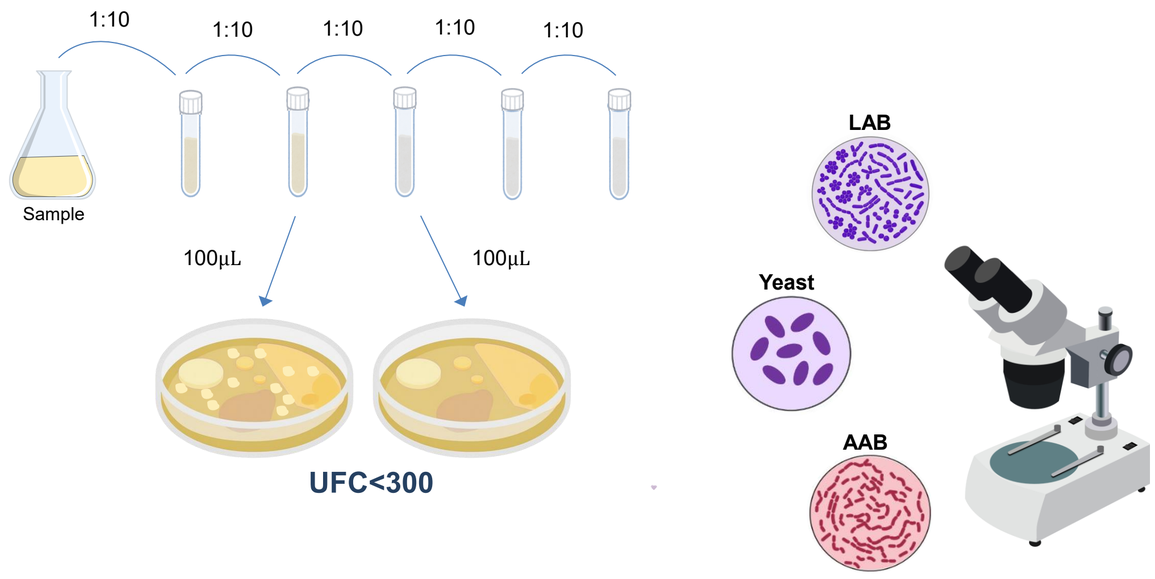

Figure 2: Serial dilutions and spread plating were performed to isolate and enumerate target bacteria and yeasts on selective media. Microscopic observations were conducted for a preliminary evaluation of the microbial targets. Gram staining was performed to distinguish between bacteria (LAB: Gram-positive; AAB: Gram-negative), while yeasts were observed to identify their morphologies.

RESULTS & DISCUSSION

Preliminary findings show higher microbial counts in EW and P than CW without irradiation. Irradiation progressively reduced microbial loads from partial reductions (i.e. 0.5 kGy) to marked reduction of microorganism across all media (i.e. 2.0 kGy).

Figure 3. Preliminary significant results showing mean total viable counts (CFU/mL) on PCA medium for LNAWs produced with different experimental vinification methods (EW1, EW2, EW3) and without sulfur dioxide (NSD). Irradiation at 2 kGy resulted in a lower microbial load compared to 0.5 kGy, with a dose-dependent reduction in the microbial population.

CONCLUSION & FUTURE WORK

- X-ray technology appears promising for stabilising LNAWs while preserving quality, offering a potential substitute for sulfur dioxide.

- Future work will explore process optimisation and scale-up of X-ray treatment, assessing its impact on wine composition and shelf-life.

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