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Monitoring the Fermentation of cv. Kalamata Natural Black Olives with LAB Starter Cultures Using Raman Spectroscopy

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INTRODUCTION & AIM

Spontaneous fermentation can promote the growth of undesirable strains, leading to abnormal fermentation and off-flavors. The use of suitable starter cultures, such as lactic acid bacteria (LAB), can minimize the risk of spoilage and ensure a controlled and stable fermentation [1]. The objective of this study was to evaluate the effectiveness of Raman spectroscopy as a rapid and non-invasive technique to monitor both spontaneous and inoculated fermentation of cv. Kalamata black olives.

METHOD

Kalamata natural black olives were subjected to Greek-style processing according to the traditional anaerobic method, in 7% brine with 50% NaCl substitution by KCl.

Three fermentations were conducted:

- ✓ **Spontaneous fermentation with the indigenous microbiota**
- ✓ **Inoculated fermentation with the commercial product VegeStart-60 (*Lactiplantibacillus plantarum*)**
- ✓ **Inoculated fermentation with the probiotic starter culture *Lactiplantibacillus pentosus* B281 (FMCC, AUA)**

- ✓ All fermentations were carried out in duplicate at controlled room temperature (20-22 °C) for 145 days.

Raman Spectra Acquisition

- ✓ Raman spectra were acquired twice a week from the surface of fermenting olives.
- ✓ Twelve spectra were captured from the surface of olive drupes (3 olives × 4 sample points/olive).
- ✓ Spectra were averaged and the area from 1800 to 900 cm^{-1} was considered for further analysis.

Ortho Partial Least Squares Discriminant Analysis (OPLS-DA)

- **Fermentation Day 0**
- **Fermentation Day 145**

RESULTS & DISCUSSION

The plot of scores in Figure 1, illustrates on the horizontal axis the class separation, against the within class variation depicted in the vertical axis.

- ✓ Distinct class separation was observed across the horizontal axis between the samples corresponding to the beginning and end of fermentation, with no overlapping at the 95% confidence interval level.

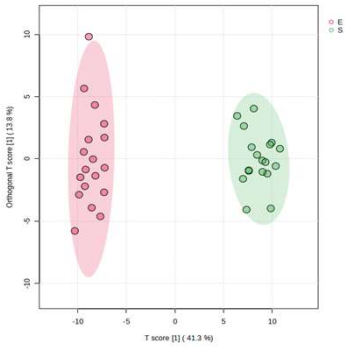

Figure 1. OPLS-DA Scores Plot discriminating cv. Kalamata table olives at the beginning (S) and end (E) of fermentation. Ellipses indicate the 95% confidence interval for each group.

Table 1. Best performance metrics of Calibration and Cross-Validation for the PLS-R Models. Statistical outputs include Latent Variables (LVs), R^2 for Calibration (R^2_C), Cross-Validation (R^2_{CV}), and Root Mean Squared Error (RMSE) of calibration and validation.

Model	LAB	LVs	R^2_C	$RMSE_C$	R^2_{CV}	$RMSE_{CV}$
LAB	Raw data	6	0.6045	0.6154	0.2113	0.7739
Yeasts	SNV[1]	4	0.5243	0.8298	0.3453	0.6250
pH	Raw data	7	0.8076	0.2409	0.6740	0.3151
Acidity	SG[2]	6	0.7745	0.1245	0.7475	0.1321

[1] Standard Normal Variate, [2]Savitsky-Golay 2nd der. (window 11, polynomial order 2)

- ➢ PLS-R models for pH and acidity demonstrate robust performance, providing a substantive foundation for further analysis and exhibiting considerable potential for enhancing model efficiency.
- ➢ PLS-R models regarding microbial evolution of LAB and yeast exhibited less robust results.

CONCLUSION

Overall, the results obtained in this work provide a promising perspective for the use of Raman spectroscopy as a rapid and non-invasive technique to monitor table olive fermentation. However, substantial model improvement through further studies is required before this method can be implemented on an industrial scale.

FUTURE WORK / REFERENCES

1. Bonatsou, S.; Tassou, C. C.; Panagou, E. Z.; Nychas, G.-J. E. Table Olive Fermentation Using Starter Cultures with Multifunctional Potential. Microorganisms 2017, 5 (2), 30. https://doi.org/10.3390/microorganisms5020030.