

ACETIC OR LACTIC BACTERIA CONTAMINATION? ASCA HAS THE ANSWER

D. Schorn-García¹, J. Ezenarro¹, M. Mestres¹, L. Aceña¹, O. Busto¹, B. Giussani², R. Boqué³

daniel.schorn@urv.cat

¹Sens Group. QAQO Dpt. Universitat Rovira i Virgili, Tarragona, Spain

²SmartChemoLab Group. DiSAT Dpt. Università degli Studi Dell'Insubria, Como, Italy

³GQqIN Group. QAQO Dpt. Universitat Rovira i Virgili, Tarragona, Spain

www.isens.urv.cat/en/

Introduction

Alcoholic fermentation of grape must is a biochemical process in which yeasts mainly transform sugars into ethanol to obtain wine. Like any bioprocess, its complexity requires exhaustive monitoring to prevent the action of undesirable microorganisms, such as lactic acid and acetic acid bacteria, which can slow down the fermentations and generate unwanted substances.

The common analytical control in wineries implies daily measurements of temperature, density and pH but, when more information is needed, supplementary analyses are performed in off-site laboratories. This externalization is time-consuming and it implies a delay in obtaining results with the subsequent delay in the application of possible corrective measures. Therefore, the use of fast and portable techniques such as the IR-spectroscopy can be very useful to obtain real-time information when monitoring the alcoholic fermentation process [1,2].

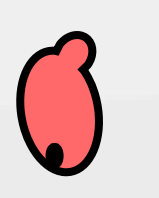
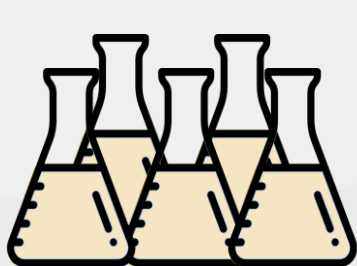
Aim of study

Combine FTIR-ATR spectroscopy and chemometric techniques as a control tool following the PAT (Process Analytical Technologies) guidelines [3].

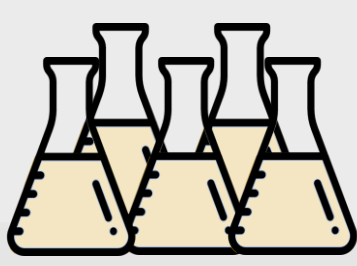
Apply ANOVA - Simultaneous Component Analysis (ASCA) to factorize the alcoholic fermentation variability sources, such as the process evolution and the contamination with lactic acid or acetic acid bacteria.

Materials and Methods

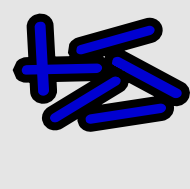
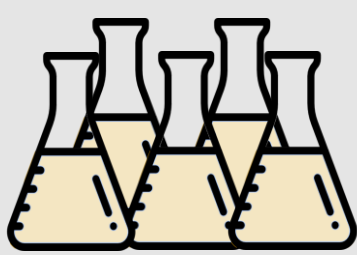
24 small-scale alcoholic fermentation batches were monitored (white must → white wine)



15 batches in Normal Operation Conditions (NOC)



5 out-of-control batches by acetic acid bacteria contamination (AAB)



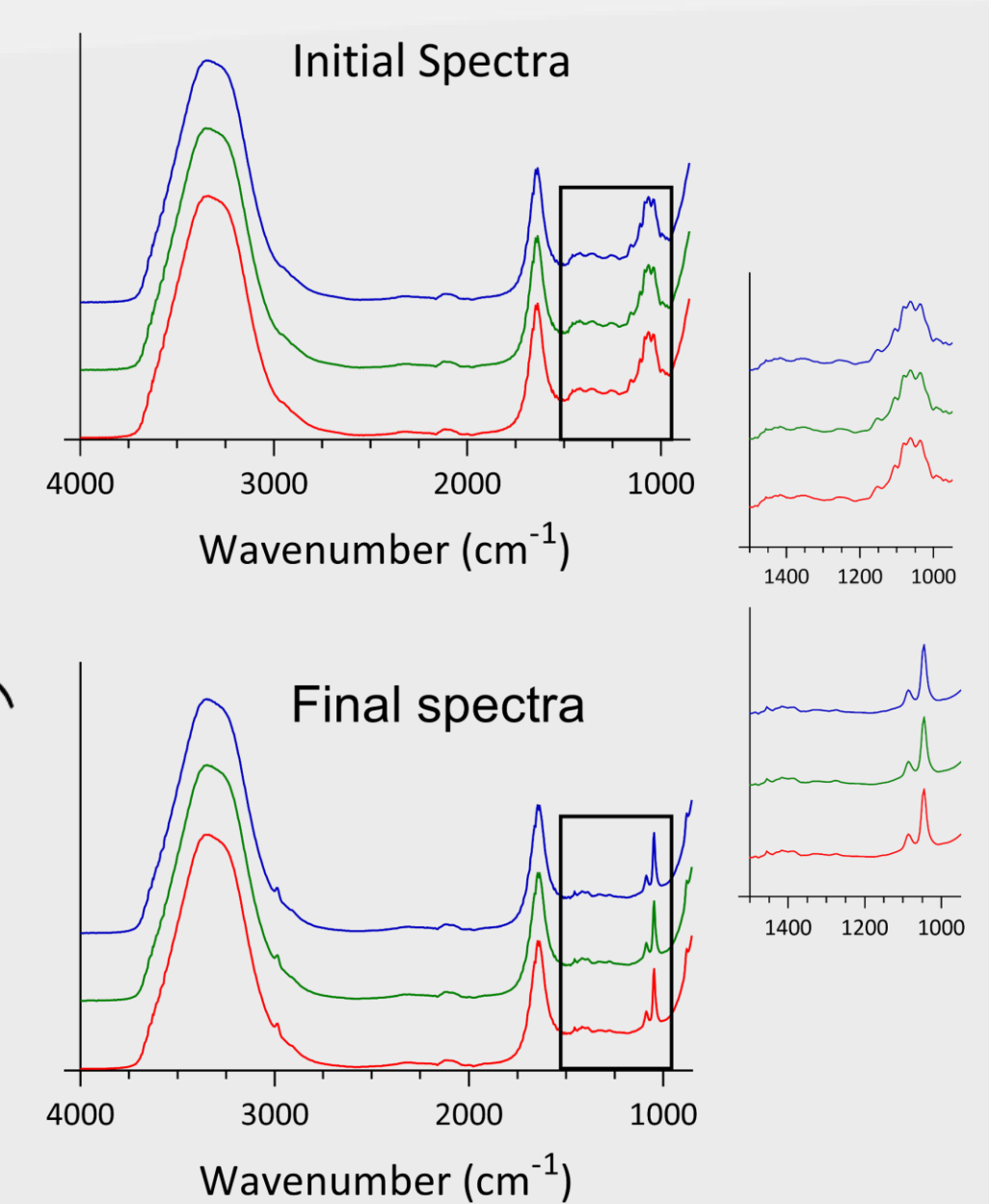
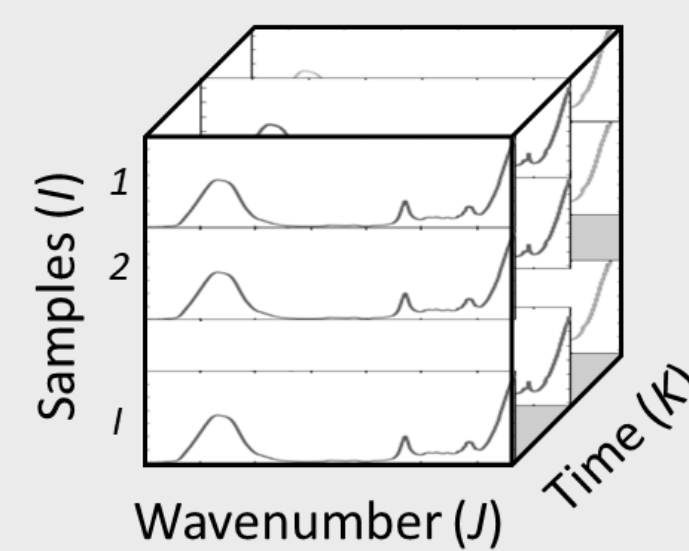
4 out-of-control batches by lactic acid bacteria contamination (LAB)

Usual oenological parameters (density and pH) were also daily analysed

Portable Attenuated Total Reflectance (ATR) Fourier Transform Infrared (FTIR) spectrometer

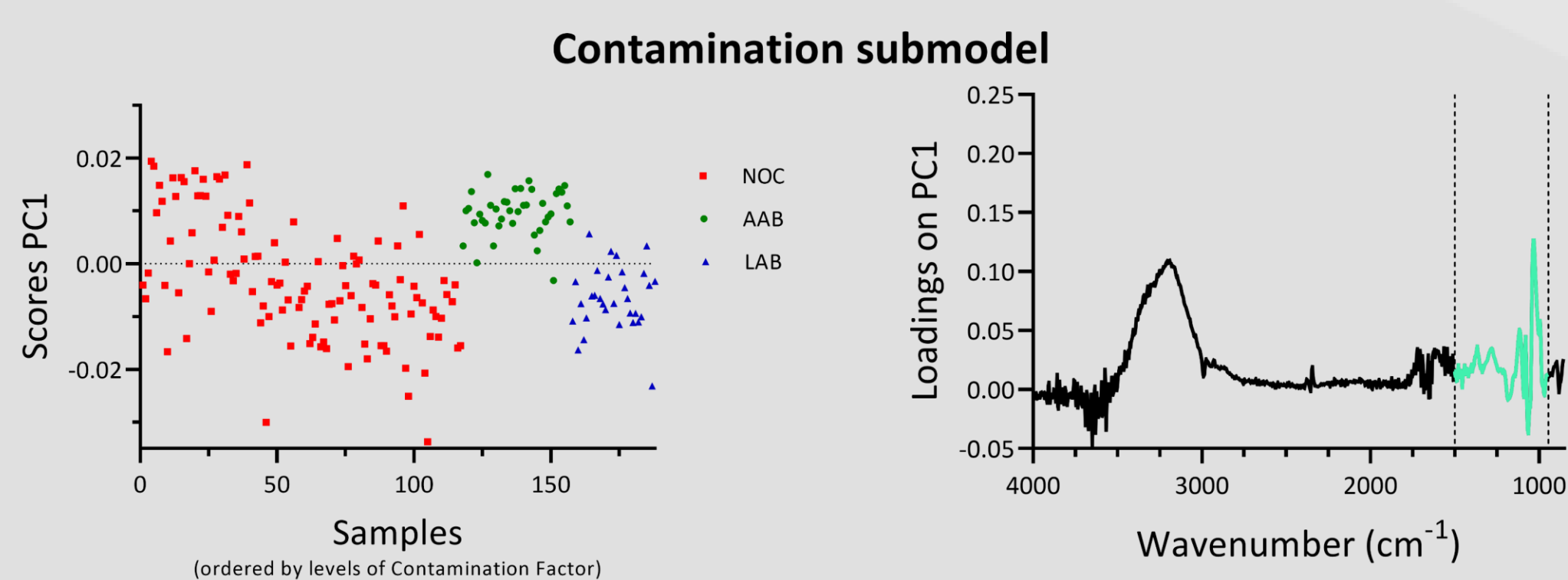


4000 – 850 cm⁻¹
(845 variables)
3 replicates
32 scans
8 cm⁻¹ resolution

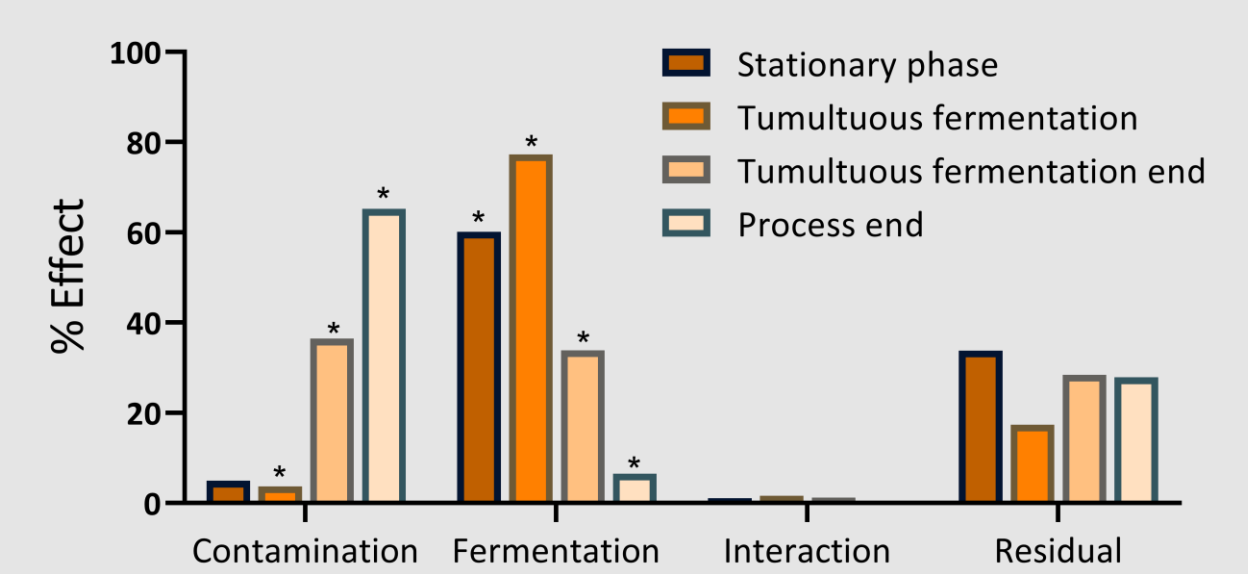


Results

ASCA in IKxJ (Sample-Time x Wavenumber) matrix

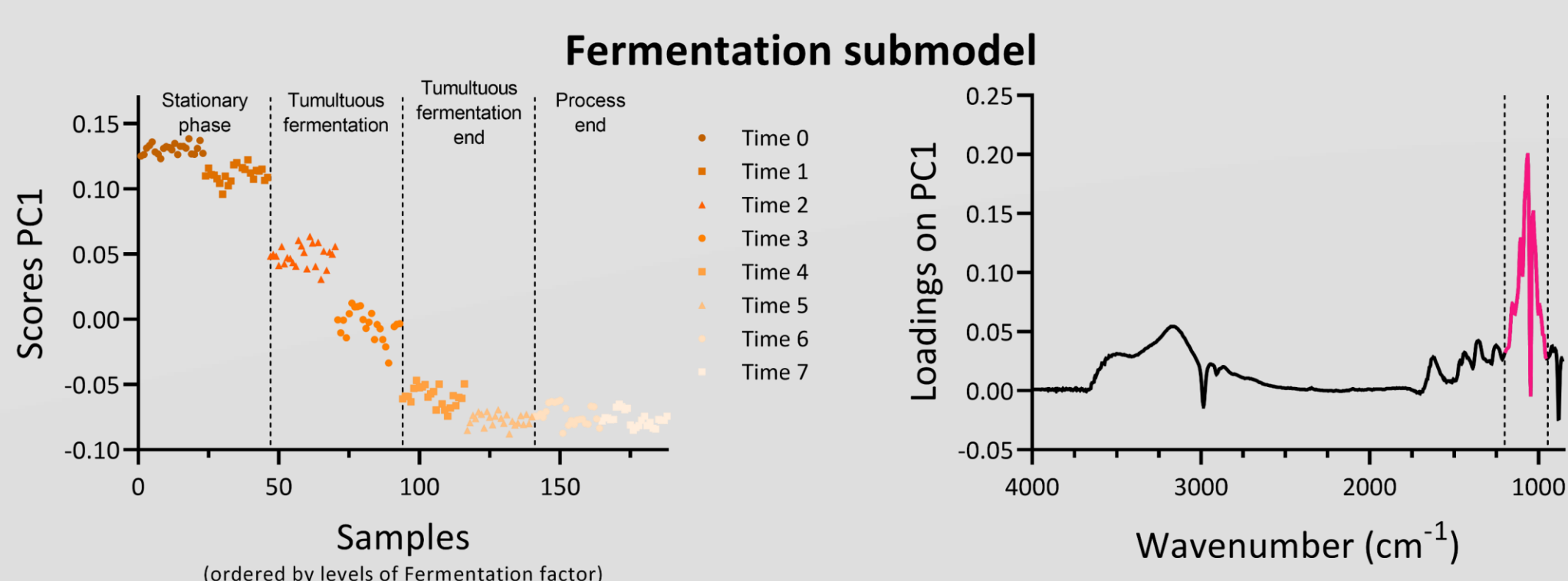


Selection of a specific region related to bacteria contamination and subtraction of the region more related to alcoholic fermentation



Division of the process into different phases due to their characteristics

As the process is ending, it is possible to focus on the "Contamination" factor

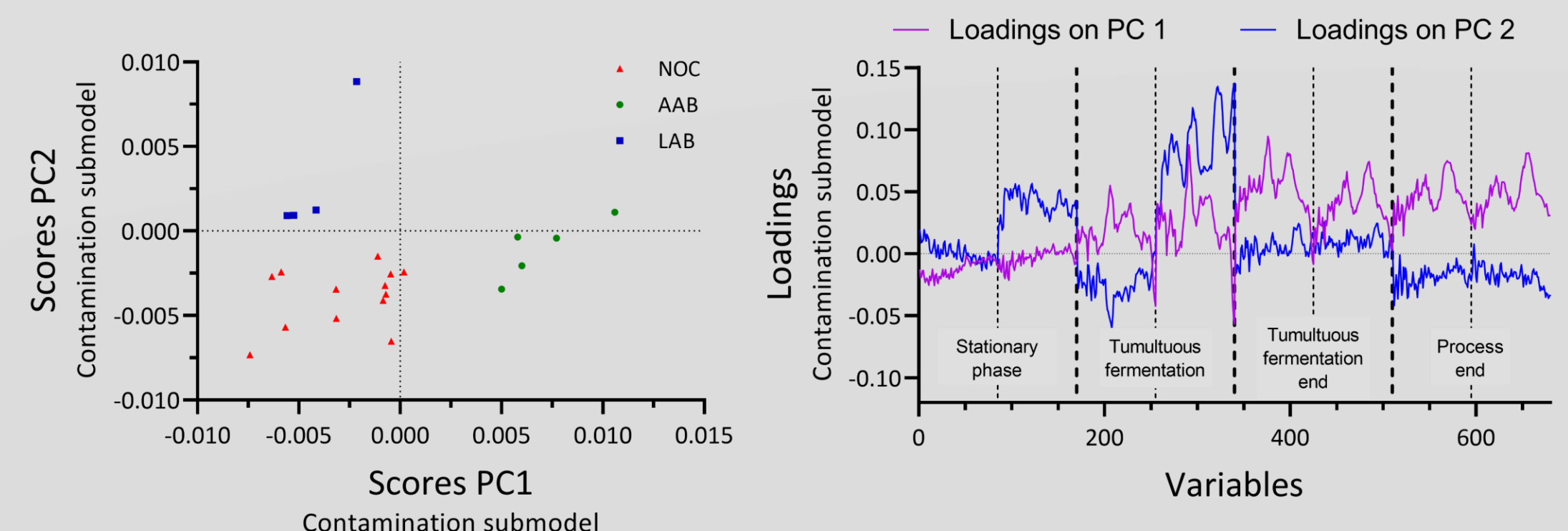


Factor	% Effect
Contamination	0.64*
Fermentation	95.61*
Factor interaction	0.70*
Residual	3.05

*: p-value < 0.05 meaning the factor is significant.

Alcoholic fermentation comprises the main variability source in the matrix. It should be noted that despite its small percentage effect, the contamination factor is significant.

ASCA in IxJK matrix



Factor	% Effect
Contamination	28.36*
Residual	71.64

IxJK unfolding allows to focus on a specific factor of interest and study it individually

Conclusions

- There are different approaches to perform ASCA models, such as different ways to arrange the data, focus on specific regions related to the factor of interest or divide the process in phases taking into account their characteristics.
- Different ASCA models have been built to study the effects of acetic acid or lactic acid bacteria contamination, showing that both contaminations are detected and even discriminated.

Bibliography

- [1] D. Cozzolino, *Appl. Spectrosc. Rev.* **51**(4) (2015) 302-317.
 [2] D. Schorn-García, J. Cavaglia, B. Giussani, O. Busto, L. Aceña, M. Mestres, R. Boqué. *Microchem J.* **166** (2021) 106215.
 [3] FDA Off. Doc., Guidance for Industry PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance (2004) 16.

Acknowledgements

Grant PID2019-104269RR-C33 funded by MCIN/AEI/ 10.13039/501100011033. This poster has been possible with the support of the Secretaria d'Universitats i Recerca del Departament d'Empresa i Coneixement de la Generalitat de Catalunya (2020 FISDU 00221) (Call 2020).

