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The use of Raman spectroscopy, HPLC and black box modelling in a complex media matrix in bioprocesses

Introduction

- Yeast extracts are highly complex media components
- Great influence on microbial behavior
- Complexity of the analysis because of the many compounds
- High product to product and batch to batch variability
- Various inputs, here specifically HPLC and Raman spectroscopy

Goals

- Goal 1: Identification of singular influential components
- Goal 2: Black box models to predict yeast extracts performance

Despite the strong influence of yeast extract on Raman spectra,...

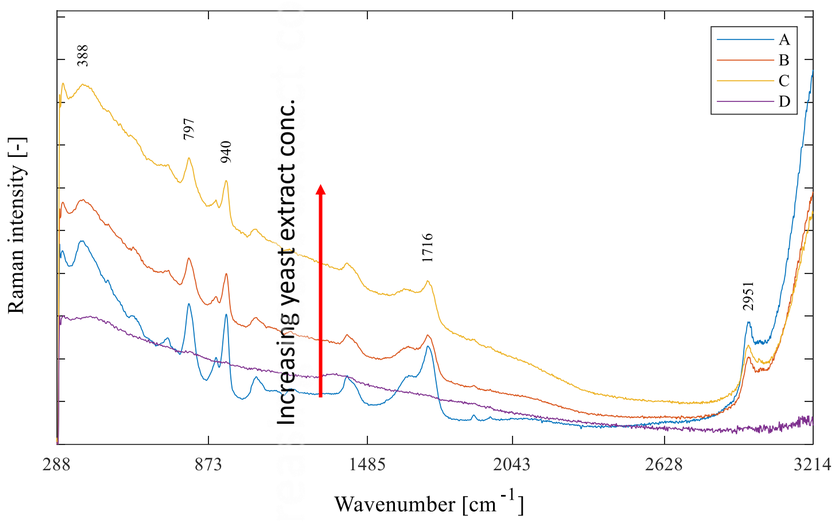

Fig. 1: Raman spectrum of citric acid in four different media. A: 100 gL^{-1} citric acid in demineralized water; B: 100 gL^{-1} citric acid in 0.5% yeast extract; C: 100 gL^{-1} citric acid in 1% yeast extract; D: 90 gL^{-1} citric acid in 0.5% Yeast Extract + Yeast Cells (OD = 42.5). Figure courtesy of Jan Steinhart.

The complex media matrix of yeast extracts hinders the analysis of singular components as it interacts with the analytical methods.

However, Raman spectroscopy can be used for various singular components, even in cell-containing media, as well as NIR and 2D-fluorescence. It was possible to calculate various components like carbon sources, total protein concentrations, cell density, acids, ethanol, etc. with success

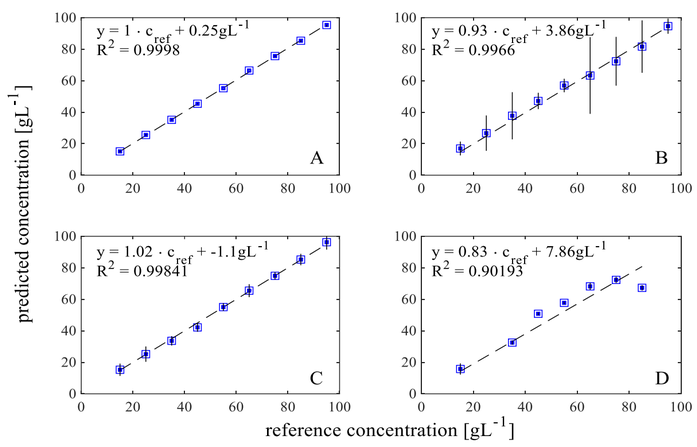

Fig. 2: Reference concentration of the validation data set in gL^{-1} plotted against the predicted concentration from the model in gL^{-1} for citric acid. Figure courtesy of Jan Steinhart.

...the calculation of the concentration of single components is still possible

But to calculate all singular, influential components of yeast extracts would be time-consuming and expensive...

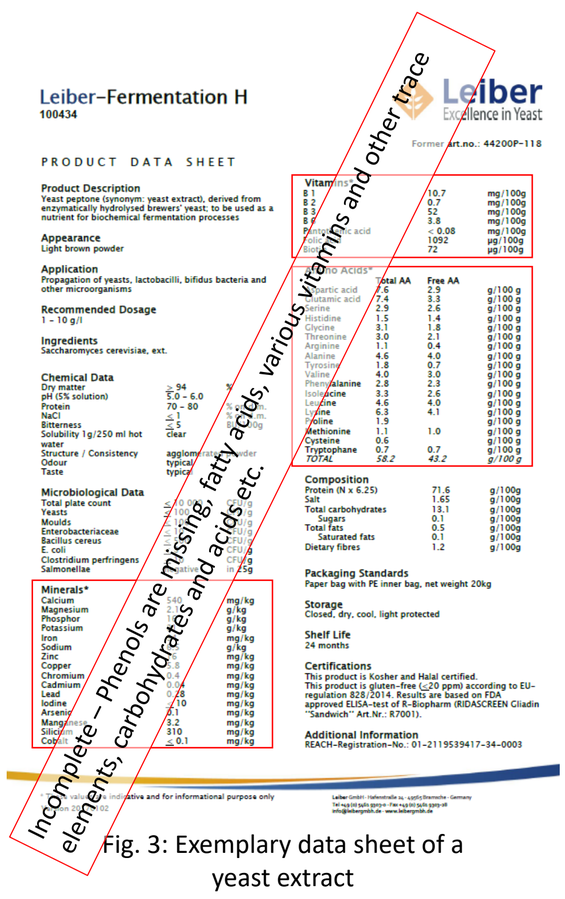

Fig. 3: Exemplary data sheet of a yeast extract

Various inputs and modeling approaches have been combined to find a way to judge the yeast extracts influence without falling back on singular component analysis.

As inputs various analytical methods which create huge amounts of data have been used – mainly HPLC (fast, easy, low cost) and Raman spectroscopy (fast, easy, high cost)

As output either the yeast extracts were categorized in good (high growth) or bad (low growth), for support vector machines, or the cell yield on yeast extract $y_{X/HE}$ with non-limiting carbon source, phosphate and nitrogen was calculated.

For the creation of the models half of the yeast extracts were used for training, and half for validation and testing of the models.

For *Pichia pastoris* and *Escherichia coli* the modelling predicts very well, especially with Raman spectra and carbohydrate HPLC chromatograms.

Bacillus licheniformis shows promising results with Raman spectra.

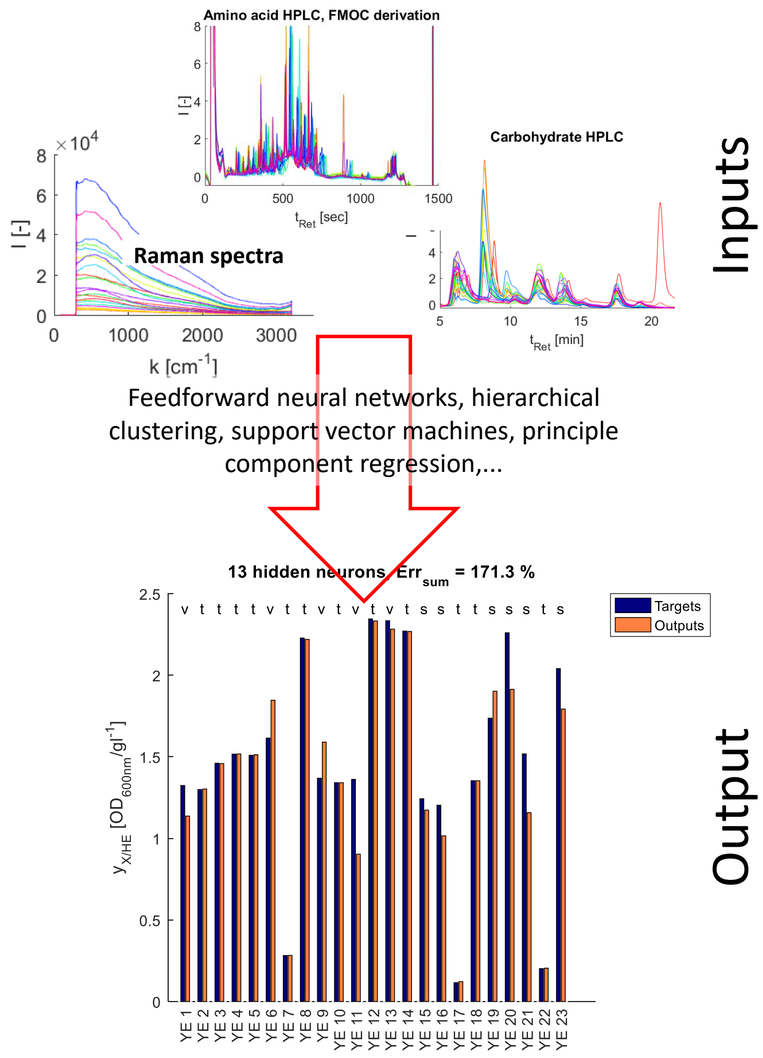

Fig. 4: Basic creation of a black box model

...simple black box models have been shown to be promising candidates to discern yeast extract quality.

Conclusion

- Despite the media complexity, Raman spectroscopy can detect singular components
- Can be used to calculate concentrations of singular components
- Raman spectra are powerful tools in the creation of data-driven black box models
- HPLC chromatograms can also be used to predict the influence of the yeast extracts on microbial growth
- Most useful black box modelling approaches were used for support vector machines and feedforward neural networks

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