Application of a segmented analysis by MCR-ALS on ^{1}H-NMR spectroscopy for the identification of adulterations in brown sugars

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INTRODUCTION

Different types of brown sugar available in the market (Beet, Cane and Coconut) could be susceptible to food fraud given their similar organoleptic character and differences in their prices [1]. Certain minor organic compounds in addition to sucrose usually remain in the final product, which can be identified and used as markers for the differentiation of these sugars. The identification of a wide range of compounds in a complex matrix can be analyzed by nuclear magnetic resonance (NMR) spectroscopy; however, the information contained in an NMR spectrum can be a challenge given the complexity of its spectral interpretation [2]. **The objective of this study,** is to improve the differentiation of types of brown sugars, mixtures and extracts of sugar beet through a **PCA analysis** by integrating and undirected resolution of resonance signals in a ^{1}H-NMR dataset by **Multivariate Resolution Curve-Alternating Least Squares (MCR-ALS)** as an independent preprocessing method, which consists of dividing the data set into spectral windows containing between one and three resonances, where each independent window is resolved by MCR-ALS [3,4], as well as to **compare** the results obtained with a more common spectral preprocessing of dimensionality reduction, such as **Binning.**

METHODOLOGY:

I) Samples

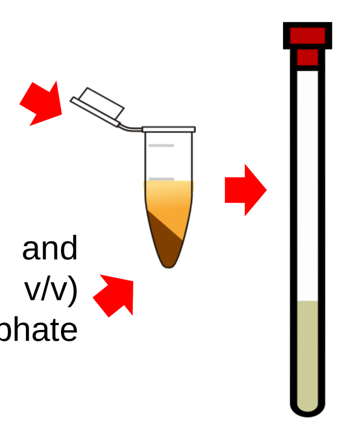
4 Samples of Coconut Brown Sugars — 5 Samples of Cane Brown Sugars → 4 Mixed Brown Sugars (Coconut and Cane)

4 Sugar Beet from Ñuble Region, Chile — 4 Sugar Beet from Bío-Bío Region, Chile → Slices Stored at -20°C

II) Preparation of Samples

300 ± 0,1 mg of each sample with 800 µL of D_2O at pH 7.4 (Phosphate Buffer) with 0,1% p/v of TSP-d4

150 ± 0,1 mg of each sample freeze-dried and extracted with 1000 µL of $MeOH/H_2O$ (1:1 v/v) solution and 800 µL of D_2O at pH 7.4 (Phosphate Buffer) with 0,1% p/v of TSP-d4



600 µL of the supernatant in a 5 mm NMR tube

III) Spectral Acquisition and Preprocessing

MestreNova v.12.0 (Mestrelab Research,Spain)

Acquisition:
Liquid Probe
B_0 :400,13 MHz
Spectral Width: 6393 MHz
Scans: 256
Temperature: 293 K

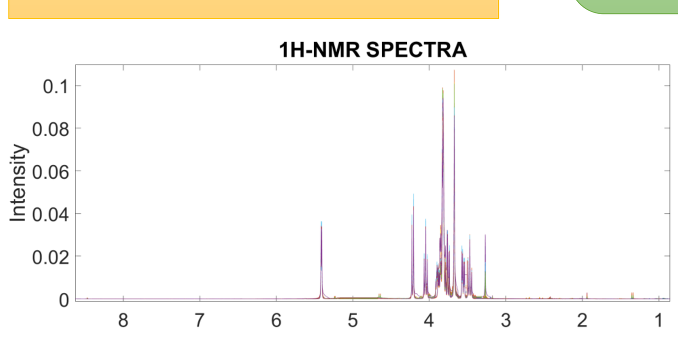
Spectral Preprocessing:
δ TSP-d4 = 0,00 ppm
Phase Correction
Baseline Correction: 3rd order polynomial

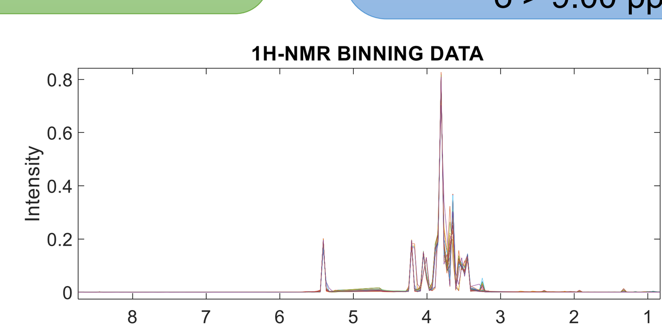
Spectral Regions Removed:
δ < 0,20 ppm
δ 3.32 – 3.38 ppm (CH_3OH)
δ 4.68 – 5.17 ppm (H_2O)
δ > 9.00 ppm

Alignment of resonance signals using "icoshift" [5] algorithm

Two different kind of ^{1}H-NMR data: **Raw and Binning**

Both data were **normalized**

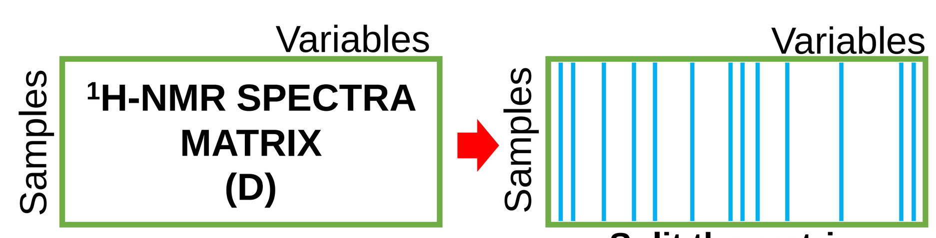




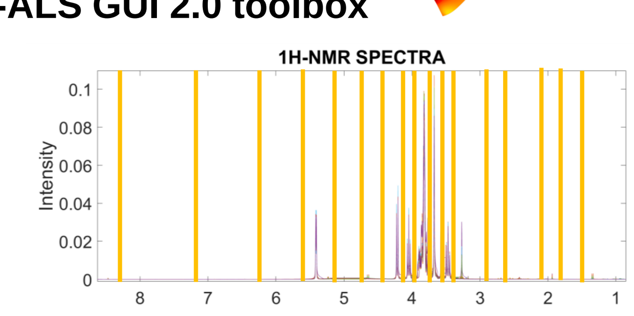
Bucket Data with 0.04 ppm

IV) MCR-ALS Processing

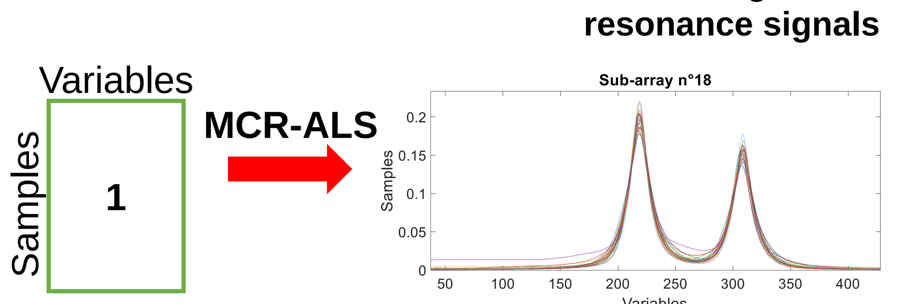
Workflow DTC-MCR-ALS [4] and MCR-ALS GUI 2.0 toolbox — MATLAB



Split the matrix according to its resonance signals

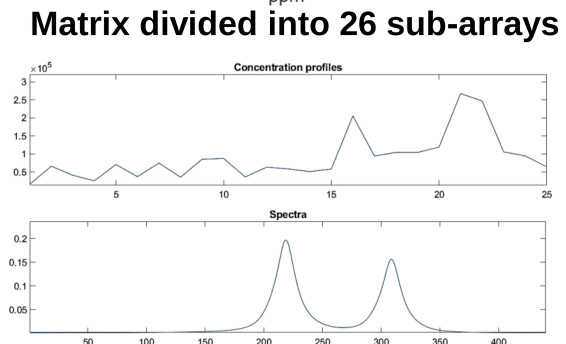


Matrix divided into 26 sub-arrays

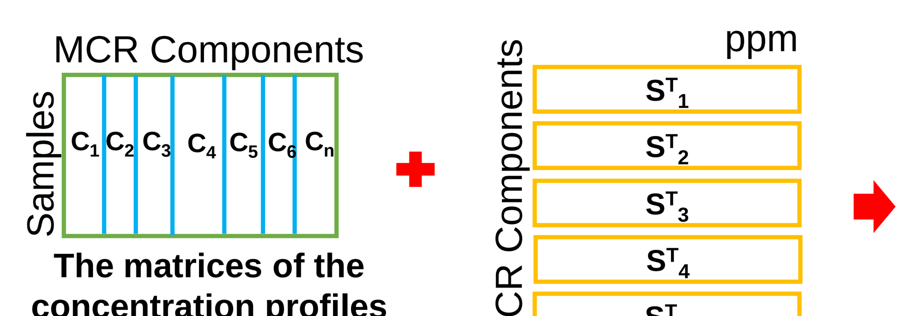


Apply MCR-ALS to each sub-array separately

Doublet associated with sub-array n°18

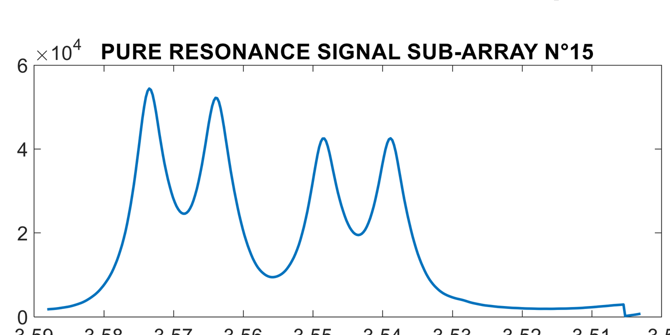


Non-negativity constraints with the algorithm "ffnls" in both modes: Concentration and Pure Spectra



The matrices of the concentration profiles (C_n) for each sub-array are joined in a new augmented matrix

For each concentration profile obtained, its pure resonance spectral signal is resolved



MCR-ALS Analysis:
- **26** sub-matrices C_n with their respective **26** sub-matrices S^T_n
- **32** components were needed to resolve all the resonance signals
- Convergence between **4-80**

V) PCA Analysis

MATLAB

Preprocessing

Conc. Profile Data Matrix (MCR-ALS)

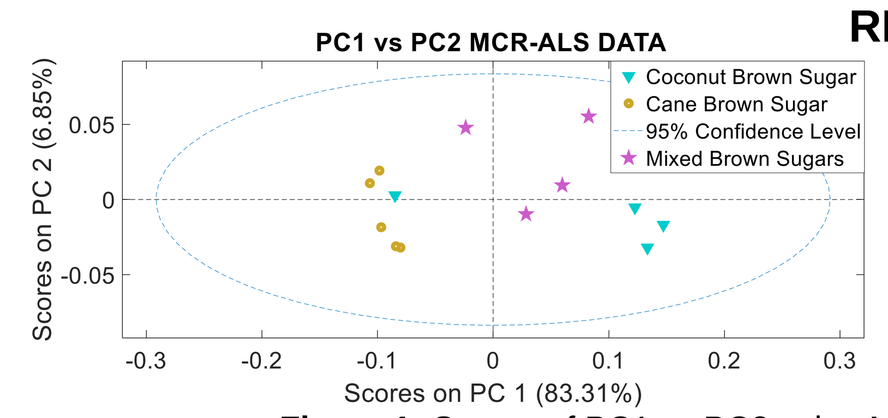
Binning Data Matrix

Mean Center *(Previously Normalized Data)*

PCA

PLS Toolbox 9.0 (Eigenvector Research Inc.)

RESULTS:



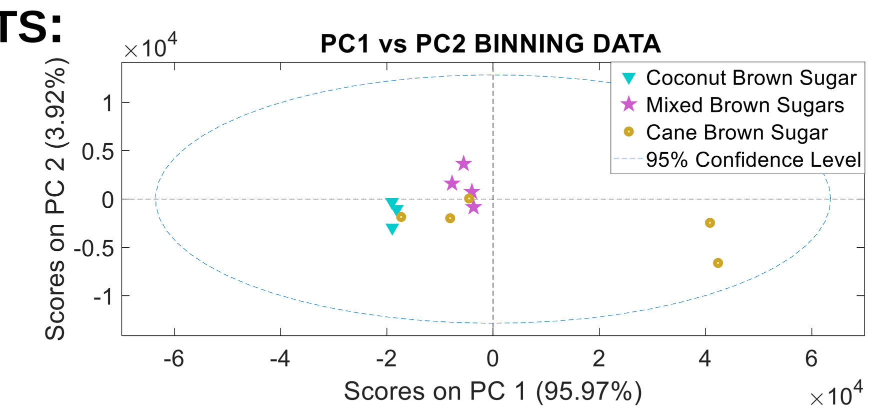


Figure 1: Scores of PC1 vs PC2 using MCR-ALS and Binning Data to evaluate Brown Sugars

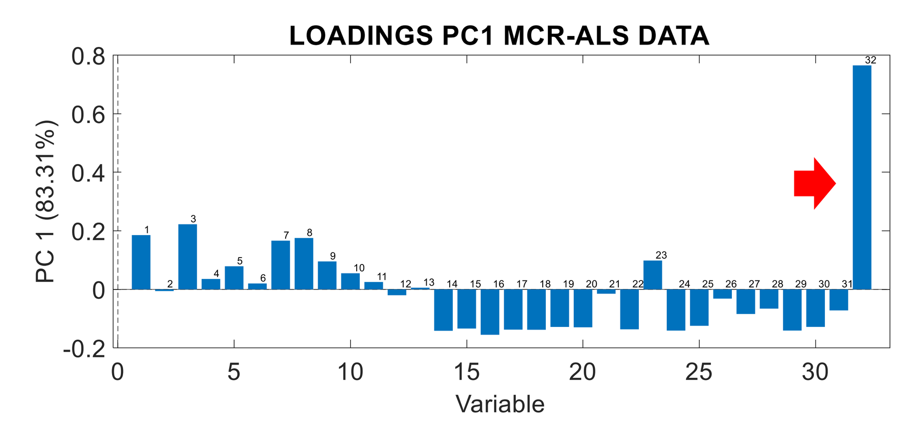


Figure 2: Loadings of PC1 using MCR-ALS Data

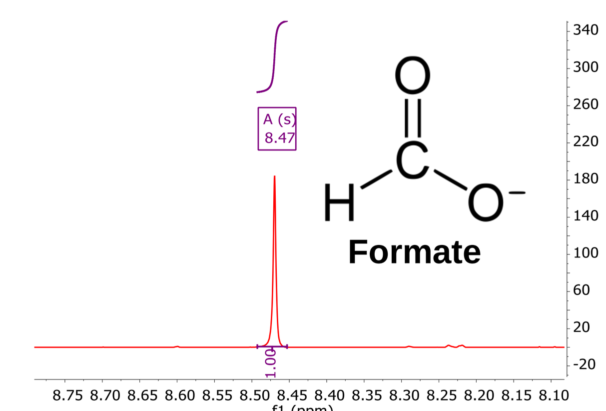


Figure 3: Pure resonance signal sub-array n°32

Integration of the singlet at δ= 8.47 ppm obtained by the MCR 32 component

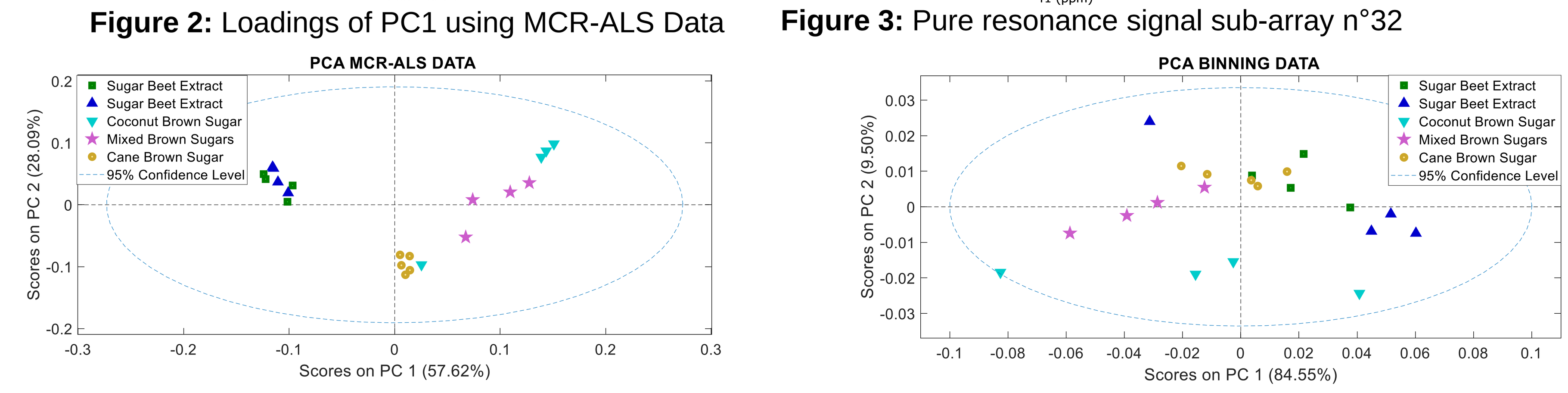


Figure 4: Scores of PC1 vs PC2 using MCR-ALS and Binning Data to evaluate Brown Sugars in addition to Sugar Beet extracts

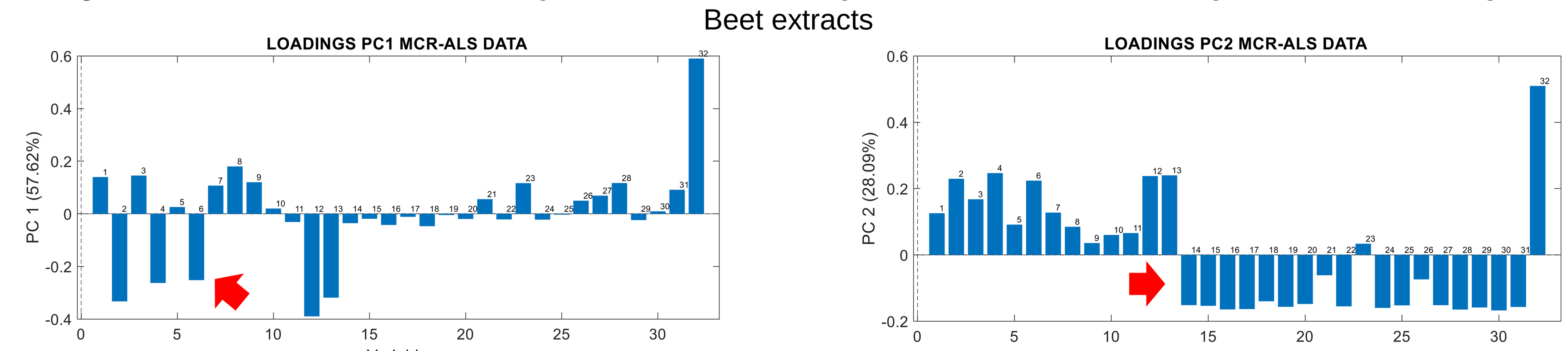


Figure 5: Loadings of PC1 and PC2 using MCR-ALS Data to evaluate Brown Sugars in addition to Sugar Beet extracts

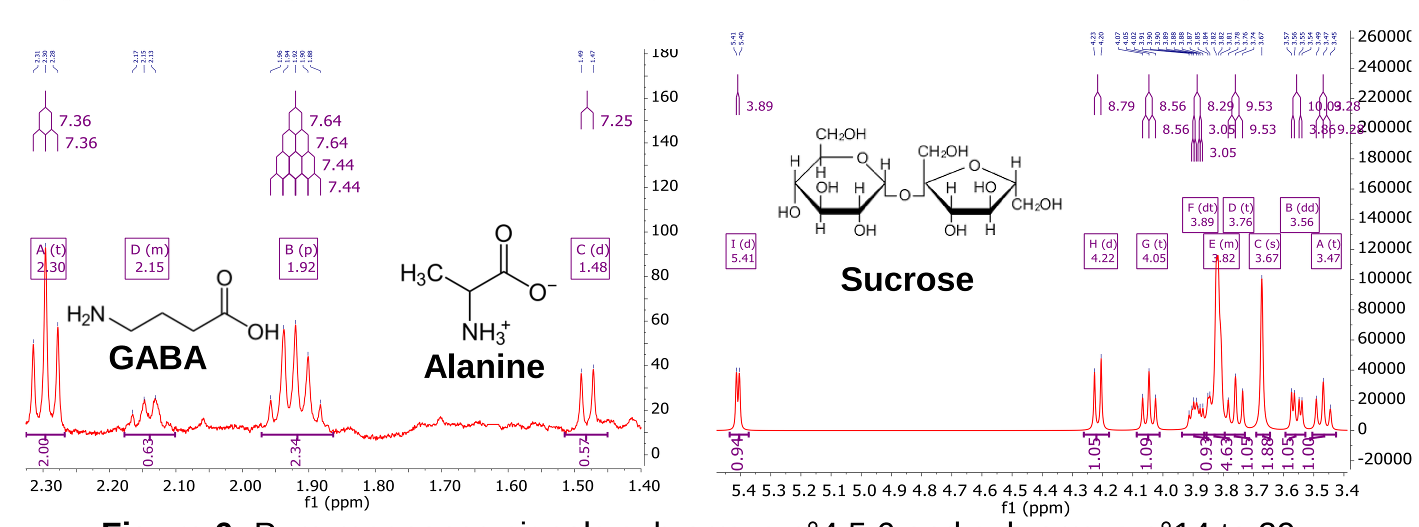


Figure 6: Pure resonance signals sub-arrays n°4,5,6 and sub-arrays n°14 to 29

The coupling constants (J), chemical shifts (δ) and the integration of resonance signals, are possible to evaluate by MCR-ALS with the purpose of a preliminary identification of those compounds that are responsible for the variability between samples.

Table 1: Example of resonance signals obtained compared with bibliography

Compound	δ (ppm) The.	δ (ppm) Exp.	Multiplicity The.	Multiplicity Exp.	J (Hz) The.	J (Hz) Exp.
Alanine	1.47	1.48	d	d	7.1	7.25
GABA	1.92 ; 2.30	1.92 ; 2.30	p ; t	p ; t	7.62 ; 7.35	7.64 – 7.44 ; 7.36

CONCLUSIONS:

- The application of MCR-ALS as a preprocessing method allowed to resolve the concentration profiles and resonance signals of polar compounds present in sugar samples, allowing the identification of characteristic spectral features of the study system.
- The MCR-ALS preprocessing show a greater selectivity to small variations associated with resonance signals compared to other preprocessing such as Binning.
- The applied methodology allows evaluating and studying resonance signals of unknown compounds which can be used for a preliminary characterization of the study matrix.
- Consequently, better results were obtained through a PCA based on the differentiation of sugar sources.

References:

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