¹H-NMR profiling of tomato samples with a benchtop spectrometer

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Metabolic phenotyping or metabolomics of tomato fruit is well documented and easily monitored for fruit sampling at a given stage of development especially for whole fruit or for pericarp tissue.
A detailed characterization of fruit development by quantitative **NMR-based** metabolomics of cultivated tomato fruit tissues[1] and pericarp of tomato fruit mutant lines[2] has been published recently at high field NMR.

To widen the interest of such an NMR-based approach, **decreasing the analytical cost and increasing the analytical throughput** are of interest.

The main objective of this work was **to test the ability of benchtop NMR to discriminate two tissues of tomato fruit (pericarp and locular tissue) at several stages of fruit development** and **to quantify the major soluble sugars and organic acids.**

Tomato fruit samples

Solanum lycopersicum, cv. Moneymaker grown in a greenhouse

Mature-green   Breaker   Orange   Red-ripe

Pericarp
Locular tissue
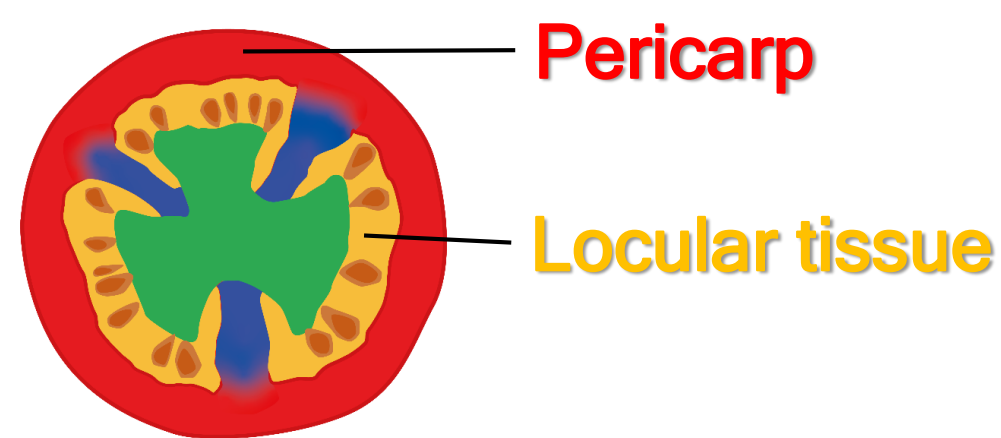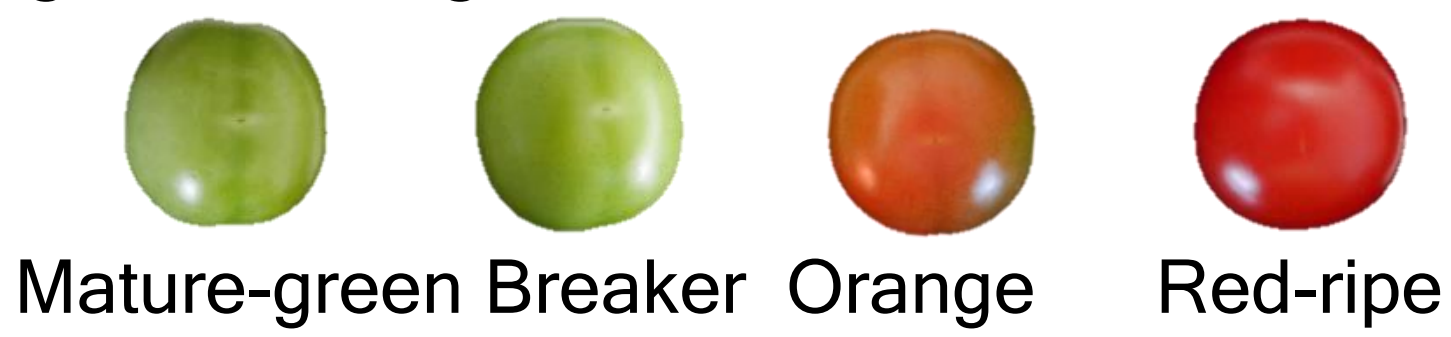
Hydro-methanolic extraction of lyophilized powder[3]
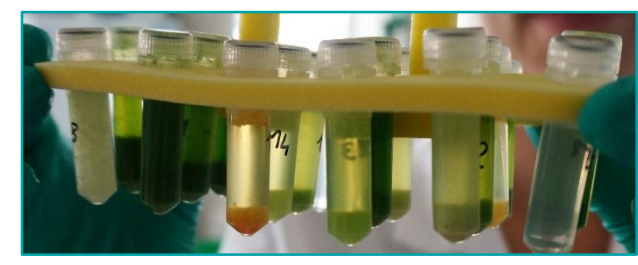
pH adjusted: 6.20 +/- 0.02

1D-¹H-NMR acquisitions

Avance III
500 MHz
Bruker
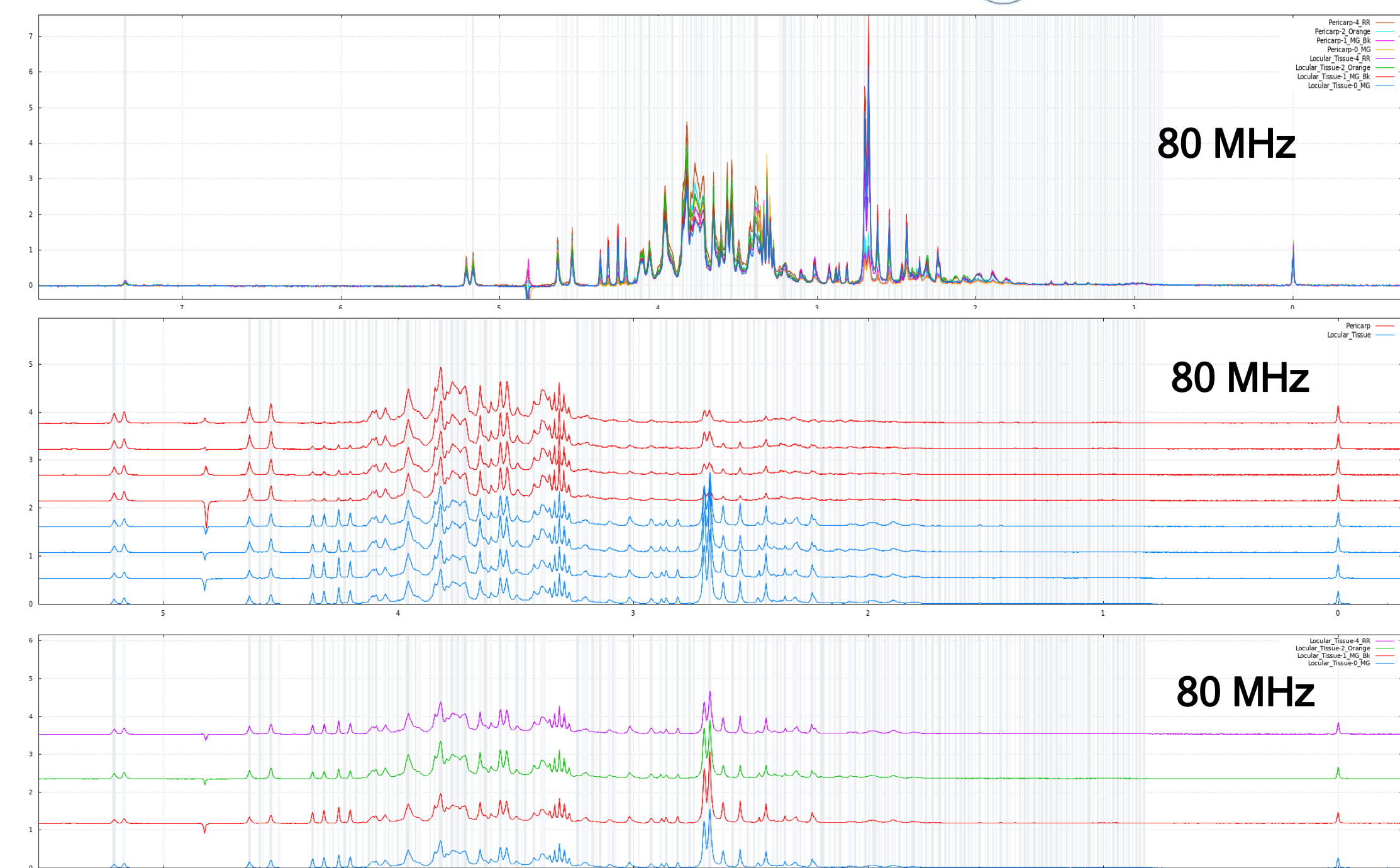(Wissembourg, France)
ATMA-BBI probe
5-mm tubes

Spinsolve 80 ᵁˡᵗʳᵃ ᶜᵃʳᵇᵒⁿ
80 MHz
Magritek
(Aachen, Germany)

5-mm tubes

Spectra acquired with water presaturation at:
- 80 MHz (WET SUP, 256 scans, 43 min)
- 500 MHz (zgpr, 32 scans, 11 min)

Spectra processing — NMRProcFlow

80 MHz
80 MHz
80 MHz

Spectra processed with NMRProcFlow[4] (nmrprocflow.org) and ERVA[5] method for bucketing and external calibration method for quantitation.
Data analyzed with univariate or multivariate (UV-scaling) statistical analyses (biostatflow.org).
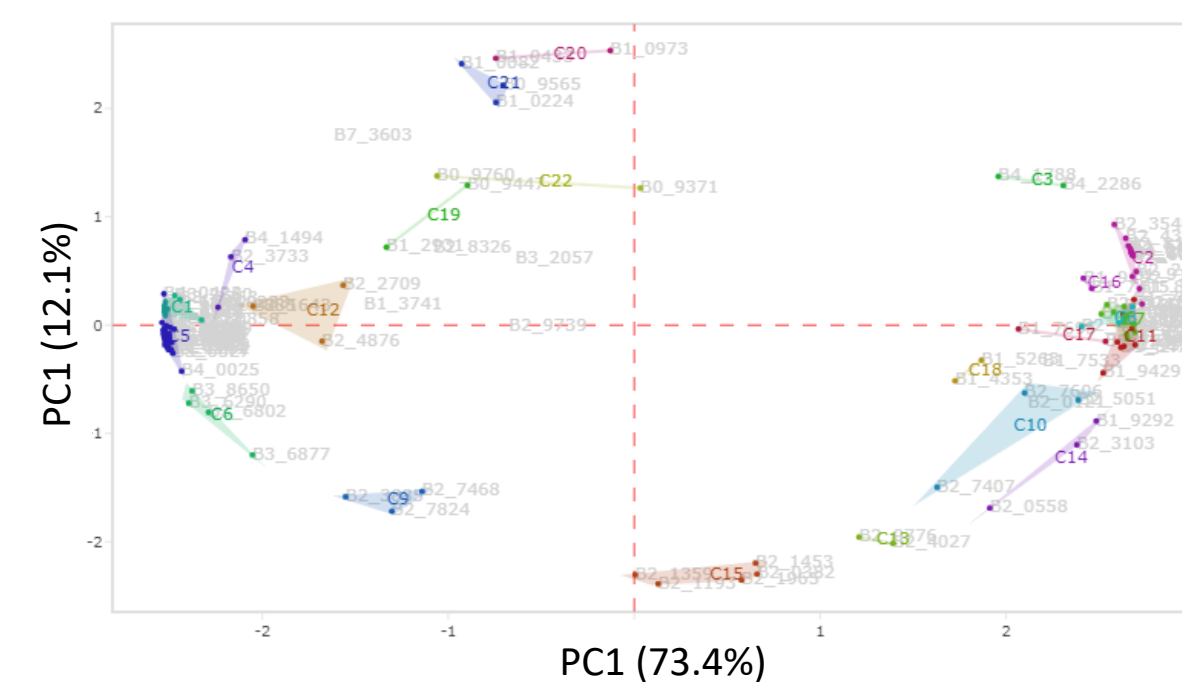
Untargeted analysis by each spectrometer clearly separates the two tissues

Multidimensional scaling (MDS) : constant-sum normalization, 8 samples, Euclidian distance

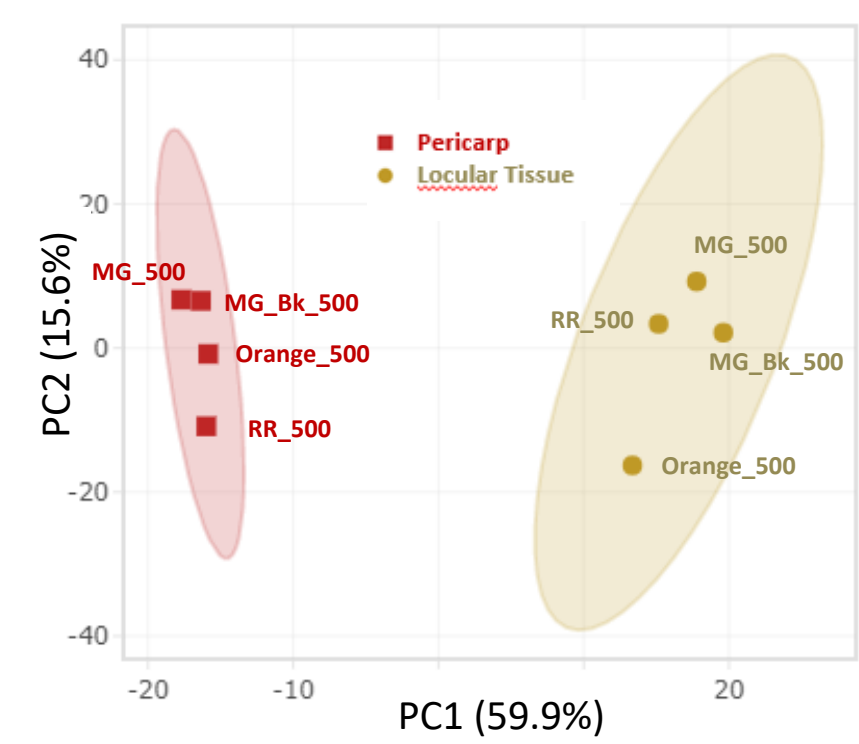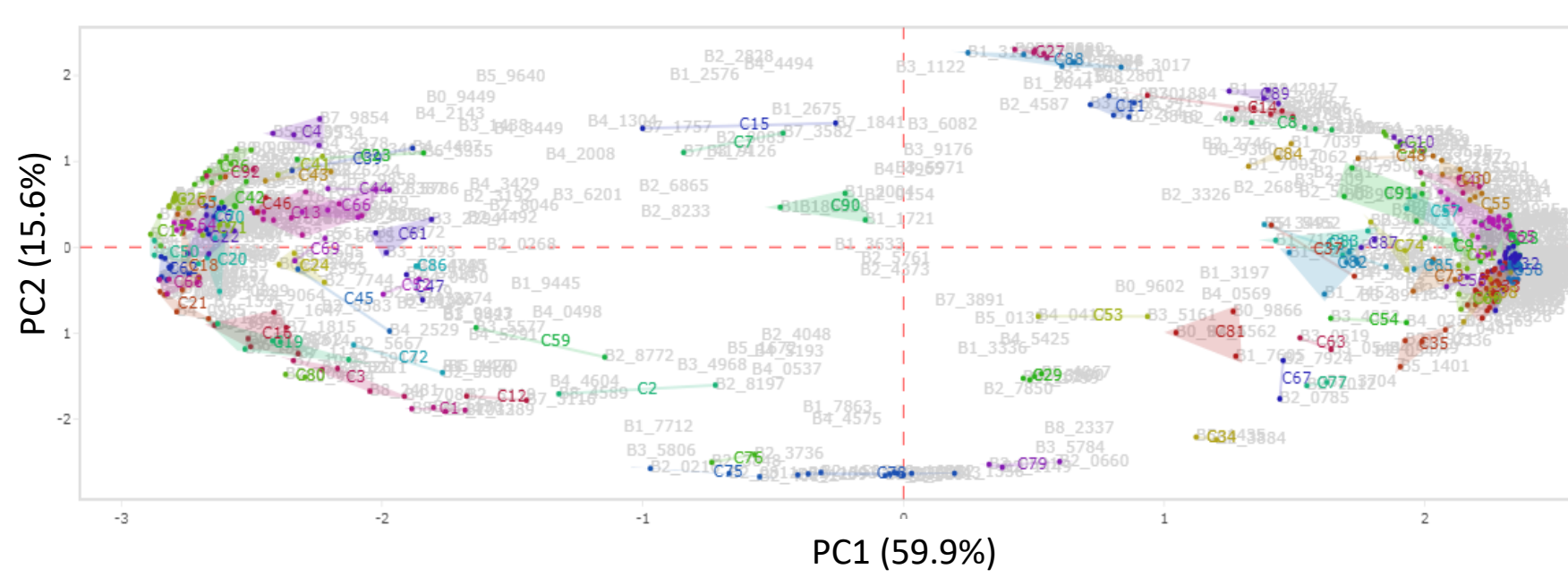Scores plot   Loadings plot

80 MHz
127 buckets
(SNR >5)
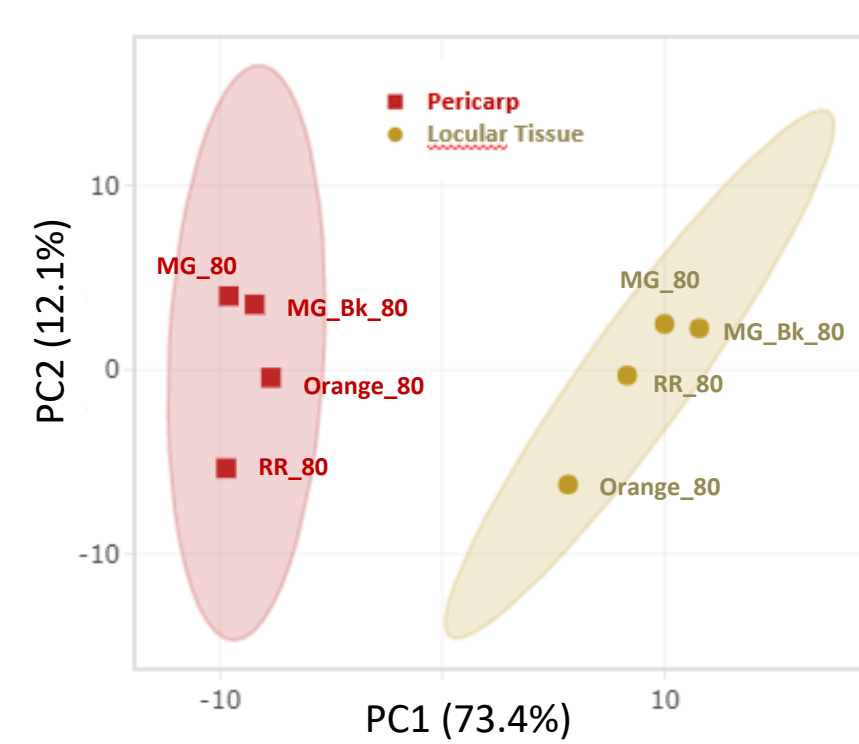
MDS loadings : 127 buckets grouped into 22 clusters in HCA (cut tree 0.26)
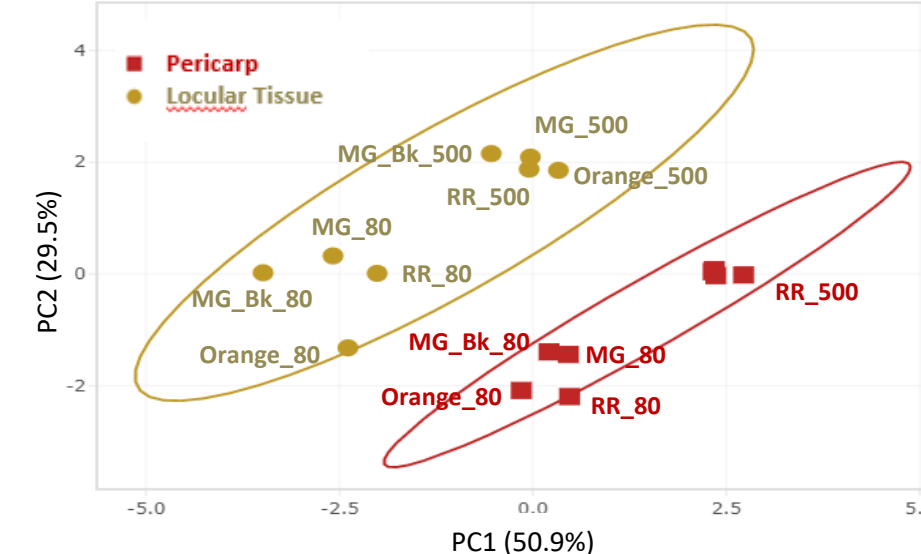
500 MHz
524 buckets
(SNR >10)

MDS loadings : 524 buckets – grouped into 92 clusters in HCA (cut tree 0.18)
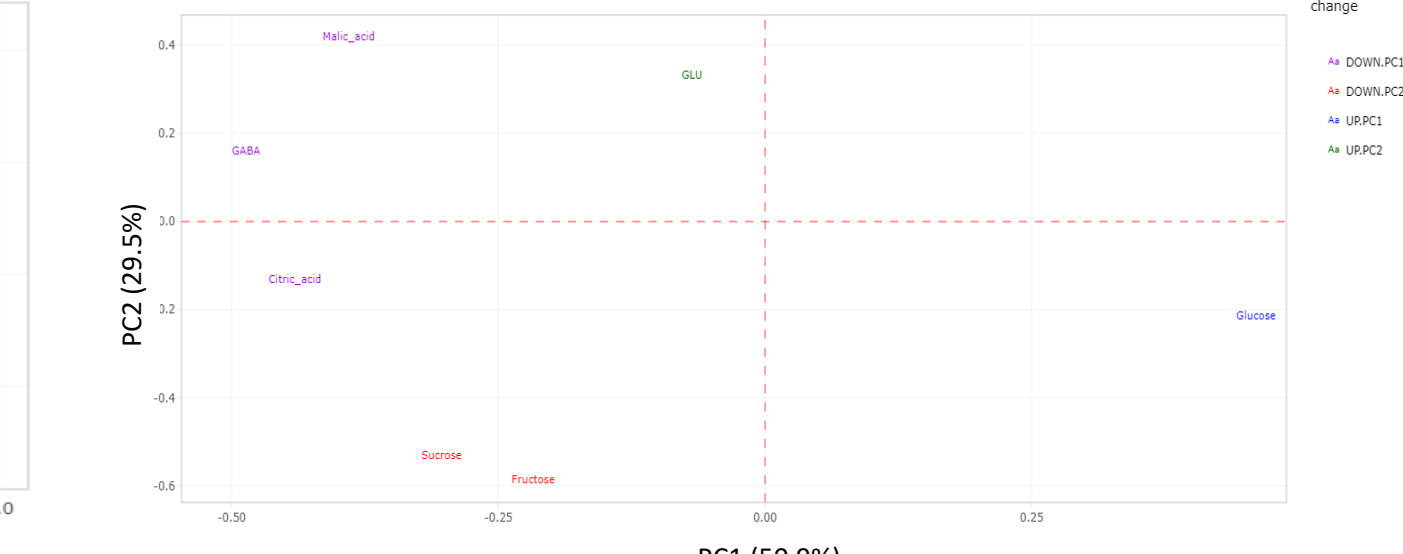
Targeted analyses at 80 MHz allow quantifying several metabolites crucial for fruit quality

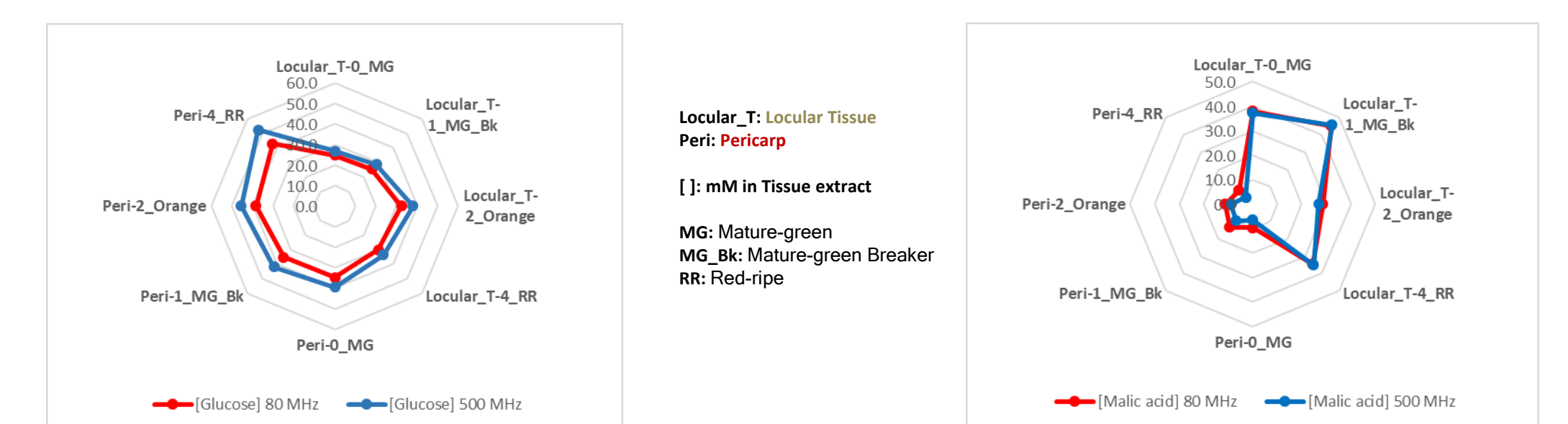PCA: UV scaling, 8 samples at 80 & 500 MHz, 7 metabolites

Scores plot   Loadings plot

Exemplary radar plot of metabolite concentration

Locular_T: Locular Tissue
Peri: Pericarp
[ ]: mM in Tissue extract
MG: Mature-green
MG_Bk: Mature-green Breaker
RR: Red-ripe

Although absolute quantification data significantly differ for most metabolites (paired Student's T test, *P*<0.05), both spectrometers revealed the same biological tendencies for several major compounds.

Conclusion - Perspectives

Specific deconvolution or model-based analysis[6] are under development to deal with benchtop reduced spectra resolution.
Benchtop NMR analysis of fruit tissue extracts could be proposed to biologists studying tomato or other fleshy fruits, to characterize fruit development of wild-types and mutants in a greenhouse, or to phenotype large series of genotypes.

References

(1) Lemaire *et al.* 2019 doi:10.3390/metabo9050093, (2) Musseau *et al.* 2020 doi:10.1105/tpc.20.00245, (3) Deborde *et al.* 2019 doi:10.1007/s11306-019-1488-3, (4) Jacob *et al.* 2017 doi:10.1007/s11306-017-1178-y, (5) Jacob *et al.* 2013 doi:10.1007/s00216-013-6852-y, (6) Matviychuk *et al.* 2021 doi:10.1016/j.aca.2021.338944

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