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To dissect or not to dissect for fruit spatial metabolomics: Tissue profiling or MRI?


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Complementary NMR approaches to characterize tomato fruit tissues

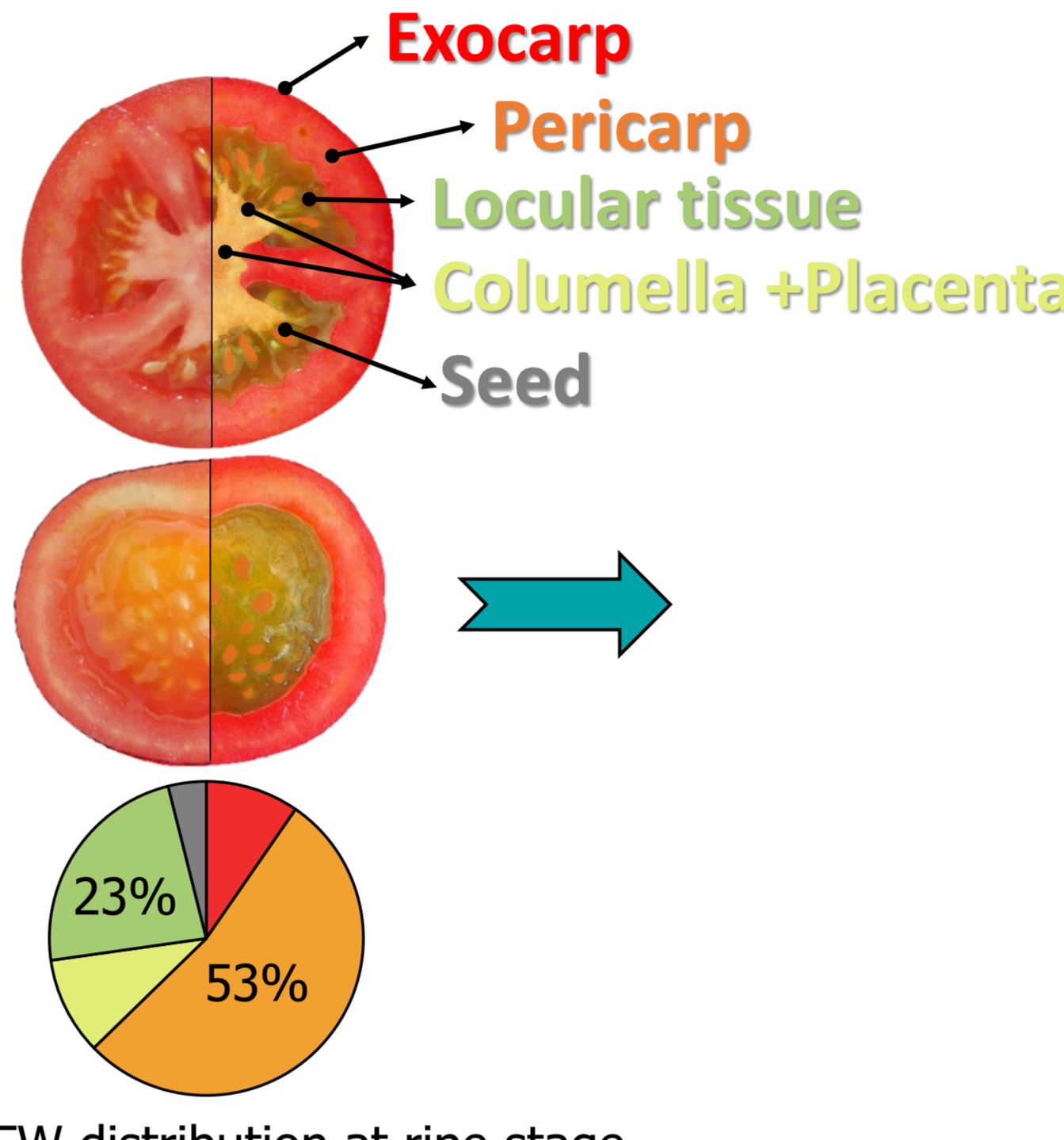
Fruit is a complex organ that protects and feeds the developing seeds and allows their dispersal at maturity. It is constituted of several interconnected tissues with specific roles. However, the majority of biochemical studies about fruit development concern the entire fruit or its largest tissue, pericarp. Here we used two strategies to investigate tissular composition: metabolomics and imagery.



Liquid NMR

Native locular tissue:
450 µL in 5-mm tube
144 µL in 3-mm tube
+ 10% (v/v) deuterated water
+ TSP-d₄ + EDTA-d₁₂


¹H-NMR analysis of extracts of dissected tissues
Polar extracts
Bruker AVIII 500 MHz spectrometer
Inverse (BBI) probe, 5 mm NMR tube
Calibration for quantitation



Exocarp
Pericarp
Locular tissue
Columella + Placenta
Seed

Tissue FW distribution at ripe stage

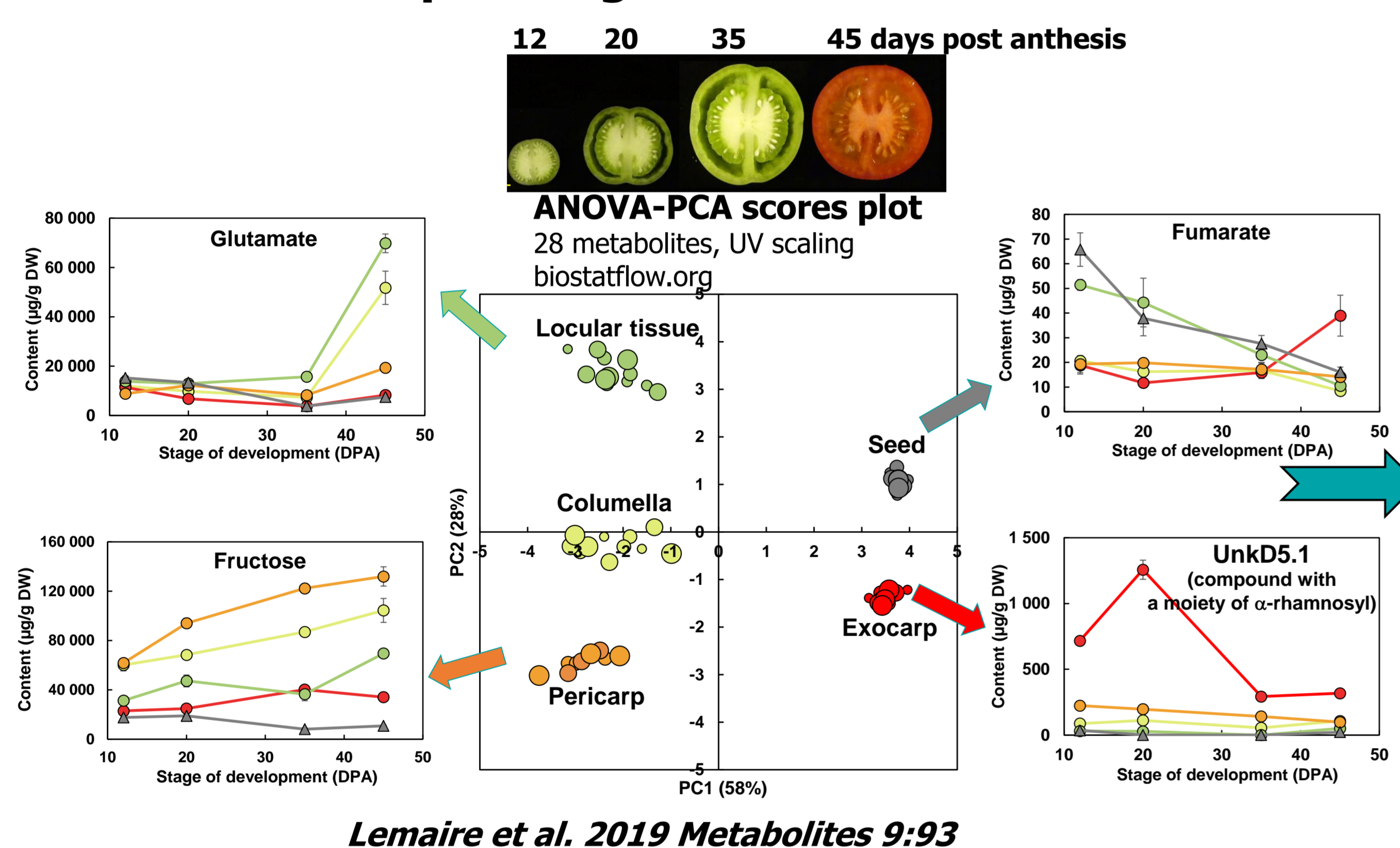
In vivo MRI



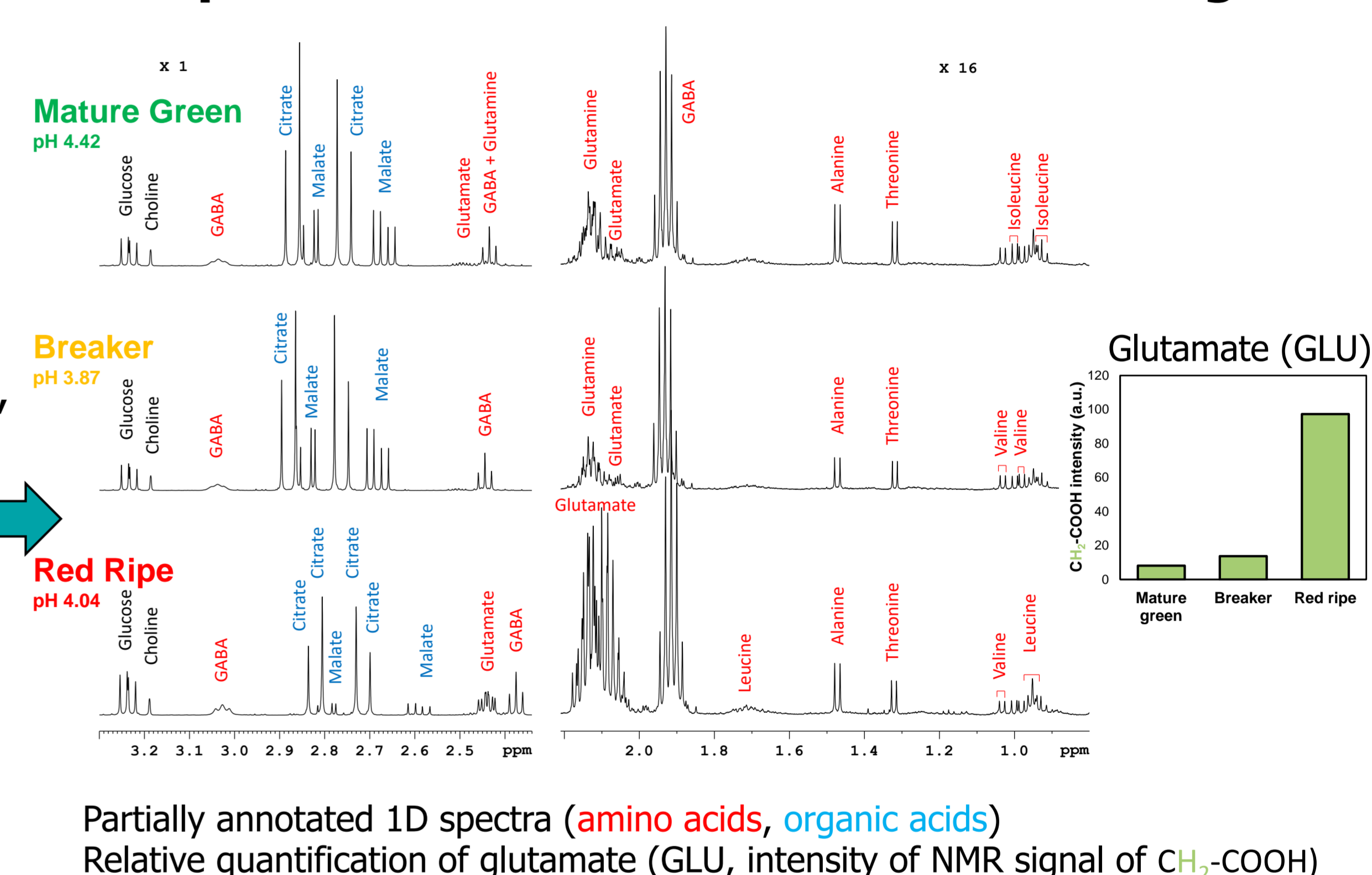
MRSI: Magnetic Resonance Spectroscopy Imaging, one NMR spectrum per pixel
CEST: Chemical Exchange Saturation Transfer, image specific of one chemical exchangeable moiety
Bruker BioSpec 500 MHz spectrometer
72 mm i.d. volume coil for both excitation and reception

¹H-NMR profiling of fruit tissues

1D ¹H-NMR profiling of extracts of isolated tissues

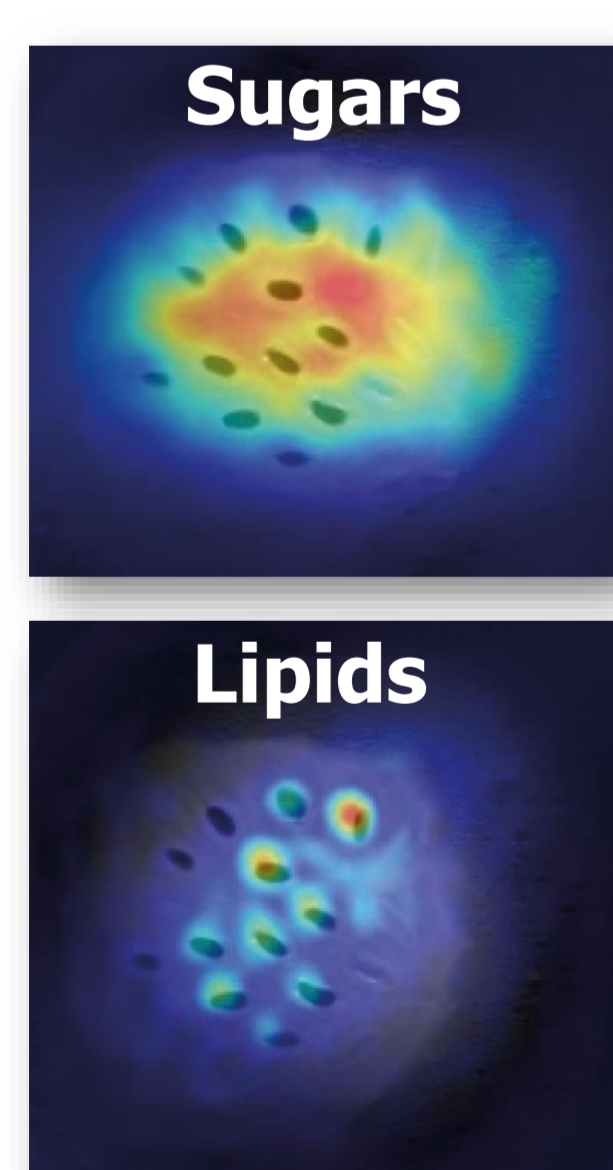


¹H-NMR spectra of native locular tissue at 3 stages



Fruit MRI

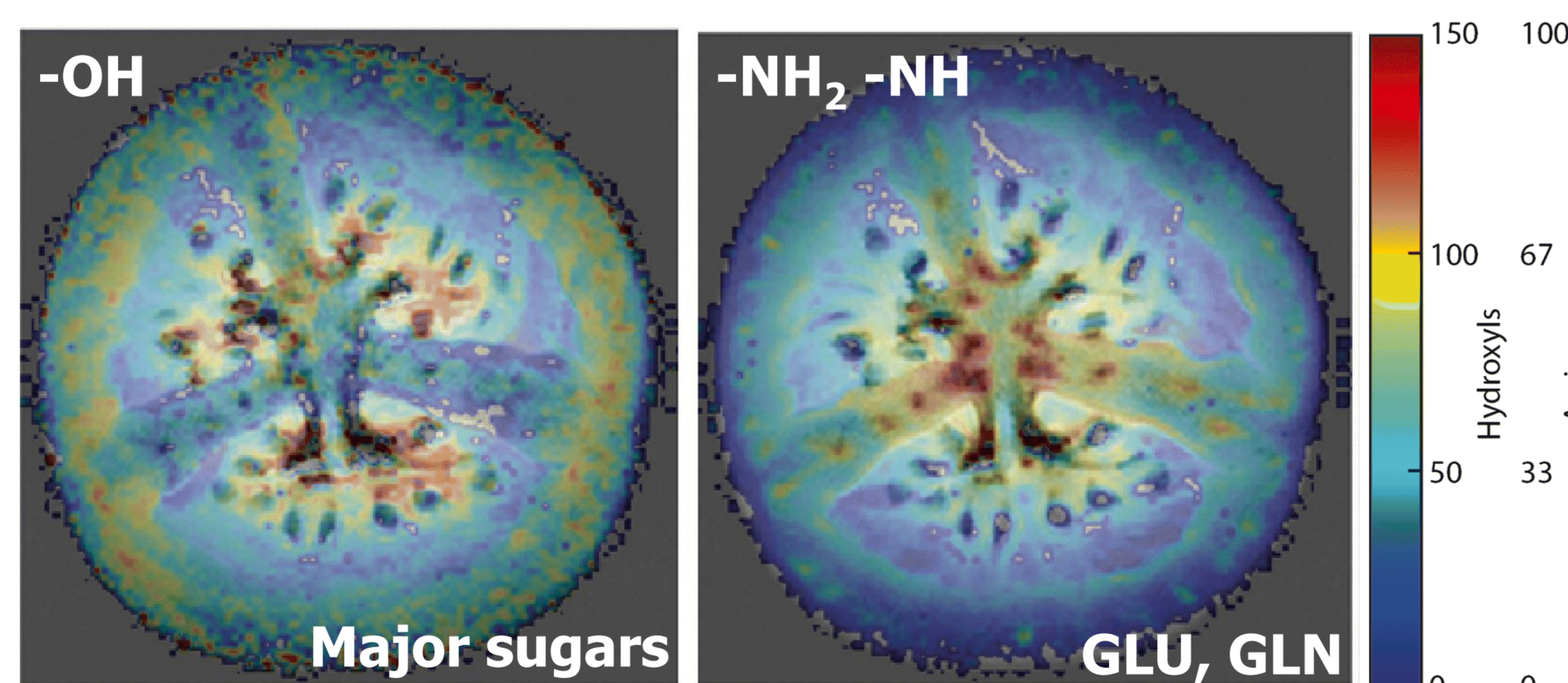
MRSI in ripe tomato



Good for locular tissue
But repeatability issues due to magnetic inhomogeneities

CEST contrasts in ripe tomato

CEST images are overlapped with the anatomical image obtained by the FLASH protocol (gray scale)



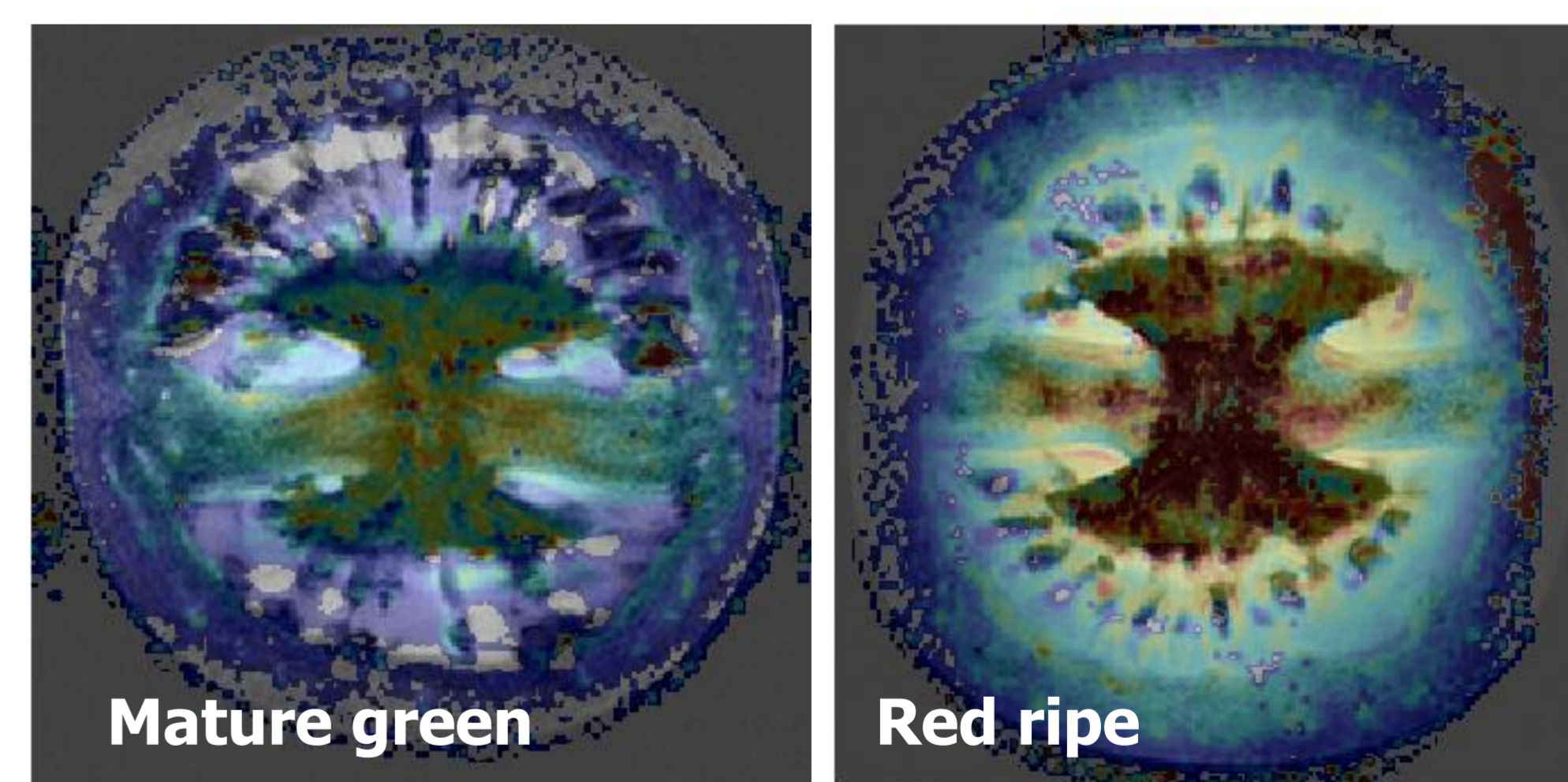
CEST-MRI principle

Sensitive to a chemical function (-OH, -NH₂, -NH)
Indirect method: effect of the exchange on the water signal
Using prior knowledge about the sample to assign CEST to metabolites
Semi-quantitative

Pages et al. 2021 *Anal Bioanal Chem* 413:1251

-NH₂ -NH CEST contrast for 2 stages

CEST images on two different tomato fruits at two maturation stage: mature green and red ripe



Metabolite spatial repartition changes

With ripening, the GLU-GLN spatial distribution changes:
- At mature green stage, GLU-GLN concentration is higher in fruit columella, radial pericarp and seed
- At a red ripe stage, GLU-GLN concentration is increased in columella, radial pericarp and locular tissue, and is particularly high in columella
In addition, CEST images highlight the fruit vasculature

Conclusions & Perspectives

- Specificities of dissected tissues are clear with ¹H-NMR profiling but **gradients within a tissue** cannot be revealed
- CEST MRI** appears more informative than MRSI regarding the distribution of major metabolites in tomato fruit
- To interpret CEST images, prior knowledge about metabolite concentration is mandatory
- LC-HRMS** of semi-polar extracts is currently being used for the characterization of minor metabolites of dissected locular tissue and seed
- MS imaging**, on a smaller part of the fruit compared to MRI, will complement MRI for the distribution of minor compounds

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Région Aquitaine (project No. 20051303006ABC and a Ph.D. grant to F.M.), EUSOL Integrated Project (grant No. FOOD-CT-2006-016214), MetaboHUB (ANR-11-INBS-0010) and IB2019_GelSeed project of INRAE BAP division for financing Patricia Ballias, Aurélie Honoré and Isabelle Atienza for growing the tomato plants
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