Spatial Localization of Metabollites In Fruits By NMR : Tissue Dissection and Metabolic Profiling Or Non-Invasive Whole Fruite Imaging ?

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[4] Bordeaux Metabolome, PMB-Metabolome, INRAE, 2018, doi:10.15454/1.5572412770331912E12, MetaboHUB, IBVM, Centre INRAE de Nouvelle Aquitaine - Bordeaux, F-33140 Villenave d'Ornon,

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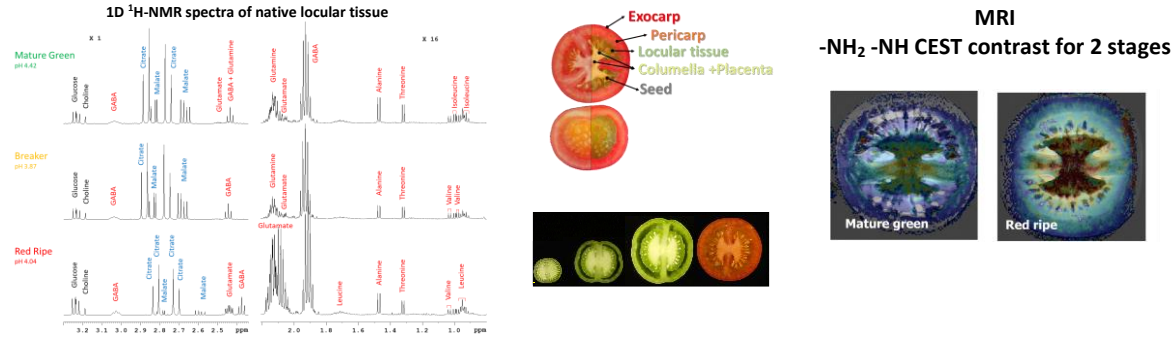
Fruit development allows seed maturation and dispersion. Fruit is a complex organ containing seeds and several interconnected tissues with specific roles. However, the majority of biochemical studies about fleshy fruit development concern the entire fruit or its larger tissue, pericarp. To study tomato (*Solanum lycopersicum*) fruit spatial composition, we used tissue dissection followed by multi-step hydroalcoholic extraction or *in vivo* imaging.



For dissection, we isolated the seeds, exocarp, mesocarp, columella with placenta, and locular tissue, and analysed their extracts individually using 1D ^{1}H-NMR profiling at 500 MHz for the quantification of major polar metabolites [1]. We also analysed the native locular tissue using 1D ^{1}H-NMR.

For the *in vivo* approach, we used magnetic resonance imaging (MRI) at 500 MHz with two MRI contrasts: Magnetic Resonance Spectroscopy Imaging (MRSI) and Chemical Exchange Saturation Transfer (CEST) [2]. For MRSI, one NMR spectrum per pixel is obtained. In CEST, the image is specific of one chemical exchangeable moiety that may come from several metabolites. For MRSI, the NMR spectrum quality was heterogeneous from one pixel to the other leading to non-exploitable data. These variations seemed to result from a significant magnetic field heterogeneity within the slice. For CEST images, we focused on frequency ranges from 0.4 to 1.6 and 2.4 to 3.6 ppm from the water frequency, leading to an image contrasted for hydroxyl and amino moieties, respectively. Based on [1] we concluded that glucose/fructose and glutamine/glutamate were imaged. The clear differences in the metabolite spatial distribution observed for glucose higher in the locular tissue, and glutamate higher in the columella, were in agreement with the data of dissected tissues. For tomato fruit, CEST MRI appears more informative than MRSI regarding the distribution of major metabolites.

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References:
[1] Lemaire-Chamley, M., Mounet, F. et al., 2019, Metabolites 9:93. doi: 10.3390/metabo9050093
[2] Pagés, G., Deborde, C. et al., 2021, Anal. Bioanal. Chem. 413, 1251-1257, doi: 10.1007/s00216-020-03101-w