



USING METABOLOMIC APPROACHES AND MODELLING OF CENTRAL METABOLIC TO STUDY FLESHY FRUIT QUALITY

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MetaboHUB (<http://www.metabohub.fr/>) is the French multi-site metabolomics and fluxomics infrastructure launched in 2013. The four technological platforms of MetaboHUB (Bordeaux, Toulouse Clermont-Ferrand and Saclay-Paris) develop and share tools and expertise for basic and applied projects related to human health and nutrition, food production and quality, sustainable agriculture and environment, green and white biotechnologies. Each platform has its own field of application. Thus, MetaboHUB Bordeaux, which is devoted to plant research, has been successfully involved in fruit systems biology.

Fruits, our major source of vitamins and anti-oxidants, represent an important market around the world. However, fruit production is relentlessly challenged by environmental challenges, but also by changes in the consumer's demand for taste and nutritional value, thus requiring constant efforts to ameliorate varieties, but also agricultural practice. Our aim is to better understand metabolism and the way it influences accumulation and quality of plant biomass, through a systems biology approach centered on fruit development. Flux estimations calculated with metabolic models of metabolism represent a valuable alternative to flux measurements in complex systems such as fruits. We performed a series of phenotyping experiments, in which we collected a range of climatic and ecophysiological data as well as a large set of samples that have been analysed for enzyme activities, metabolome and structural traits (Biais et al., *Plant Physiol.* 2014, Benard et al., *J. Exp. Bot.* 2015). In collaboration with mathematicians we developed and validated two models describing fruit development.

The first model is a small-scale kinetic model with ordinary differential equations (ODE) describing realistic rate equations of each step of the metabolic network of interconversion and storage of sugars, taking into account the subcellular volumes and the enzyme capacities experimentally determined. Fits of the measured sugar contents allowed the description of developmental changes in the sucrose uptake and its partitioning within the network and, most interestingly, pinpoint the strong control exerted by vacuolar carriers on the fruit storage capacity. One of the major findings evidenced by this model was the high accumulation of soluble sugars within the vacuole together with organic acids, enabling the osmotic-driven vacuole expansion that occurs during cell division (Beauvoit et al., *Plant Cell* 2014).

The objective of the second model was to determine the fluxes in the central metabolic network during the tomato fruit development with a medium-scale stoichiometric model including the balance of cofactors and energy. The measured concentrations of the main biomass components and the accumulated metabolites in the pericarp were fitted in order to calculate the corresponding external fluxes used as constraints to solve the model. The distribution of the calculated fluxes of central metabolism were then displayed and compared with known metabolic behaviours. A striking output of the model was the energetic balance indicating that excess ATP is dissipated just before the onset of ripening, supporting the concept of the climacteric crisis (Colombié et al., *Plant J.* 2015).

Importantly, both models have been cross-validated by comparing several fluxes including the carbon-demand of the fruit. The apparent gap between low values of calculated fluxes compared with measured enzyme capacities suggest a complex reprogramming of the metabolic machinery during fruit development.

In the frame of the MetaboHUB project and to refine the stoichiometric model, we intend (i) to enlarge the model with a metabolic model reconstructed from genomic data and (ii) to introduce quantitative metabolomics and thermodynamic data to better constrain the system and improve the flux modelling, and extend it to several other fleshy fruit species.

Biais et al. (2014). *Plant Physiol.* 164: 1204-1221; Benard et al. (2015). *J. Exp. Bot.* in press;

Beauvoit et al. (2014) *Plant Cell* 26:3224-3242; Colombié et al. (2015) *Plant J.* 81:24-39