

Depth profiling of tomato fruit cuticle during development using Confocal Raman Microscopy

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The plant cuticle is defined as the first barrier between the aerial non-lignified organs of land plants and the environment. Some pivotal roles are ascribed to this outermost layer such as acting as support to the plant, protecting it against radiation, pathogens, water loss or mechanical injuries as well as controlling the gas exchange with the surroundings or the proper development of the plant during first stages of development [1]. Although the composition of tomato fruit cuticle has been thoroughly analysed [2-3], the spatial distribution of its components has received little attention due to the spatial resolution limitation of conventional techniques [4].

Herein, Confocal Raman Microscopy has been employed to locate the microspatial distribution and changes throughout development of different tomato fruit cuticle components. Results have shown the cutin matrix as a homogeneous layer without any detectable gradient between the outer and inner sides of the cuticle. A combination of uni and multivariate approaches has allowed to selectively locate the phenolic acid and flavonoid fractions. Comparison of the cuticle Raman spectra with a reference library has additionally allowed to identify esterified *p*-coumaric acid and free *p*-hydroxybenzoic acid as the main phenolic acids present during growth. Both showed a similar distribution during fruit development, mainly located to the outermost layer of the cuticle and within pegs, which changed to a more homogenous distribution of esterified *p*-coumaric acid during ripening. Changes in flavonoid composition and location were also detected during ripening.

Based on the results, we propose three models of spatial location of cuticle components during fruit development. During the earliest stages of development phenolics are homogeneously distributed along the whole depth of the cuticle. Further in development, phenolics are restricted to the outermost side of the cuticle and the middle region of the pegs. Finally, during ripening, phenolic acids are located across the entire cuticle and flavonoids started to accumulate displaying a heterogeneous distribution along cuticle width, with a higher accumulation to the inner side.

References

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