## P-012: From the soft to the hard walnut shell: Changes in microchemistry revealed by Confocal Raman Microscopy

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In a common sense, a nut is a fruit composed of a hard shell and a seed. The nutshells give protection to the seeds and the embryo, which are crucial for the next tree population. Walnut shells are very hard materials composed of polymeric components such as cellulose, hemicelluloses and up to 33% of lignin. A detailed understanding of the microstructure and the chemical changes during their growth process is the basis for identifying the structural features that are most important for answering the question on how nutshell design is optimized to bring up these fascinating life protecting materials.

In this study, extensive characterization of the walnut shell during the growing and maturation period (from July to October) was carried out by Confocal Raman microscopy. Revealing intrinsic chemical and morphological principles based on Raman imaging helped to highlight differences and similarities within and between developmental stages. The results show that nuts collected in July have reached the final shell thickness and lignification has already started. Across the shell a gradient in cell wall thickness and lignin content pictures the ongoing development; while for the mature state (nuts sampled in October) thick-walled highly lignified cells were found across the entire shell. Another remarkable and important feature imaged within the cells was numerous pits, which in the mature state became filled up with highly fluorescing extractives.

Our results clearly indicate the potential of Confocal Raman spectroscopy to gain new insights into chemical changes of the nutshell microstructure of different development stages of walnut. In the long term, we aim at revealing important structure-function relationships of the nutshell design during development to result in possible applications in biomimetic research.

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