

Raman Spectroscopy applied to the characterization of rough plant surfaces of romaine lettuce

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Romaine lettuce is one of the most widely consumed vegetables worldwide, which is susceptible to pathogen contamination. In this study, we explore the application of advanced microscopy and spectroscopy techniques to characterize the surface of romaine lettuce, with the aim of identifying its structural and physico-chemical properties. Raman spectroscopy was used to identify specific functional groups and spectra, could potentially indicate the chemical compounds present, generally associated with plant protection against multiple biotic and abiotic stress factors [1,2]. Given that Raman spectroscopy is a non-destructive technique, it allows for the identification of chemical compounds in plants without altering their structure, making it an essential tool for studying the physico-chemical properties of plant surfaces. In this study, Raman mapping was also performed on the stomatal areas to investigate their heterogeneous composition. Their two surfaces, abaxial (lower) and adaxial (upper) sides, were compared. The results indicated similar chemical functional groups on both surfaces. However, the variation observed in Raman intensities was systematic, being generally smaller on the abaxial side. These areas were also investigated by advanced Atomic Force Microscopy techniques, providing information about the location of hydrophilic areas. FTIR studies provided the surface leaf composition with a broader spatial scale. Understanding the surface properties of romaine lettuce is crucial, suggest that increased adhesion is associated with increased pathogen binding-on the edible parts of the leaves [3]. These areas are of special interest due to their role in gas exchange and transpiration. Our findings revealed the presence of carbohydrates, carotenoids, and hydrophilic nano-areas, which can influence microbial adhesion. This approach offers a comprehensive understanding of the physico-

chemical and biological properties of the plant surface, highlighting their role in self-protection against biotic and abiotic factors.

By analyzing the surface properties of various species and organs, such as rose petals [4] and olive leaf [5], we can gain a deeper understanding of the interaction between plant surfaces and their environment. These characteristics not only affect pathogen adhesion but also reveal the heterogeneity of the chemical components of leaf surfaces, as defense mechanisms against pathogens and many other stress factors.

References

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- [5] Fernández, V., et al., Chemical and structural heterogeneity of olive leaves and their trichomes. *Communications Biology*, (2024), vol 7, no 1, p. 352.

Figures

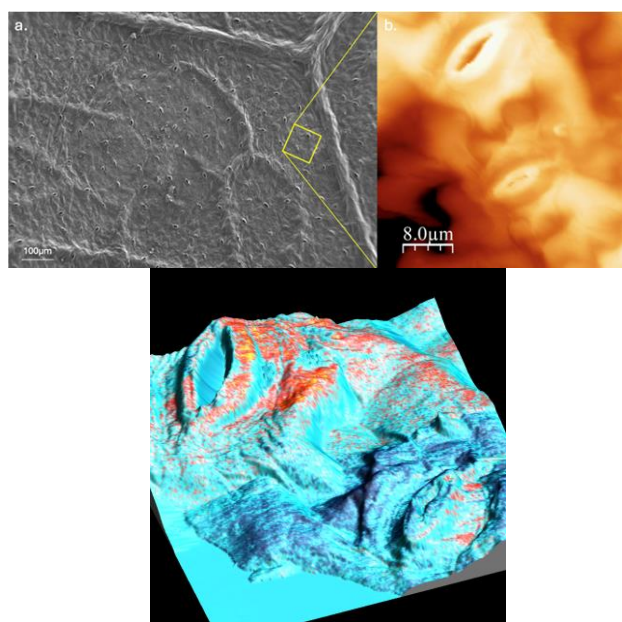


Figure 1. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) images of the stomatal zones of romaine lettuce.

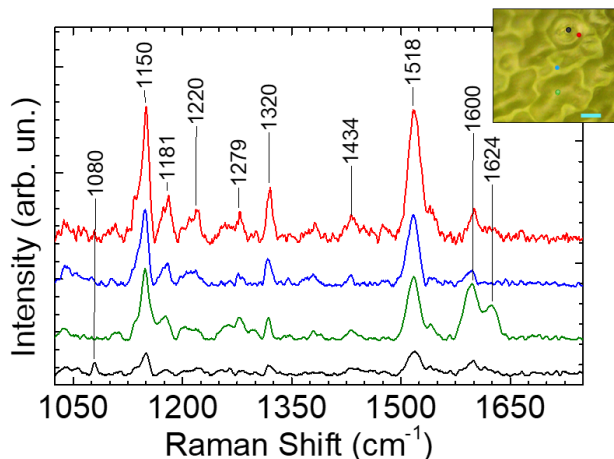


Figure 2. Raman spectroscopy spectra of four distinct regions within the stomatal zones of romaine lettuce, highlighting key chemical components. Notable differences between areas are observed in the peaks: 1080cm^{-1} (carbohydrates, VM: $\nu(C-O)+\nu(C-C)+\delta(C-O-H)$); 1150cm^{-1} (carotenoids with VM: $\nu(C-C)$, $\nu(C-O-C)$); 1320cm^{-1} (cellulose, aliphatics, phenylpropanoids, VM: $\delta(CH_2)$); 1434cm^{-1} (alkanes, waxes with $\nu(CH_2)$); 1518cm^{-1} (carotenoids, VM: $\nu(C=C)$ in-plane), and $1600-1624\text{cm}^{-1}$ (phenylpropanoids, VM: $\nu(C-C)$ in the aromatic ring + $\sigma(CH)$). VM: Vibrational Modes).

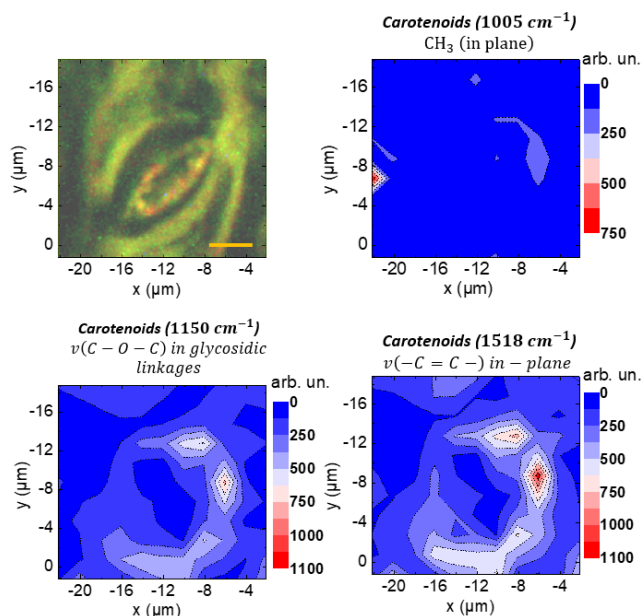


Figure 3. Raman spectroscopy maps showing the intensity of the modes corresponding to different functional groups in the stomatal regions of romaine lettuce. Each map represents different functional groups, with the color scales varying between maps. The yellow reference line represents a scale of $4\ \mu\text{m}$.