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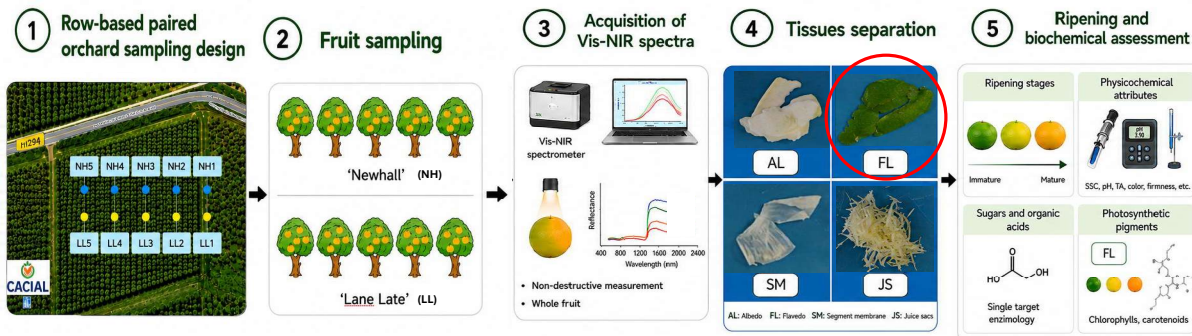
## INTRODUCTION

Navel orange cultivars (*Citrus sinensis* (L.) Osbeck) exhibit an inter-varietal gap in ripening onset, despite similar phenology up to fruit set. Later-ripening cultivars exhibit an extended phase II (cell expansion), slower progression of internal quality attributes (middle phase II – phase III), and a delayed color break (phase III – ripening) (Pires *et al.*, 2023). The visible and near-infrared (Vis-NIR) reflectance spectra of plant tissues are divided into three main wavelength ranges: i) 400–700 nm, associated with pigment content; ii) 800–1300 nm, associated with light scattering effects promoted by tissue structure; iii) wavelengths >1400 nm, associated with the presence of water, proteins, soluble solids, and cellulose.

## AIM

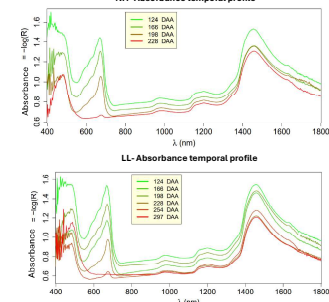
The aim of this study was to evaluate the potential of visible and near-infrared reflectance spectroscopy (Vis-NIRS) to characterize ripening dynamics and identify spectral signatures associated with key physicochemical changes in two navel orange cultivars throughout fruit development.

## PROCEDURES

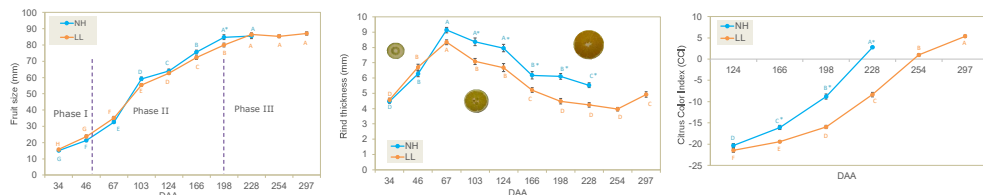


**Figure 1.** Measurements were performed on 5 independent replicates (5 fruits/tree/cultivar) of early-season NH and mid-season LL navel orange cultivars from 34 days after anthesis (DAA) until full ripeness. Regarding ripening, DAA was only considered from 124 DAA onwards. Biochemical analysis was only performed on FL. The effects of time (DAA) and variety were tested for each parameter using a two-way ANOVA, and the multiple comparison test applied was the Student–Newman–Keuls test at a significance level of  $p < 0.05$  (SigmaPlot 13.0, Systat Software, Inc., USA). Statistically different means ( $p < 0.05$ ) over time and between varieties are indicated by uppercase letters and an asterisk (\*), respectively.

## RESULTS



**Figure 3.** For each cultivar, reflectance spectra were acquired in intercanopy mode in the 400–1800 nm range from 124 DAA until full ripening (MI > 6.5). The spectrometers used were the following: AvaSpec Mini2048CL-V125 (Visible) and AvaSpec NIR256-1.7EC (Infrared) [Avantes B.V., The Netherlands].



**Figure 2.** Growth and fruit ripening in NH and LL cultivars. Their respective fruit size, average rind thickness, dry mass percentage (% DM), Citrus Color Index (CCI), Maturity Index, and total soluble solids content (TSS) were determined over time (DAA – days after anthesis) according to Cavaco *et al.* (2018)

## CONCLUSIONS

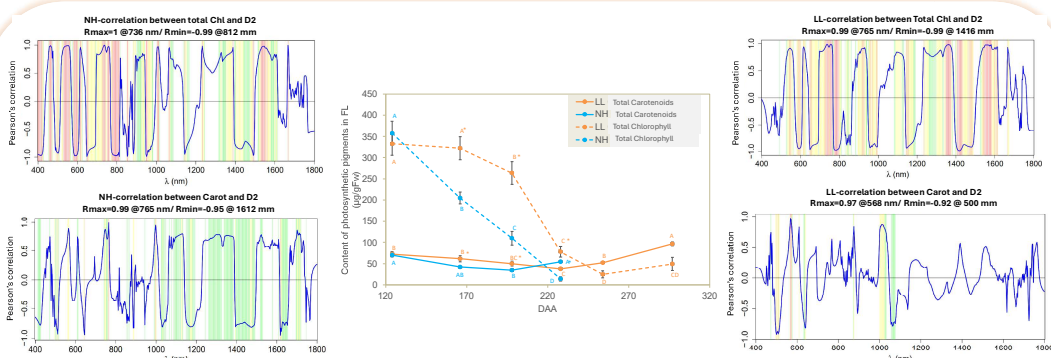
Pearson's correlation analysis applied between the various fruit quality attributes and their respective Vis-NIR spectra, revealed a high level of complexity. In some cases, indirect correlations with specific compounds were further observed.

Structural changes in the peel (roughness and thickness) acted as an additive constant across the entire spectral range. After removing this effect using multiplicative scatter correction (MSC), it was possible to identify, in the 400–700 nm region, the influence of declining Total Chl and increasing Carot content through ripening.

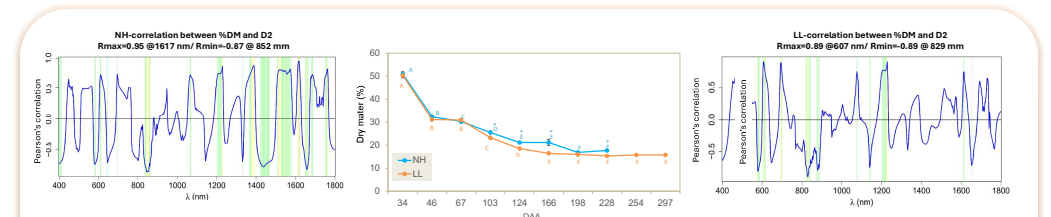
The decline of % DM, paralleled by the increment of water content through ripening also affected the spectra in the water-related wavelengths (1470 nm). Yet, this was less consistent than pigment-driven changes in the visible region.

Moreover, it was difficult to associate any spectral changes with increasing SSC.

Although preliminary, these results indicate that it is possible to establish spectral signatures throughout ripening in these orange cultivars, using refined analytical approaches.



**Figure 4.** Pearson's correlation ( $p < 0.005$ ) between the photosynthetic pigment content in the FL and the second derivative (D2, obtained using the Savitzky–Golay filter) of the reflectance spectra acquired from 124 DAA until full ripening of both cultivars. Wavelengths with  $R > 0.7$  are indicated by vertical lines using the following colour code: red –  $R > 0.95$ ; orange –  $R$  between 0.9 and 0.95; yellow –  $R$  between 0.8 and 0.9; green –  $R$  between 0.7 and 0.8. Total content of chlorophyll (Total Chl) and carotenoids (Carot) were extracted from in fruits' FL in cold methanol according to Cavaco *et al.* (2003). Concentration values of Total Chl and Carot were calculated according to Lichtenthaler (1987).



**Figure 5.** Pearson's correlation ( $p < 0.005$ ) between % DM (dry matter percentage) and the second derivative (D2, obtained using the Savitzky–Golay filter) of the spectra acquired from 124 DAA until full maturation of each variety. Wavelengths with  $R > 0.7$  are indicated by vertical lines using the following colour code: red –  $R > 0.95$ ; orange –  $R$  between 0.9 and 0.95; yellow –  $R$  between 0.8 and 0.9; green –  $R$  between 0.7 and 0.8.

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