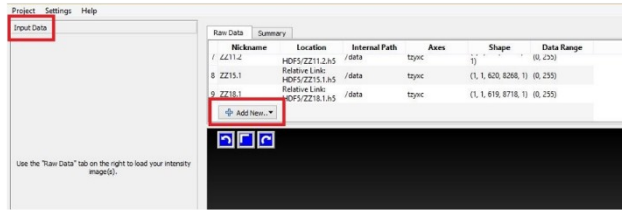


Supplementary Material

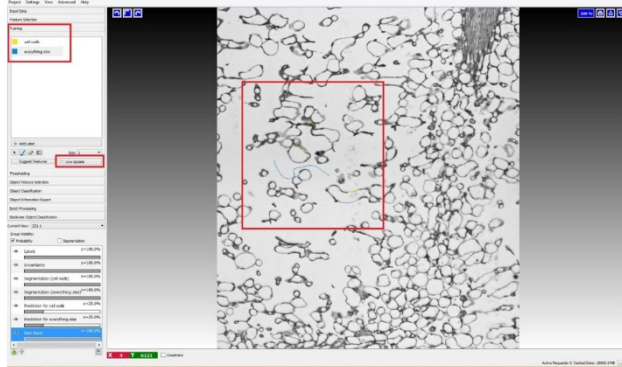
Analysis of the peel structure of different Citrus spp. via light microscopy, SEM and μ CT with manual and automatic segmentation

In *ilastik*, firstly a project was created for each species by selecting Pixel Classification + Object Classification and saving the project. Then all 10 sections of the thin sections of a species were added to the project (Figure 1A). Following, all features (Color/Intensity, Edge and Texture) were selected in Feature selection. In the Training tab, the two labels (cell wall and everything else) were created (Figure 1B). Dark pixels were selected and assigned to the label "cell wall" and light pixels inside the cell and in the intercellular space were selected and assigned to the label "everything else". The Live Update function was used to see probabilities of the labels (only few label sections were selected (about 5 selections for each label)). In the next tab, the Threshold and Filter settings were set (Figure 1C). For species with very distinct intercellular spaces (citron and pomelo) the Size Filter was adjusted to a larger size. In the tab Object Feature Selection select Features All excl. location was selected. In Object Classification 3 labels (Cells, Intercellular Spaces and Background) were created. By clicking on the respective areas of the thin section, they were assigned to the label. The view function was used to check how the classification worked and to adjust it accordingly. After training the software on one image, the algorithm was applied to the remaining 9 images of the dataset and the segmentation was checked. This was done using Object Information Export (Figure 1D). The respective image could be selected and checked under Prediction - Preview. If necessary, the parameters were adjusted in Training and Object Classification. Finally, the individual segmented images were exported and measured using the Threshold function of the *ImageJ/Fiji* software. The oil glands and edges of the image were segmented using the magic wand tool and subtracted with the cell tissue from the total area, resulting in the area of the intercellular spaces.

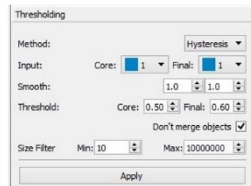
A



B



C



D

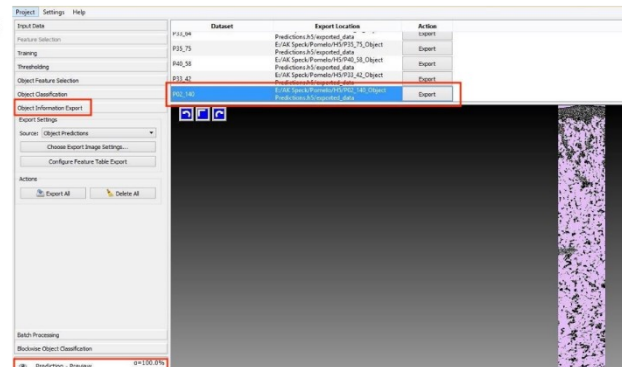


Figure 1. ilastik software settings (version 1.3.2) using thin sections of pomelo peel to characterize the intercellular spaces of the peel. (A) Import of the total dataset of thin sections. (B) Labeling of the Areas within the peel. (C) Threshold settings and (D) Export of the dataset.

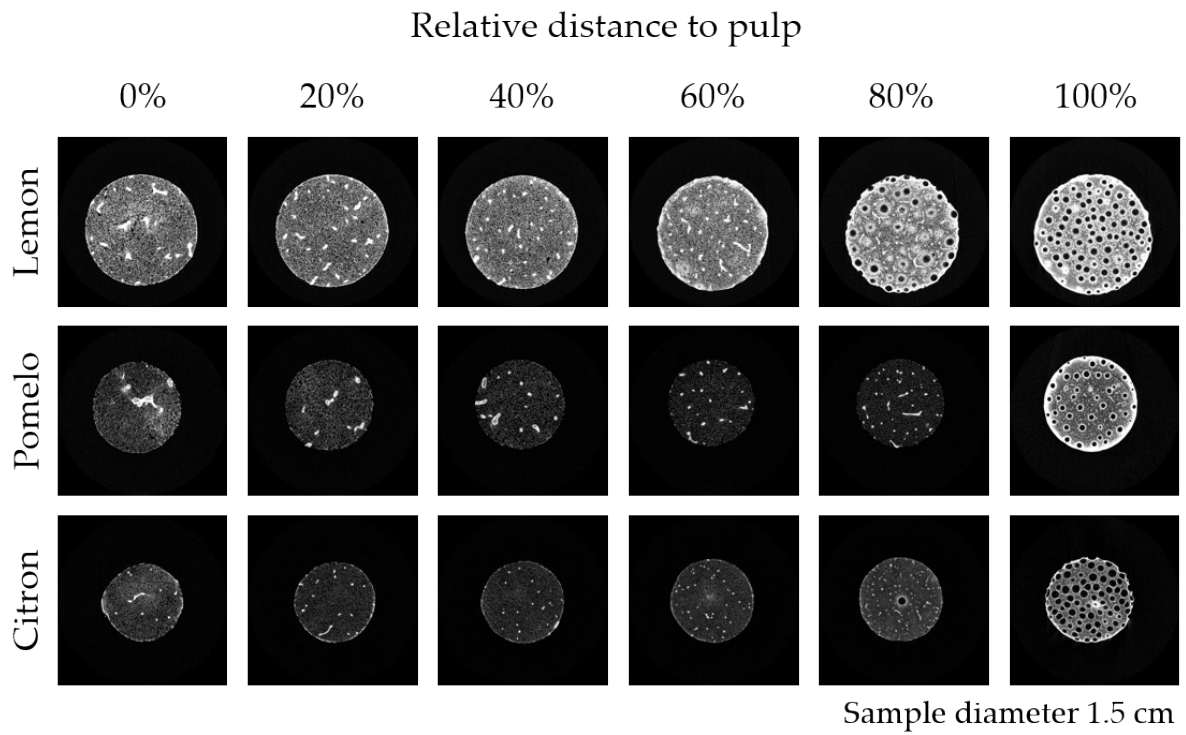


Figure 2. μ CT scans of the peels of Citrus spp. in different distances to pulp. Visualization of the vascular bundles and the outer oil glands. Sample diameter 1.5 cm.

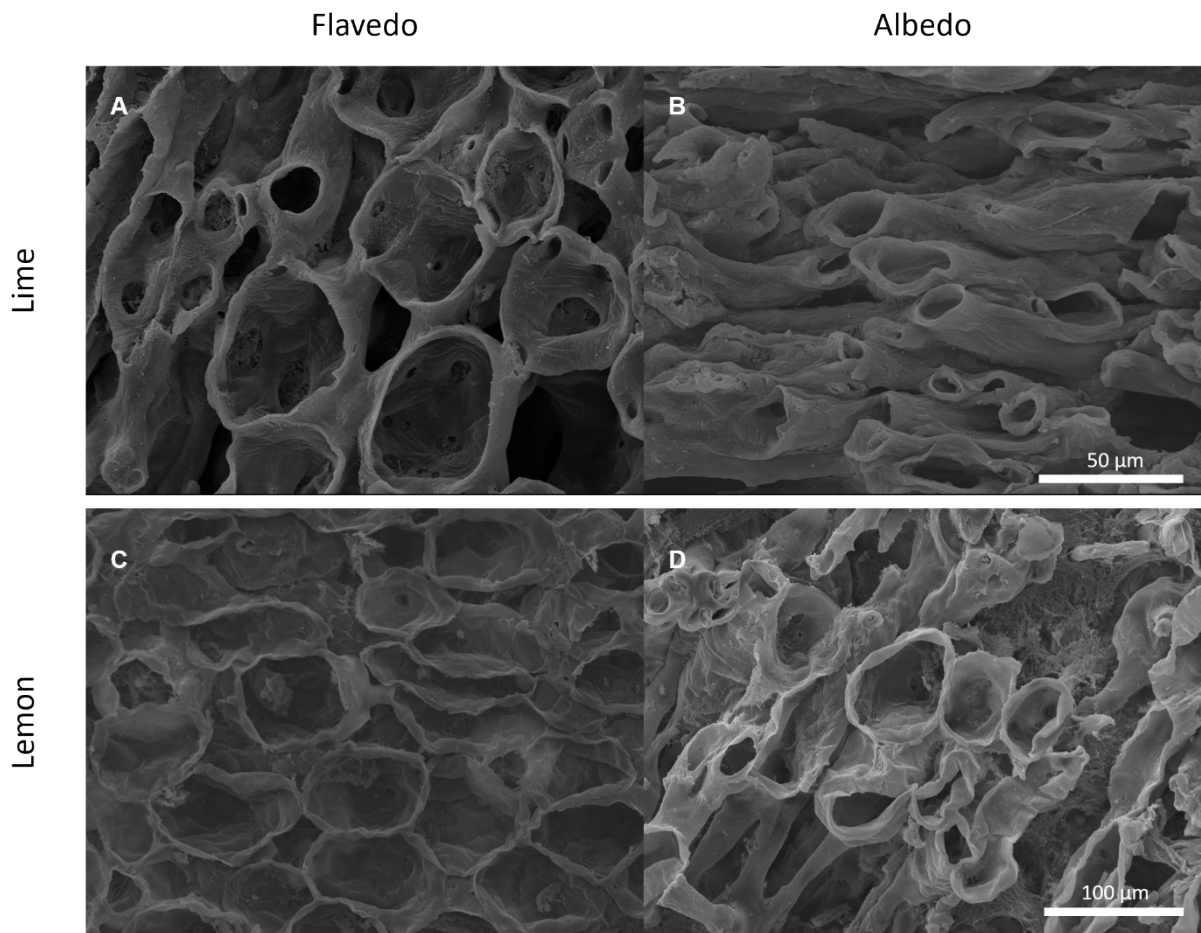


Figure 3. SEM images of the peel tissue of lime and lemon peel. Outer flavedo (A) and inner albedo cells (B) of Lime and outer flavedo (C) and inner albedo cells (D) of lemon (equal magnification for the images of Lime (A and B) and equal magnification for the images of lemon (C and D)).