Unravelling the nanostructure of strawberry fruit pectins by atomic force microscopy

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Atomic force microscopy (AFM) allows the analysis of individual polymers at nanostructural level with a minimal sample preparation. This technique has been used to analyse the pectin disassembly process during the ripening and postharvest storage of several fleshy fruits. In general, pectins analysed by AFM are usually visualized as isolated chains, unbranched or with a low number of branchs and, occasionally, as large aggregates. However, the exact nature of these structures is unknown. It has been suggested that pectin aggregates represent a mixture of rhamnonogalacturonan I and homogalacturonan, while isolated chains and their branches are mainly composed by polygalacturonic acid. In order to gain insight into the nature of these structures, sodium carbonate soluble pectins from ripe strawberry (*Fragaria* x *ananassa*, Duch.) fruits were subjected to enzymatic digestion with endo-Polygalacturonase M2 from *Aspergillus aculeatus*, and the samples visualized by AFM at different time intervals. Pectins isolated from control, non-transformed plants, and two transgenic genotypes with low level of expression of ripening-induced pectinase genes encoding a polygalacturonase (APG) or a pectate lyase (APEL) were also included in this study. Before digestion, isolated pectin chains from control were shorter than those from transgenic fruits, showing number-average (L_N) contour length values of 73.2 nm vs. 95.9 nm and 91.4 nm in APG and APEL, respectively. The percentage of branched polymers was significantly higher in APG polyuronides than in the remaining genotypes, 33% in APG vs. 6% in control and APEL. As a result of the endo-PG treatment, a gradual decrease in the main backbone length of isolated chains was observed in the three samples. The minimum L_N value was reached after 8 h of digestion, being similar in the three genotypes, 22 nm. By contrast, the branches were not visible after 1.5-2 h of digestion. L_N values were plotted against digestion time and the data fitted to a first-order exponential decay curve, obtaining R^2 values higher than 0.9. The half digestion time calculated with these equations were similar for control and APG pectins, 1.7 h, but significantly higher in APEL, 2.5 h, indicating that these polymer chains were more resistant to endo-PG digestion. Regarding the pectin aggregates, their volumes were estimated and used to calculate L_N molecular weights. Before digestion, control and APEL samples showed complexes of similar molecular weights, 1722 kDa, and slightly higher than those observed in APG samples. After endo-PG digestion, size of complexes diminished significantly, reaching similar values in the three pectin samples, around 650 kDa. These results suggest that isolated polymer chains visualized by AFM are formed by a HG domain linked to a shorter polymer resistant to endo-PG digestion, maybe xylogalacturonan or RG-I. The silencing of the pectate lyase gene slightly modified the structure and/or chemical composition of polymer chains making these polyuronides more resistant to enzymatic degradation. Similarly, polygalacturonic acid is one of the main component of the aggregates.

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