

The preparation of the cell wall material (CWM) from the olive pulp by treatment with aqueous solutions of SDS, ball-milling and treatment with solution of PAW is the most efficient procedure for the obtention of material, free of intracellular contaminants necessary for the study of olive pulp cell walls.

The abundance of proteins and phenolic compounds co-precipitated with the cell walls when alcohol insoluble residues were used prevented the utilisation of this procedure in this type of tissue.

Olive cell walls were very rich in pectic substances, didn't present significant amounts of xyloglucans and were also constituted by glucuronoxylans. Glycoproteins rich in hidroxyproline and (gluco)mannans were also present in this type of tissue.

A significative fraction of phenolic substances is integrant part of olive pulp cell wall. It was responsible for the slight green colour of the CWM and for it low total sugars content (62%).

A detailed analysis to the material solubilised during the preparation of the CWM allowed to see that the ball-milling with 0.5% SDS solubilised some cell wall polymers, namely, ester linked xylan-pectin complexes and arabinogalactan-rich glycoproteins.

The pectic polysaccharides, rich in uronic acid and arabinose, differed greatly in their ease of extraction from the cell wall matrix. The majority was extracted from the CWM with CDTA and sodium carbonate; significant amounts appeared also in 1M and 4M KOH and principally after neutralisation and dialysis of the α -cellulose residue. Significant amounts of pectic polysaccharides remained in the α '-cellulose residue and part was removed by chlorite/acetic acid treatment.

Although the differences in extractability presented, the pectic polysaccharides of olive pulp are a range of polymers structurally similar in which the relative proportions of the variously linked arabinosyl residues were highly comparable. The relative proportions were:

$$\text{T-Araf} : (1\rightarrow3)\text{-Araf} : (1\rightarrow5)\text{-Araf} : (1\rightarrow3,5)\text{-Araf} = 2.1 : 1.0 : 2.6 : 2.8$$

The arabinose residues that occur in pectic polysaccharides of olive pulp have some single characteristics as the presence of (1→3)-linked arabinosyl residues and terminally linked residues in anomeric configuration β .

In olive pulp (1→4)-linked xylosyl residues were detected in fractions rich in pectic substances, as described for pectic polysaccharides from the cell wall of other soft tissues. It's possible that these residues are part and parcel of this type of polysaccharides and not contaminant hemicellulosic material.

In the cell walls of olive pulp are also present acidic xylans. One glucuronoxytan was easily isolated and characterised, showing to be constituted by (1→4)- β -D-Xylp, (1→2,4)- β -D-Xylp, and terminally-linked 4-O-Me- α -D-GlcPA. The xylan showed to have 8% of branching points and a degree of polymerisation of 200 xylosyl residues. This type of xylan have been found in tissues where lignified cells (sclereids) are dispersed in the parenchymatous tissues of the pulp, and it is most probable that the xylan would have been derived from the lignified stone cells and not from the parenchymatous cells.

The xyloglucans are not abundant in olive pulp and the small amounts isolated were associated with xylans. This is in contrast to the significant amounts of pure xyloglucans that have been isolated from other soft tissues from vegetables and fruits.

Two distinct populations of xylan-xyloglucan complexes, with apparent molecular weight of 2000 Kd and 100 Kd, were isolated and characterised. The polymers with higher molecular weight were acidic xylans with short chain (1→4)-, terminally- and a small amount of (1→4,6)-linked glucosyl residues as side chains. The polymers with lower molecular weight were very branched xyloglucans and short chain acidic xylans, possibly as side chains. In this second population were detected (1→2)-linked xylosyl residues and terminally-linked arabinosyl and galactosyl residues.

A small but significant amount of hydroxyproline rich cell wall glycoproteins was isolated from the 4M KOH extracts; from these extracts and also by treatment of the α '-cellulose were isolated glycoprotein-rich fractions that also contained pectic material associated. It's possible that these polymers are associated by phenolic linkages to the hydroxyproline rich glycoproteins.

Some olive pulp cell wall polysaccharides, although the different origin, are associated by linkages strong enough to allow their isolation in the form of complexes, as the xylan-xyloglucan xyloglucan-xylan complexes. Although the small amount in which they occur, it's possible that they have an active role in the organisation of the olive pulp cell wall.

The softening of the olive pulp after processing is mainly due to degradation of pectic polysaccharides. Similar degradation of the pectic polysaccharides was obtained by treatment of the CWM with hot water (method frequently used for the extraction of pectin), provoking de depolymerisation of pectins by β -elimination reactions and giving rise to small amount of arabinose-rich oligosaccharides. The hemicelluloses, although were not altered by the processing, were more easily extracted, probably due to the hydrolysis of some cross-linking linkages in the cell wall during the processing.

The study of the highly lignified tissues of olive seed showed a very lignified cell wall. After exhaustive treatment with chlorite and acetic acid it was possible to isolate glucuronoxylans, xylan-pectic polysaccharide complexes associated with phenolic material and small but significant amounts of pectic polysaccharides that were virtually free of xylans. Lignin (30-35%), cellulose (30%), glucuronoxylans (30%) and pectic substances (2-3%) are the principal polymers present in the cell walls of this tissues.

The most abundant xylan isolated from the lignified tissues had a degree of polymerisation of 89 xylosyl residues and 7% of branching points. All the glucuronic acid residues present appeared to be in the form of 4-*O*-Me- α -D-GlcpA in the proportion of 1:14 xylosyl residues, in a regular structure.

The work done allowed the elucidation of the major polymers constituent of olive pulp and seed cell walls. Several points may be further explored as a basis for future research:

- The nature of the linkage between xylan and xyloglucan in the xylan-xyloglucan complexes;
- The nature of the linkage between xylan and pectin in olive pulp extracted in fraction 0.5% SDS;

- The nature of the linkage between carbohydrates and lignin in olive pulp and how it compares with the nature of the linkages in the lignified tissues.
- How the pectic fraction obtained from the lignified tissues of seed hull compares with the fractions obtained from the pulp;
- What are the detailed structure of hydroxyproline-rich glycoproteins and mannans from olive pulp.

The study of olive cell walls may be applied to the systematic improvement and diversification of products from the olive industry, namely:

- Control of the conditions of fermentations;
- Control of the changes in texture provoked by the alkali treatment, pasteurisation or storage.

This study may also be used as a investigation basis for the development of new olive products and sub-products such as:

- olive bagasse for eventual dietary fibre production;
- olive seed de-lignification for xylose production, as proposed for the highly lignified tissues of almond seed (Pou-Ilinas *et al.*, 1990);
- excess olive profit to various applications including gels.