ABSTRACT

Experimental procedures were developed for the isolation and characterisation of cell wall polymers from olive pulp. The same type of procedure was applied to the study of processed green and black olives. In addition, experimental procedures that allowed the isolation and characterisation of the polymers from the highly lignified tissues of the olive seed were developed.

In preliminary studies alcohol insoluble residue (AIR) was used as the source of cell wall material but this residue was not found to be suitable for detailed fractionation studies due to the presence of large amounts of co-precipitated protein and phenolics. Therefore, cell wall material (CWM) was prepared by blending the olive pulp in dilute aqueous solution of sodium dodecylsulphate (SDS), wet ball-milling in 0.5% SDS and treating with phenol:acetic acid:water (PAW) (2:1:1, w/v/v). This procedure gave a material free of intracellular contaminants such as oil, phenolics and proteins.

From the 0.5% SDS extract a xylan-pectic polysaccharide complex possibly cross-linked by ester linkages and some proteoglycans rich in arabinose, galactose and hydroxyproline were isolated.

Polymers were solubilised from the CWM by sequential extraction with aqueous solutions of CDTA, Na_2CO_3, 1M KOH, 4M KOH and 4M KOH + borate to leave a cellulose-rich residue (RC1). Neutralisation and dialysis of RC1 resulted in the solubilisation of a fraction rich in pectic material leaving the residue RC2. A short treatment of RC2 with chlorite / acetic acid solubilised pectic polysaccharides and some glycoproteins to give the final residue (RF).

The polymers from the various extracts were fractionated by graded precipitation with ethanol prior to anion-exchange chromatography, and selected fractions were submitted to detailed analysis using techniques such as sugar and methylation analysis and ^{13}C NMR spectroscopy.

Closely related pectic polysaccharides very rich in arabinose were the major components of the cell wall and they differed in their ease of extraction from the wall matrix. The relative proportions of the different linkage-types of the arabinosyl residues were similar in all the pectic arabinose-rich

fractions. NMR spectroscopy showed that 30 to 40% of the L-arabinofuranosyl residues had the β anomeric configuration.

Significant amounts of acidic xylans were isolated from the precipitates obtained on neutral-isation of the 1M KOH extracts. The major acidic xylan had a chain length of 250 residues of which 13% were substituted on C-2 with terminally-linked 4-O-Me-Glc$p$A; terminally-linked glucuronic acid was not detected.

The xyloglucans had xylans associated with them and no pure xyloglucans could be isolated by the chromatographic methods used. Two distinct types of xylan-xyloglucan complexes, with apparent molecular weights of 100 and 2 000 kD, were isolated and partially characterised. The polymers with lower molecular weight were highly branched xyloglucans having short chains of acidic xylans and the polymers with higher molecular weight were acidic xylans having short chains xyloglucan moieties.

Significant amounts of hydroxyproline-rich cell wall glycoproteins were detected in the neutral fractions of the 4M KOH and 4M KOH + borate extracts.

From the analysis of fresh and processed olive pulp (both green and black olives) it could be inferred that the softening was mainly due to the degradation of pectic polysaccharides. Similar degradation of the pectic polysaccharides was also observed on treatment of the CWM with hot water (a method frequently used in the past for the extraction of pectins). The glucuronoxylans, however, were not altered by processing.

Olive seed hulls were found to be very heavily lignified. The cryo-milled hull was delignified by exhaustive treatment with warm chlorite / acetic acid. The holocellulose was found to contain mainly glucuronoxylans and small amounts of xylan-pectic polysaccharide complexes associated with phenolic material; a small amount of pectic polysaccharides virtually free of acidic xylans was also isolated.