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Retention of procyanidins by apple cell walls limits their diffusion ?

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Apples are rich in polyphenols and more precisely in procyanidins contained in vacuoles of cells. Cell walls are composed of a network of polysaccharides such as cellulose, hemicellulose and pectins which define the structure and the texture of cells. It has already been shown that procyanidins interact with polysaccharides and that the affinity of procyanidins for pectins is higher than for hemicelluloses when cells are ruptured [1]. The aim of this study is to understand the impact of leaching process on the fate of procyanidins in apple. We present here the influence of the osmotic pressure on removal of procyanidins from apple pieces and their associations with cells walls by binding isotherms and with commercial pectins by isothermal titration calorimetry.

Apple parenchyma (cv. Granny Smith) was immersed in water for 6h at room temperature and changes of osmotic pressure via different concentrations of mannitol (0 to 0.6M) in external medium were used. Samples were removed each hour and procyanidins content was analyzed by HPLC-DAD. For association analyses, procyanidins with intermediate degree of polymerization (DP 8) were extracted from freeze-dried pulps of apple (cv. Golden delicious) as described in [2]. Cell walls were isolated from fresh ripe apples (cv. Ariane) using the phenol: buffer method (PB) as described in [1]. For binding isotherms, procyanidins (5 g/L) were incubated for 1h with the cell wall suspension (PB) or commercial pectins (20 g/L), in citrate/phosphate buffer pH 3.8 [1,3]. After incubation the solution and the cell wall-polyphenol complexes were separated by filtration under vacuum. The binding isotherms were interpreted according to the Langmuir formula [3]. For isothermal titration calorimetry, commercial apple pectin (30 mM galacturonic acid equivalent) dissolved in citrate/phosphate buffer pH 3.8 was titrated by procyanidins (30 mM (-)-epicatechin equivalent) in VP-ITC instrument (Microcal®, GE Healtcare) [2].

The higher the mannitol concentration, the lower the diffusion of procyanidins: some procyanidins were lost from the apple tissue when there was a difference in osmotic pressure between parenchyma cells and outer medium, none when osmotic pressures were equilibrated. Therefore in living tissue loss of procyanidins was only possible when cells were ruptures. With cooked tissues, procyanidin loss was observed whatever the osmotic pressure but remained partial, in contrast to monomeric polyphenols. This could be explained by the association between cell walls and procyanidins as it showed by binding isotherms. From cell walls, pectins associated with procyanidins with an affinity on the order of 10^3 M^{-1} through hydrophobic interactions and hydrogen bonds.

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