ENDO-XYLOGALACTURONAN HYDROLASE

A novel enzyme for fruit processing


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Abstract

Commercial pectinases used in apple juice manufacturing contain a mixture of pectin-degrading enzyme activities. Nevertheless, fragments of branched pectic molecules (or pectic hairy regions) are resistant to degradation, and can cause membrane fouling in the final ultrafiltration step of concentrated apple juice. The pectic hairy regions contain both rhamno- and xylogalacturonan. Whereas rhamnogalacturonan-degrading enzymes were already identified, no enzyme was known for a fast degradation of xylogalacturonan. Apparently, these enzymes are very minor components of technical pectinases. Since Aspergillus strains are able to completely degrade pectin, including xylogalacturonan, we decided to use expression cloning to obtain xylogalacturonan-degrading enzymes.

A cDNA library of Aspergillus tubingensis was constructed in the yeast Kluyveromyces lactis, using a carbon source rich in xylogalacturonan. The library was screened using a hairy regions preparation from apple, and xylogalacturonan prepared from gum tragacanth as substrates. A novel endo-xylogalacturonase was found, XGH. The enzyme specifically degrades xylose-substituted galacturonic acid backbones. In lab scale filtration experiments, XGH was able to decrease membrane fouling caused by hairy regions from apple.

1. The role of enzymes in fruit juice processing

Clear apple juice is one of the most popular fruit juices, and worldwide apple is the most important raw material for the production of clear juice. In 2000 around 10 Mio tonnes of the apples were processed into concentrate, which is about 20% of the world production of apples. Juice concentrates are shipped all over the world, from apple-growing countries to juice-consuming countries. Cell wall polysaccharides play an important role in fruit juice manufacturing. For instance, fruit contains pectin, which due to its viscous nature and gel-forming properties can cause problems during processing. Application of enzyme preparations in fruit processing is already a quite old practice. In particular, pectinase complexes have been used to facilitate processing.
Often, these technical pectinase preparations are derived from the food grade fungus *Aspergillus niger*. These preparations contain a mixture of enzymes aimed at the optimal degradation of pectin.

There is a great variety in fruits, and in processing methods (reviewed in Grassin and Fauquembergue, 1999). Of these, the production of apple juice concentrate is probably the most important one. This process (Fig. 1) starts by grinding the apples. After addition of the enzyme preparation, containing a complex of pectin-degrading enzymes, the pulp is incubated for 30-60 min at room temperature. This step is called maceration. Maceration is followed by pressing and flash pasteurization of the raw juice. After cooling to 50 °C, pectinase is added again; the purpose of this is to degrade the pectin in the raw juice, and to promote the flocculation of cell wall particles, necessary for clarification of the juice. In the beginning of the season amylase is also included in the technical preparations in order to degrade the starch present in unripe apples. If the starch is not removed aggregation of starch polymers (retrogradation) may take place in the concentrate, causing undesirable hazes. After approximately 2 hours of depectinization and clarification the juice is ready for ultrafiltration followed by concentration.

Pectinases have several roles in juice production. (i) During the maceration step these enzymes degrade the pectins in the apple cell wall. As a result, the cell walls lose their strength, which enhances the efflux of the juice from the cell. In addition, the solubilized pectin in crushed fruits can form a highly viscous mass, which is difficult to press. Pectinases play an important role in reducing the viscosity of this mass. These two effects together greatly improve the pressability of the juice. (ii) During the depectinization step pectin is degraded, resulting in clarification of the juice as described above. (iii) Last but not least, complete removal of pectin is needed to facilitate concentration of the juice, since gellification of residual pectin prevents efficient concentration.