

Megazyme

ARABINAN ASSAY PROCEDURE

ARA 6/01



In the processing of apples and pears, the yield of juice can be dramatically improved by using enzymes to degrade the pulp polysaccharides, together with more exhaustive extraction of the pulp with diffusion equipment. Of course, these processes also significantly increase the amount of partially degraded polysaccharide which is solubilised. This polysaccharide material may be soluble as extracted, but subsequent changes in temperature and pH conditions can directly lead to precipitation (or crystallisation), or to chemical modification followed by precipitation. Such a problem can be experienced in the production of clear apple or pear juice, in which case, an arabinan haze material is produced. This material was shown to be microcrystalline 1,5- α -L-arabinan.

Arabinans, as present in cell-wall pectic-substances, have been shown to consist of a main chain of 1,5- α -linked L-arabinofuranosyl residues to which other L-arabinofuranosyl residues are linked 1,3- α and 1,2- α in either a comb-like or a ramified arrangement.

It is generally accepted that the best solution to this "arabinan haze" problem is to use pectinase enzyme preparations containing high levels of both α -L-arabino-furanosidase and *endo*-1,5- α -L-arabinanase. The combined action of these two enzymes reduces arabinan mainly to arabinose.

In the currently described procedure for the measurement of arabinan in juice concentrates, arabinan is separated from arabinose and low degree of polymerisation oligosaccharides by gel filtration. The arabinan is then hydrolysed to arabinose by the combined action of α -L-arabinofuranosidase and *endo*-arabinanase. The released arabinose is then measured using galactose dehydrogenase (which also acts on arabinose).

MATERIALS SUPPLIED:

1. **Arabinofuranosidase / endo-Arabinanase (Megazyme International Ireland Limited):**

Mix the enzyme thoroughly and dilute 0.2 ml of the suspension (in ammonium sulphate) to 2.0 ml in 0.1 M sodium acetate buffer (pH 4.0) to give final concentration of ;

α -L-arabinofuranosidase 40 U/ml.

endo-arabinanase 2 U/ml.

Store this diluted solution at -20°C between use.

2. **Pear Juice Concentrate: (4.2 mg/ml arabinan)**

An aliquot (1.0 ml) of this juice is diluted to 10 ml with distilled water and 2 ml of this is fractionated on a PD-10 column to separate arabinan from free arabinose. The arabinan content of the original concentrate (calculated using the equation below) is 4.2 mg/ml.

3. **Pharmacia PD-10 column:**

After use, this column should be washed with 50 ml of distilled water and then with 10 ml of 0.02% sodium azide as a preservative. The column should be clamped, sealed and stored at room temperature.

MATERIALS NOT SUPPLIED:

1. **NAD (β -Nicotinamide adenine dinucleotide)**

Dissolve 0.1 g NAD (Boehringer Mannheim cat. no. 127965) in 10 ml of distilled water. Store frozen between use.

2. **β -Galactose Dehydrogenase**

Obtained from Roche Diagnostics (Cat. no. 662046). Adjust the concentration of this enzyme to 70 Units/ml by diluting the enzyme as supplied with ammonium sulphate solution (50% w/v). Store at 4°C.

SAMPLE PREPARATION:

Juice concentrate (10 ml) is diluted to 100 ml with distilled water, (with the control sample supplied, dilute 1.0 ml to 10 ml with water) mixed thoroughly, and heated to 70°C for 10 min (to dissolve any insoluble arabinan material). The solution is cooled to room temperature.

An aliquot (2 ml) of this diluted solution is added to a pre-washed Pharmacia PD-10 column and allowed to percolate into the gel. The column is then washed 2 times with 2 ml of distilled water. The entire elution solution (6 ml) is collected for arabinan analysis.

The PD-10 column is washed with 50 ml of distilled water in preparation for the next sample.

MEASUREMENT OF ARABINAN:

Samples (0.1 ml) of the eluate are treated with an *endo*-arabinanase /arabinofuranosidase preparation (0.1 ml of diluted preparation) in sodium acetate buffer (0.1 M, pH 4.0) at 40°C for 1 hour (**Reaction Solutions**).

Samples are then treated with Tris/HCL buffer (2.5 ml, pH 8.6, 0.2 M); NAD (0.1 ml, 10 mg/ml) and galactose dehydrogenase [10 microlitres, 70 U/ml, Roche Diagnostics (Cat. no. 662046)] at 40°C for 1 hour.

Blank absorbance values are determined by adding 0.1 ml of sample to 0.1 ml of acetate buffer (0.1 M, pH 4.0) and then treating with Tris/HCL, NAD and galactose dehydrogenase as for the Reaction solution. (This determination measures **free arabinose** in the sample eluting from the PD-10 column).

The absorbance of Reaction solutions and Blanks are measured at 340nm against distilled water.

CALCULATIONS:

$$\begin{aligned} \text{Arabinan (mg/ml)} &= \Delta E \times F \times 10 \times \frac{60}{2} \times \frac{1}{1000} \times \frac{132}{150} \\ &= \Delta E \times F \times 0.264. \end{aligned}$$

ΔE = absorbance reaction - absorbance blank.

F = $\frac{50 \text{ (micrograms of arabinose)}}{\text{absorbance for 50 micrograms of arabinose}}$

10 = dilution factor.

$\frac{60}{2}$ = volume correction factor (2 ml of solution was passed through the column and diluted to 6 ml, of which 0.1 ml was analysed).

$\frac{1}{1000}$ = conversion from micrograms to milligrams.

$\frac{132}{150}$ = adjustment from free arabinose to anhydro-arabinose (as occurs in arabinan).

Note: The juice concentrate sample is chromatographed on the Pharmacia PD-10 (Sephadex G-25) column to remove low molecular weight arabino-oligosaccharides which would otherwise be analysed as arabinan.

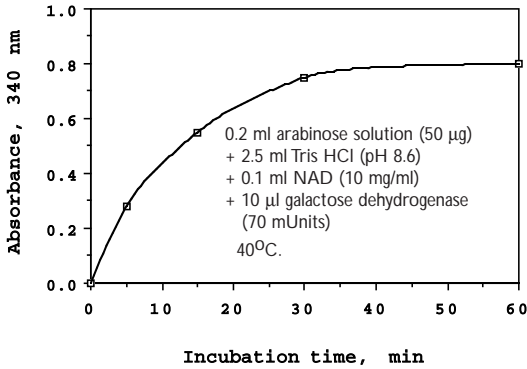


Figure 1. Absorbance (340nm) increase on incubation of galactose dehydrogenase with arabinose

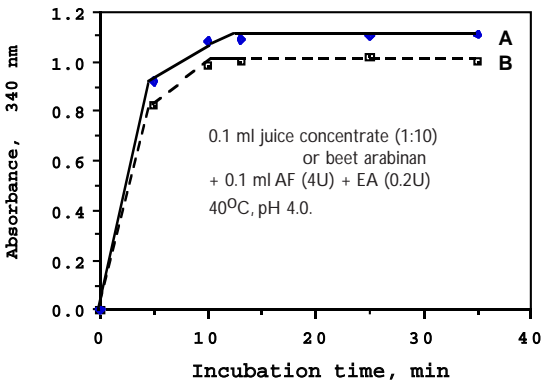


Figure 2. Hydrolysis of arabinan to arabinose by *endo*-arabinanase plus α -L-arabinofuranosidase.
A. sugar beet arabinan. **B.** arabinan in pear juice concentrate.

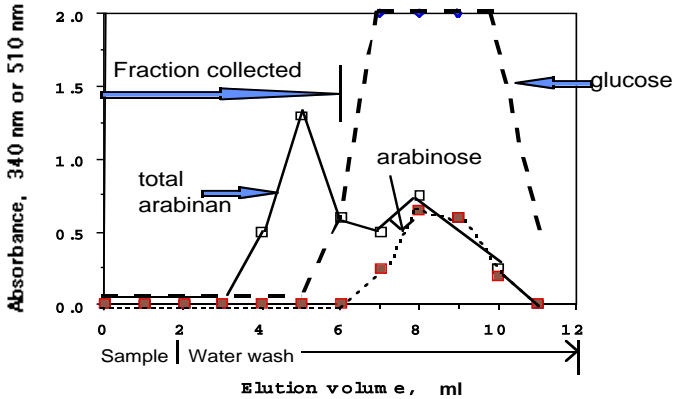


Figure 3. Chromatography of pear juice concentrate on a PD-10 column. The eluate was analysed for arabinose, arabinan and glucose. Sample; 2ml of concentrated pear juice diluted 1:10. Eluant; water.



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