Megazyme

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ARABINAN

ASSAY PROCEDURE

K-ARAB 08/18

(100 Assays per Kit)

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INTRODUCTION:

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.

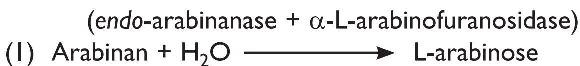
Arabinans, as present in cell-wall pectic-substances, have been shown to consist of a main chain of 1,5-α-linked L-arabinofuranosyl residues to which other L-arabinofuranosyl residues are linked 1,3-α and 1,2-α in either a comb-like or a ramified arrangement.

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-arabinofuranosidase and *endo*-1,5-α-L-arabinanase. The combined action of these two enzymes reduces arabinan to arabinose and lower degree of polymerisation arabino-oligosaccharides.

PRINCIPLE:

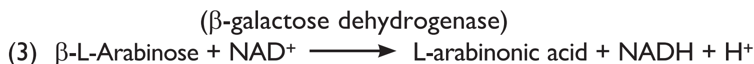
In the currently described procedure for the measurement of arabinan in juice concentrates, arabinan is separated from arabinose and low degree of polymerisation oligosaccharides by gel filtration.

Arabinan is hydrolysed by the combined action of α-L-arabinofuranosidase and *endo*-arabinanase to L-arabinose (1).



Interconversion of the α- and β-anomeric forms of L-arabinose is catalysed by galactose mutarotase (GalM) (2).

The β-L-Arabinose is oxidised by NAD^+ to L-arabinonic acid in the presence of β-galactose dehydrogenase (3).



The amount of NADH formed in this reaction is stoichiometric with the amount of L-arabinose or D-galactose. It is the NADH which is measured by the increase in absorbance at 340 nm.

KITS:

Kits suitable for performing 100 assays are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: Buffer (25 mL, pH 8.6) plus sodium azide (0.02% w/v) as a preservative (Buffer A).
Stable for > 2 years at 4°C.

Bottle 2: NAD^+.
Stable for > 5 years below -10°C.

Bottle 3: α-L-Arabinofuranosidase plus *endo*-arabinanase suspension (2.2 mL).
Stable for > 4 years at 4°C.

Bottle 4: β-Galactose dehydrogenase plus galactose mutarotase suspension, 2.2 mL.
Stable for > 2 years at 4°C.

Bottle 5: L-Arabinose standard solution (5 mL, 0.5 mg/mL in 0.02% sodium azide).
Stable for > 4 years at 4°C.

Bottle 6: Arabinan control in mannitol: Arabinan concentration on vial.
Stable for > 5 years at 4°C.

Component 7: Pharmacia PD-10 column.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1. Use the contents of bottle 1 as supplied.
Stable for > 2 years at 4°C.

2. Dissolve the contents of bottle 2 in 12 mL of distilled water. Divide into appropriately sized aliquots and store in polypropylene tubes below -10°C between use and cool during use. Stable for > 2 years below -10°C.

3. Use the contents of bottle 3 as supplied. Before opening for the first time, shake the bottle to remove any protein that may have settled on the rubber stopper. Stable for > 4 years at 4°C.

4. Use the contents of bottle 4 as supplied. Before opening for the first time, shake the bottle to remove any protein that may have settled on the rubber stopper. Subsequently, store the bottles in an upright position. Stable for > 2 years at 4°C.

5. Use the contents of bottle 5 as supplied.
Stable for > 4 years at 4°C.

6. Add 10 mL of distilled water to the vial and a 5 x 15 mm magnetic stirrer bar. Stir at room temperature for 5 min to ensure complete dissolution of the arabinan. Divide the solution into 5 mL aliquots and store below -10°C. Stable to repeated thawing and re-freezing. Use 0.1 mL of this solution in the assay procedure as shown on page 4.
Stable for > 5 years below -10°C.

7. After use, wash the PD-10 column with 50 mL of distilled water and then with 10 mL of 0.02% sodium azide as a preservative. Clamp and cap the column and store it at room temperature. Stable for > 5 years at room temperature.

BUFFER NOT SUPPLIED:

Sodium acetate buffer (100 mM, pH 4.0).
Add 5.8 mL of glacial acetic acid (1.05 g/mL) to 900 mL of distilled water. Adjust the pH to 4.0 by careful addition of 2 M (8 g/100 mL) sodium hydroxide solution. Add 0.2 g of sodium azide. Adjust the volume to 1 litre with distilled water. Stable for > 2 years at 4°C.

EQUIPMENT (RECOMMENDED):

1. Volumetric flasks (50 mL, 100 mL and 500 mL).
2. Disposable plastic cuvettes (1 cm light path, 3.0 mL).
3. Micro-pipettors, e.g. Gilson Pipetman® (20 µL and 200 µL).
4. Positive displacement pipettor e.g. Eppendorf Multipette®
   - with 5.0 mL Combitip® (to dispense 0.2 mL aliquots of Buffer 1 and 0.1 mL aliquots of NAD^+ solution).
   - with 12.5 mL Combitip® (to dispense 2.5 mL aliquots of juice concentrate and distilled water).
5. Analytical balance.
6. Spectrophotometer set at 340 nm.
7. Vortex mixer (e.g. IKA® Yellowline Test Tube Shaker TTS2).
8. Thermostated hot-block heater or water bath (set at 40°C).
9. Stop clock.
10. Whatman No. 1 (9 cm) filter papers.

METHOD:

1. Transfer 10 mL of juice concentrate (at room temperature) to a 100 mL volumetric flask and dilute to the mark with distilled water.
2. Transfer the solution to a 250 mL flask and heat to 70°C to ensure complete dissolution of the arabinan. Cool the solution to room temperature.
3. Prepare a PD-10 column by washing with 20 mL of distilled water.
4. Add 2.0 mL of the diluted juice solution to the PD-10 column and allow it to percolate into the column. Wash the column twice with 2 mL of distilled water and collect this with the initial permeate (6 mL total volume). This step removes essentially all free L-arabinose from arabinan. Free L-arabinose not separated is determined in the assay in the blank determination.
5. Use this solution in the assay described below.

PROCEDURE:

Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: optimally 40°C in a dry hot-block heater or in the spectrophotometer
Final volume: 2.54 mL
Sample solution: 4-70 µg of L-arabinose
Read against air (without a cuvette in the light path) or against water

Pipette into cuvettes	Blank	Sample
sample solution buffer (Acetate buffer, pH 4.0) solution 3 (α-L-arabinofuranosidase + EA)	0.10 mL 0.10 mL -	0.10 mL 0.10 mL 0.02 mL
Mix*, cap the cuvette and incubate at 40°C for 60 min. Add:		
distilled water solution 1 (buffer) solution 2 (NAD^+)	2.02 mL 0.20 mL 0.10 mL	2.00 mL 0.20 mL 0.10 mL
Mix*, read the absorbances of the solutions (A_1) after approx. 3 min and start the reactions by addition of:		
suspension 4 (β-GalDH + Gal-MR)	0.02 mL	0.02 mL
Mix*, cap the cuvettes and incubate at 40°C in a hot-block heater for approx. 10 min. Read the absorbance (A_2) of the solutions. Alternatively, incubations can be performed at 25°C.		

* for example with a plastic spatula or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.

CALCULATIONS:

Determine the absorbance difference (A_2-A_1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{L\text{-arabinose}}$.

The concentration of L-arabinose can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{L\text{-arabinose}} \quad [g/L]$$

where:

V = final volume [mL]
MW = molecular weight of the substance to be assayed [g/mol]
ε = extinction coefficient of NADH at 340 nm
= 6300 [$l \times mol^{-1} \times cm^{-1}$]
d = light path [cm]
v = sample volume [mL]

It follows for L-arabinose

$$c = \frac{2.54 \times 150.1}{6300 \times 1.0 \times 0.1} \times \Delta A_{L\text{-arabinose}} \quad [g/L]$$

$$= 0.6052 \times \Delta A_{L\text{-arabinose}}$$

Thus, for arabinan

$$c = 0.6052 \times \Delta A_{L\text{-arabinose}} \times 132/150.1 \quad [g/L]$$

$$= 0.5322 \times \Delta A_{L\text{-arabinose}}$$

where:

132/150.1 = Adjustment from free L-arabinose to anhydro-arabinose (as occurs in arabinan)

If the sample has been diluted during preparation, the result must be multiplied by the dilution factor, F.

When analysing solid and semi-solid samples which are weighed out for sample preparation, the content (g/100 g) is calculated from the amount weighed as follows:

Content of arabinan

$$= \frac{c_{arabinan}\text{ [g/L sample solution]}}{weight_{sample}\text{ [g/L sample solution]}} \times 100 \quad [g/100\ g]$$

> **NOTE:**
> The L-arabinose standard solution is not required for determination of the L-arabinose content of the test solutions. This solution is used simply to check assay conditions, i.e. activity of β-galactose dehydrogenase/galactose mutarotase mixture.

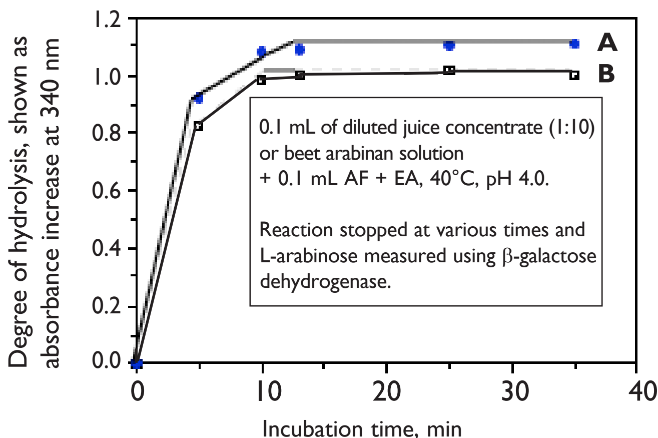


Figure 1. Hydrolysis of arabinan to arabinose by *endo*-arabinanase plus α-L-arabinofuranosidase. **A.** sugar beet arabinan. **B.** arabinan in pear juice concentrate (diluted 1:10).

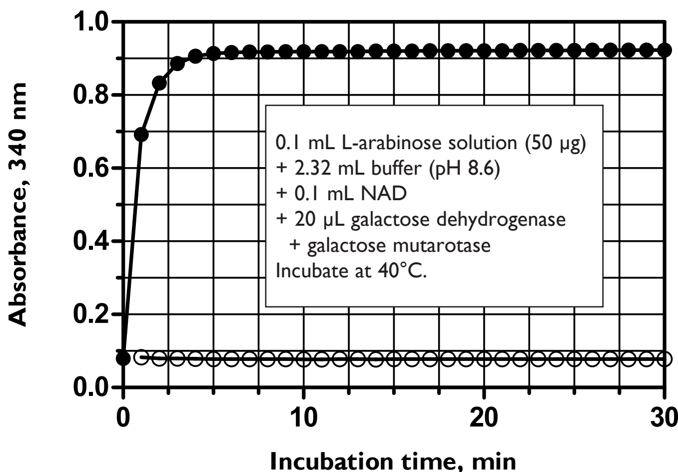


Figure 2. Absorbance increase at 340 nm on incubation of β-galactose dehydrogenase plus galactose mutarotase with L-arabinose.

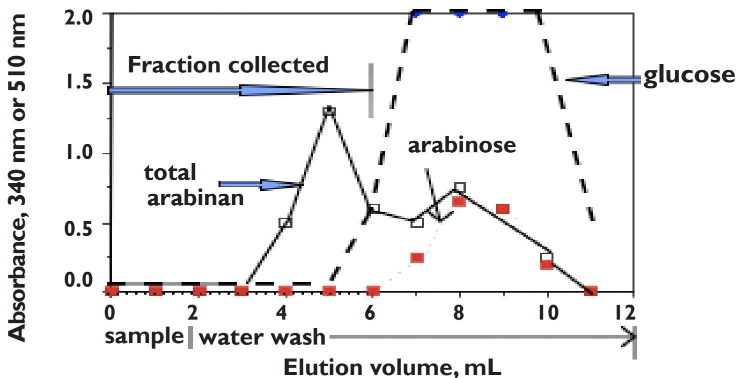


Figure 3. Chromatography of pear juice concentrate on a PD-10 column. The eluate was analysed for L-arabinose, arabinan and D-glucose. **Sample:** 2 mL of concentrated pear juice diluted 1:10. **Eluant:** water.

NOTES:

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