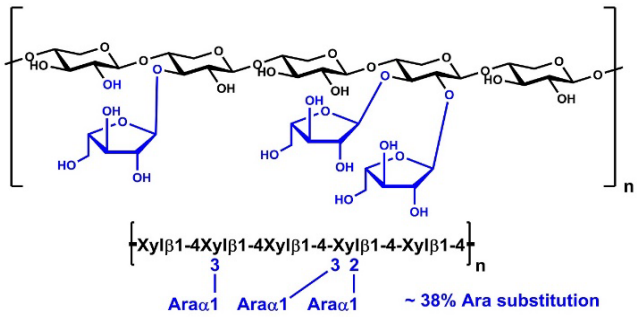
ARABINOXYLAN (Wheat Flour; for Reducing Sugar Assays)

08/23

P-WAXYRS

CAS: 9040-27-1
Source: Wheat

STRUCTURE:



Schematic representation of wheat arabinoxylan unit.

Refer to the product lot number Certificate of Analysis for lot specific properties.

PROPERTIES:
Molecular Weight: 323 Kd (MAALS)
Physical Description: White, odourless powder

STORAGE CONDITIONS:
Store dry at room temperature in a well-sealed container. Under these conditions, the product is stable for several years.

MEASUREMENT OF *endo*-1,4-β-XYLANASE USING P-WAXYRS IN REDUCING SUGAR ASSAYS:

A. NELSON-SOMOGYI REDUCING SUGAR METHOD

a) Preparation of reagents:
Solution A: Dissolve 25 g of anhydrous sodium carbonate, 25 g of sodium potassium tartrate and 200 g of sodium sulphate in 800 mL of distilled water. Dilute this solution to 1 L with distilled water and store in a sealed Duran bottle at room temperature.
Solution B: Dissolve 30 g of copper sulphate pentahydrate in 200 mL of distilled water containing 4 drops of concentrated sulphuric acid. Store the solution in a sealed Duran bottle at room temperature.
Solution C: Dissolve 50 g of ammonium molybdate in 900 mL of distilled water and add 42 mL of concentrated sulphuric acid. Separately, dissolve 6 g of sodium arsenate heptahydrate in 50 mL of distilled water and add this to the ammonium molybdate solution. Dilute the combined solution to 1 L, mix well and store in a sealed Duran bottle at room temperature.
(Reagents A to C are stable at room temperature for ~ 1 year).

Reagent D: Add 1 mL of reagent B to 25 mL of reagent A (Stable for ~ 7 days at room temperature).
Reagent E: Immediately before use, dilute reagent C 5-fold (e.g. 50 mL to 250 mL) with distilled water and use this on the day of preparation.

NOTE: Appropriate personal protecive equipment should be used as required.

b) Preparation of substrate solution:

Add 1 g of wheat flour arabinoxylan (**P-WAXYRS**) to 90 mL of 100 mM sodium acetate buffer (pH 4.5) and dissolve by stirring at approx. 50°C for 10 min on a magnetic stirrer hot-plate. Adjust the volume to 100 mL with 100 mM sodium acetate buffer (pH 4.5) and store the solution in a well-sealed Duran bottle at room temperature. Alternatively, prepare substrate in 100 mM sodium phosphate buffer (pH 6.0) using the same procedure.

c) Assay procedure:

1. Add 0.5 mL aliquots of wheat arabinoxylan solution (1.0% w/v) [in 100 mM sodium acetate buffer (pH 4.5) or 100 mM sodium phosphate buffer (pH 6.0)] into a set of test tubes (16 x 120 mm) and pre-equilibrate these at 40°C for 5 min.
2. Initiate the reaction by adding 0.2 mL aliquots of suitably diluted and pre-equilibrated (at 40°C for 5 min) endo-xylanase preparation to the substrate solution with vigorous stirring on a vortex stirrer and incubate the tubes at 40°C.
3. Terminate the reaction after various time intervals (e.g. 3, 6, 9 and 12 min) by the addition of 0.5 mL of reagent D, with vigorous stirring of the reaction mixture on a vortex stirrer.
4. Prepare reagent blanks by adding 0.5 mL of Nelson-Somogyi reagent D to 0.5 mL of substrate solution and 0.2 mL of the buffer solution as used in the assay and immediately mix the tube contents.
5. Prepare enzyme blanks by adding 0.5 mL of Nelson-Somogyi reagent D to 0.5 mL of substrate solution and mixing well. Then add enzyme solution (0.2 mL) as used in the assay and immediately mix the tube contents.
6. Prepare xylose standards by adding 0.5 mL of Nelson-Somogyi reagent D to 0.5 mL of substrate solution plus 0.2 mL of xylose standard (0-90 µg/0.2 mL; 0-0.6 µmoles/0.2 mL) with mixing.
7. Place all tubes (reaction, reagent blanks, enzyme blanks and xylose standards) in a boiling water bath at the same time and incubate for 15 min.
8. Remove the tubes from the boiling water bath and add 3.0 mL of reagent E with mixing on a vortex mixer.
9. Allow the tubes to stand for 10 min at room temperature and then mix the contents again.
10. Measure the absorbance of the reaction tubes against the enzyme blank at 520 nm. Concurrently, measure the absorbance of the xylose standards against the reagent blank.

d) Calculations:

Calculate the rate of hydrolysis as µmoles of xylose reducing-sugar equivalent released per minute. **One Unit** of *endo*-xylanase activity is defined as the amount of enzyme required to release one µmole of xylose reducing sugar equivalents per minute from the arabinoxylan substrate at pH of 4.5 or 6.0 (depending on the enzyme assayed) and at 40°C.

endo-Xylanase (U/mL of original solution)

= DA/time x F x 5 x 1/150 x Dilution

where:

ΔA/time	= Increase in absorbance at 520 nm/time of incubation
	= Factor to convert absorbance values to µmoles of xylose equivalents
	= µg of xylose / absorbance for µg of xylose
5	= Conversion from 0.2 mL as assayed to 1.0 mL
1/150	= Factor to convert from µgrams to µmoles
Dilution	= Dilution of the original enzyme preparation

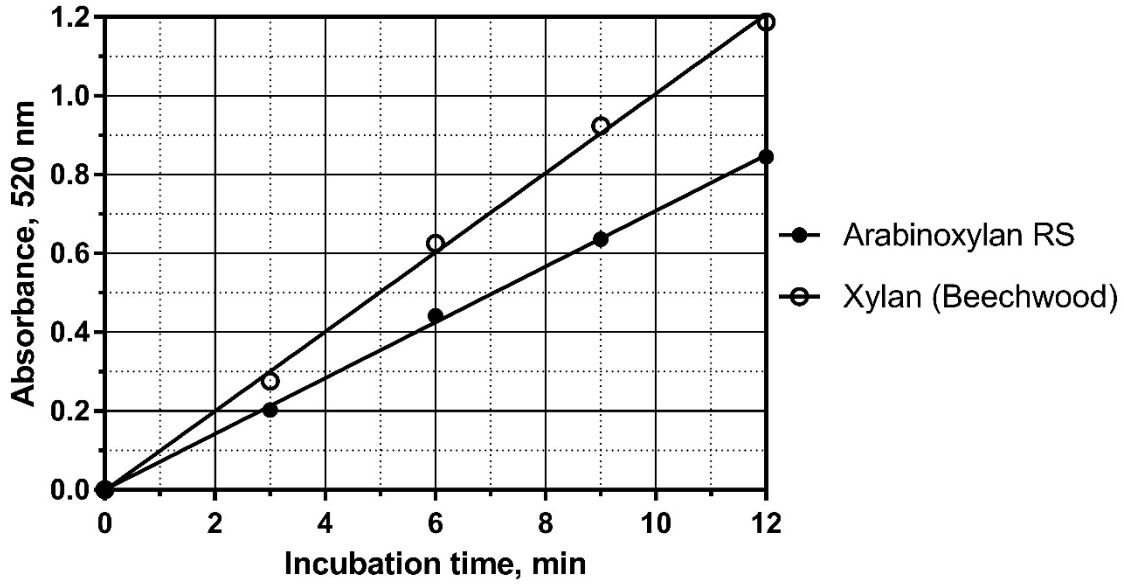


Figure 1. Relative rate of hydrolysis of Arabinoxylan (for reducing sugar assays) and Xylan (beechwood) by *A. niger endo*-xylanase determined using Nelson-Somogyi reducing sugar method.

NOTE:

Average absorbance values for Reagent/Enzymes blanks with Nelson-Somogyi reducing sugar assays are:

Arabinoxylan RS	~ 0.05
Xylan (Beechwood)	~ 0.16

B. DNSA REDUCING SUGAR METHOD

a) Preparation of reagents:

DNSA Reagent: Dissolve 10 g of 3,5-dinitrosalicylic acid, 2 g of phenol, 0.5 g of sodium sulphite and 10 g of sodium hydroxide in 800 mL of water. Adjust the volume to 1 L and store the solution in a sealed Duran bottle at room temperature (stable for > 2 months).

Rochelle's Salt solution: Dissolve 80 g of potassium sodium tartrate in 120 mL of water. Adjust the volume to 200 mL with water. Store the solution in a sealed Duran bottle at room temperature.

b) Preparation of substrate solution:

Add 1 g of wheat flour arabinoxylan (**P-WAXYRS**) to 90 mL of 100 mM sodium acetate buffer (pH 4.5) and dissolve by stirring at approx. 50°C for 10 min on a magnetic stirrer hot-plate. Adjust the volume to 100 mL with 100 mM sodium acetate buffer (pH 4.5) and store the solution in a well-sealed Duran bottle at room temperature. Alternatively, prepare substrate in 100 mM sodium phosphate buffer (pH 6.0) using the same procedure.

c) Assay procedure:

1. Add 1.8 mL aliquots of wheat arabinoxylan solution (1% w/v) [in 100 mM sodium acetate buffer (pH 4.5) or 100 mM sodium phosphate buffer (pH 6.0)] into a set of test tubes (16 x 120 mm) and pre-equilibrate these at 40°C for 5 min.
2. Initiate the reaction by adding 0.2 mL aliquots of suitably diluted and pre-equilibrated (at 40°C for 5 min) *endo*-xylanase preparation to the substrate solution with vigorous stirring on a vortex stirrer and incubate the tubes at 40°C.
3. Terminate the reaction after various time intervals (e.g. 1, 2, 3, 4 and 5 min) by adding 3 mL of DNSA reagent solution with vigorous stirring.
4. Prepare reagent blanks by adding 3 mL of DNSA reagent to 1.8 mL of substrate solution plus 0.2 mL of the buffer solution as used in the assay, and immediately mix the tube contents.
5. Prepare enzyme blanks by adding 3 mL of DNSA reagent to 1.8 mL of substrate solution plus 0.2 mL of the enzyme solution as used in the assay and immediately mix the tube contents.
6. Prepare the xylose standards by adding 3 mL of DNSA solution to 1.8 mL substrate solution plus 0.2 mL of xylose standard (0-300 µg/0.2 mL; 0-2 µmoles/0.2 mL).
7. Place all tubes (reaction, reagent blanks, enzyme blanks and xylose and standards) in a boiling water bath and incubate for 15 min.
8. Remove the tubes from the boiling water bath and immediately add 1 mL of Rochelle's salt solution and immediately mix the tube contents on a vortex mixer.
9. Allow the tubes to cool down for 15 min at room temperature and then mix the contents again.
10. Measure the absorbance of the xylose standards against the reagent blank at 540 nm. Concurrently, measure the absorbance of the reaction solutions against the enzyme blank at 540 nm.

d) Calculations:

Calculate the rate of hydrolysis as µmoles of xylose reducing-sugar equivalent released per minute.

One Unit of *endo*-xylanase activity is defined as the amount of enzyme required to release one µmole of xylose reducing sugar equivalents per minute from the arabinoxylan substrate at pH 4.5 (or pH 6.0) and 40°C.

endo-Xylanase (U/mL of original solution)

$$= \frac{\Delta A}{\text{time}} \times F \times 5 \times \frac{1}{150} \times \text{Dilution}$$

where:

ΔA/time	= Increase in absorbance at 540 nm/time of incubation
	= Factor to convert absorbance values to µmoles of xylose equivalents
	$= \frac{\text{µg of xylose}}{\text{absorbance for µg of xylose}}$
5	= Conversion from 0.2 mL as assayed to 1.0 mL
1/150	= Factor to convert from µgrams to µmoles
Dilution	= Dilution of the original enzyme preparation

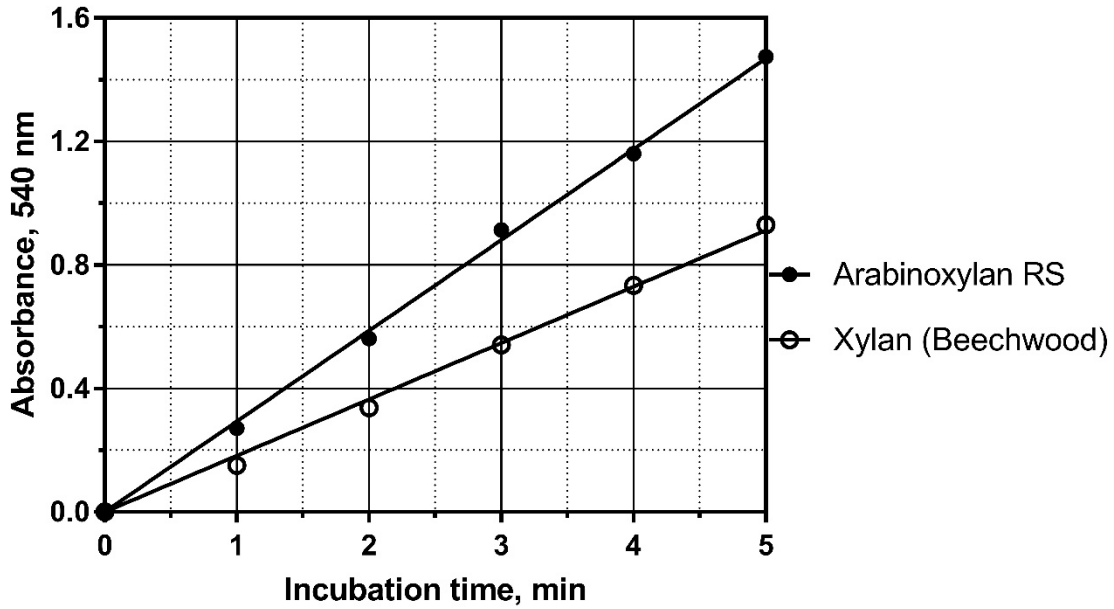


Figure 2. Relative rate of hydrolysis of Arabinoxylan (for reducing sugar assays) and Xylan (beechwood) by *A.niger endo*-xylanase determined using DNSA reducing sugar method.

NOTE:

Average absorbance values for Reagent/Enzymes blanks with DNSA reducing sugar assays are:

Arabinoxylan RS	~ 0.18
Xylan (Beechwood)	~ 0.18