COEI-1-ACTARA Determination of endo- □(1,5) arabinanase activity in pectolytic enzyme preparations

General specifications

These enzymes are usually present among other activities, within a complex enzymatic preparation. Unless otherwise stipulated, the specifications must comply with the resolution OIV/OENO 365/2009 concerning the general specifications for enzymatic preparations included in the International Oenological Codex.

1. Origin

Reference is made to paragraph 5 “Source of enzyme and fermentation environment” of the general monograph on Enzymatic preparation

The enzymatic preparations containing these activities are produced by directed fermentation of microorganisms such as *Aspergillus niger, Aspergillus Tubigensis, Aspergillus Awamori Trichoderma reesei, Penicillium funiculosum or* Arabinanases belong to the family glycohydrolases.

2. Scope / Applications

Reference is made to the International Code of Oenological Practices, OENO 11/2004; OEN 12/2004; OENO 13/2004; OENO 14/2004 and OENO 15/2004.

Arabinanases are useful for the maceration of the grapes, the clarification of musts and wines, the filterability of musts and wines since they are facilitating. the action of other enzyme activities hydrolysing the constituents of the cell wall of grape.

3. Principle

The substrate employed is Azurine-crosslinked debranched arabinan (AZCL-Arabinan). Highly purified arabinan from sugar-beet pulp is treated with □-L-arabinofuranosidase to remove 1,3- and 1,2- □ -linked arabinofuranosyl residues, leaving linear 1,5- □ -arabinan. This polysaccharide still contains a small percentage of galacturonic acid, galactose and rhamnose (6, 4 and 2 % respectively), but is resistant to attack by polygalacturonase and endo-1,4- □-D-galactanase. The polysaccharide is then dyed and crosslinked. Treatment of this substrate with a large excess of □-L-arabinofuranosidase results in a limited release of arabinose but no release of dye labelled fragments.

AZCL-Arabinan is a highly sensitive and very specific substrate for the assay of endo arabinanase, when you measure the supernatant after the reaction at 590 nm.

4. Apparatus

4.1. Glass test tubes (15 ml)
4.2. Water bath set 40 °C
4.3. Vortex tube mixer
4.4. Qualitative Filter circle, retented particle diameter : 11 µm (in liquid)
4.5. 1 cm light path cuvettes
4.6. Spectrophotometer set 590 nm
4.7. Chronometer
4.8. Pipet (500 µl, 10 ml)
4.9. pH meter
4.10. 15 ml glass test tubes
4.11. Metal rack for 15 ml test tubes
4.12. Funnel
4.13. 100 ml graduated flask

5. Reagents and products:

5.1. Arabinazyme Tablets (Megazyme, batch 60701 as an example)
5.2. Trizma base (CAS no. 77-86-1)
5.3. Glacial acetic acid (CAS No. 64-19-7)
5.4. Sodium hydroxid solution (CAS No. 1310-73-2)

6. Solutions

1. Dilution Buffer

- (Sodium Acetat buffer, 50 mM, pH 4.0)

2. Glacial acetic acid is added to 900 ml of distilled water. This solution is adjusted to pH 4,0 by the addition of 1 M sodium hydroxide solution. The volume was adjusted to 1 L with distilled water. 2 % Trizma Base Solution

- Dilute 2 g Trizma Base in 100 ml distilled water.

7. Preparation of the sample

7.1. Enzyme dilution

For most commercial pectinase enzyme preparations, a dilution of 500-fold is required. Place 200 mg of commercial preparation in a 100 ml graduated flask, make up with dilution buffer (6.1), and stir in order to obtain a homogeneous mixture.

8. Procedure

8.1. Enzymatic reaction

The test tubes are prepared at least in duplicate.

500 µl of diluted enzyme in dilution buffer (7.1) are pre-equilibrated to 40 °C for 5 min.

The reaction is initiated by the addition of an Arabinazyme tablet. Start the chronometer.

The tablet hydrates rapidly. The suspension should not be stirred.

After exactly 10 min at 40 °C the reaction is terminated by the addition of 10 ml Trizma Base solution (6.2) and stir.

After about 5 min standing at room temperature, the slurry is stirred again and filtered through a qualitative filter circle.

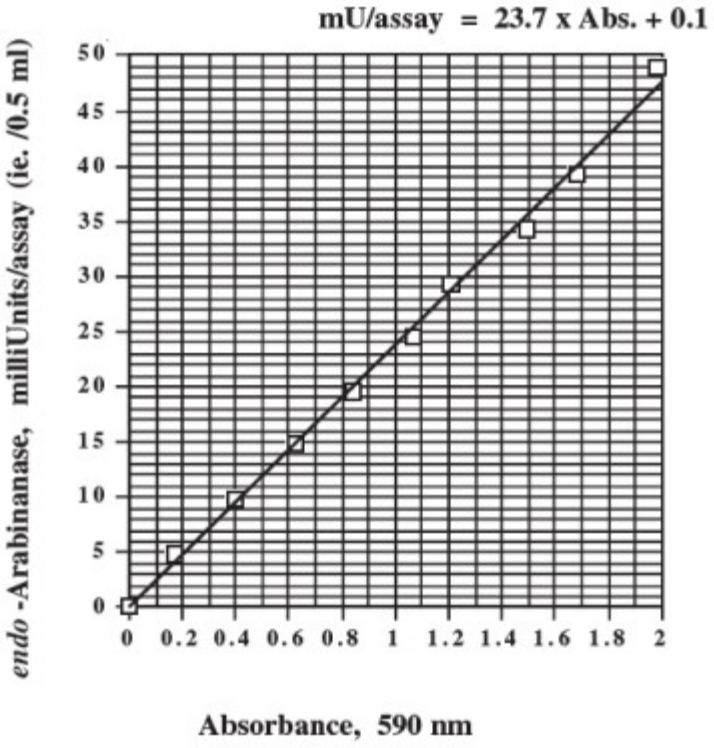
The absorbance of the reactions solutions are then measures at 590 nm against the reaction blank

8.2. Reaction blank

A reaction blank is prepared by adding 10 ml Trizma base solution (6.2) to 500 µl enzyme solution and stir before the addition of the Arabinazyme tablet.

9. Calculations

Endo-Arabinanase activity being assayed is determined by reference to the calibration curve of the test kit (i.e. Lot.No. 60701)



Where: $Y = MX + C * 2 * Fv/1000$ [U/g or ml]

- Y endo-arabinase activity (in milliUnits/assay)
- M slope of the calibration graph
- X absorbance of the reaction at 590 nm (minus the reaction blank, or read against the reaction blank)
- C intersection on the Y-axis (intercept point)
- 2 conversion from 0,5 ml enzyme dilution to 1 ml in the test
- F_V Dilution factor of the original enzyme preparation (i.e. 500-fold)
- 1000 conversion from milliUnits to Units

10. References

- http://secure.megazyme.com/downloads/en/data/T-ARZ200.pdf
- Dietrich H., Will F. (1998); Vom Phänomen der Trübung; Getränkeindustrie; 2; S. 80 – 88.