

# Catechol oxidase

## Introduction

Catechol oxidase is the enzyme responsible for the browning of fruit. It is easy to prepare from a number of different sources – bananas are particularly good – and the reaction is readily followed using a colorimeter.

## Background

Catechol oxidase has a number of alternative names, (Polyphenol oxidase, Diphenol oxidase, Tyrosinase, etc,) The reaction catalysed is the oxidation of catechol to the yellow product 1,2-benzoquinone. On exposure to air there is a further reaction in which the yellow benzoquinone is converted to dark brown melanin.

## **SAFETY**

The volumes and concentrations of catechol and its inhibitors, (if used), present minimal hazards. Good laboratory practices should be observed and the wearing of gloves is advised when preparing working solutions

## Suggestions for investigations

- Effect of pH on enzyme activity
- Effect of temperature on enzyme activity
- Effect of competitive and non-competitive inhibitors
- Enzyme kinetics

## Enzyme extraction

Catechol oxidase can be extracted from fruits that brown on exposure of their cut surfaces to air, potato and apple are commonly used. We have found banana to be easiest to use, and the extract retains its activity for weeks in the fridge.

To prepare the enzyme blend banana with two volumes of water or just squash the banana with a fork and crush it in a pestle and mortar with two volumes of water. 20g of banana with 40cm<sup>3</sup> of water will give plenty of extract for most purposes. Filter the extract through several layers of butter muslin and store refrigerated.

## Reaction mixture

For continuous reading put 0.1cm<sup>3</sup> of the enzyme extract in a cuvette and add 2.9cm<sup>3</sup> of a mixture containing 0.5cm<sup>3</sup> of catechol solution and 2.4cm<sup>3</sup> of buffer. The reaction can be performed at room temperature

The optimum pH is around 6.8 and catechol concentrations between 0.01M and 0.05M (final concentration in the cuvette) should give a good linear response when the concentration is plotted against the rate of reaction.

More details, suggestions for investigations and sample results can be viewed on the *Mystrica* website, [www.mystrica.com](http://www.mystrica.com)