Banana polyphenol oxidase

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Polyphenol oxidases catalyse the oxidation of a wide range of phenolic compounds to the

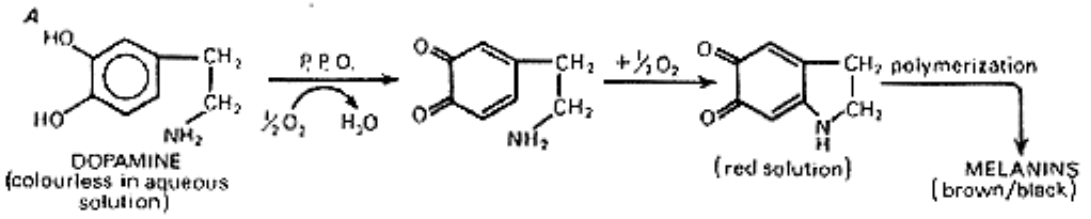
corresponding quinones, subsequent non-enzymatic reactions of which lead to the formation of melanin pigments. These events occur in the browning of damaged fruits and vegetables. Polyphenol oxidase (PPO) from potato has been suggested for use in school experiments [1, 2]. A highly active PPO can also be extracted from bananas. This enzyme has been used in tertiary level teaching [3], however it is easy to extract, comparatively stable and lends itself to a range of investigations which might be undertaken profitably by sixth formers.

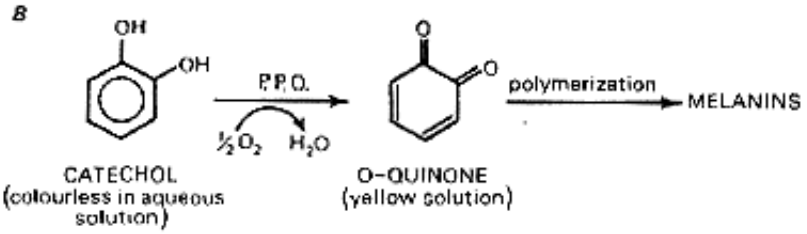
EXTRACTING THE ENZYME

8 g of banana flesh is homogenized in 30 cm^3 of deionized water in a liquidizer (or ground up using a mortar and pestle). The mixture is strained through muslin and centrifuged for 2 minutes to produce a clear supernatant. Although much more sophisticated extraction procedures have been described [4] the extract produced by the method above is highly active and retains its activity for at least 24 hours at room temperature and for several days if it is stored at 4 °C.

DEMONSTRATING PPO ACTIVITY

Suitable substrates are dopamine and catachol (benzene 1, 2-diol)*. In both cases the enzyme catalyses an initial oxidation to produce a coloured substance which is further oxidized by atmospheric oxygen through intermediate stages to melanins.





These reactions can be demonstrated by setting up the following mixtures.

For A	*For B*
1 cm^3 of enzyme extract	1 cm^3 of enzyme extract
1 cm^3 of 0.2 per cent dopamine hydrochloride solution	1 cm^3 of 2 per cent catechol solution
4 cm^3 of phosphate/citrate buffer (pH7)	4 cm^3 of phosphate/citrate buffer (pH7)

The substrates should be prepared just before use since they undergo autoxidation. At these concentrations the intermediate colours appear within a few minutes and the dense brown or black final stage is obvious within 10 minutes.

IS THE ENZYME INVOLVED AN OXIDASE?

Having first established that the production of the intermediate colour is enzymic (a reaction mixture containing boiled and cooled extract produces no colour change) the nature of the enzyme may be investigated using the Thunberg technique. Tubes set up as shown in Figure 1 are evacuated for 2 minutes and then the contents are mixed. The absence of any colour change shows that oxygen is necessary for the reaction to occur and hence that the enzyme involved is an oxidase.

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* Contact with this in *powder form* causes skin and eye irritation. It is also harmful by skin absorption. It is harmless when used in the concentrations suggested.

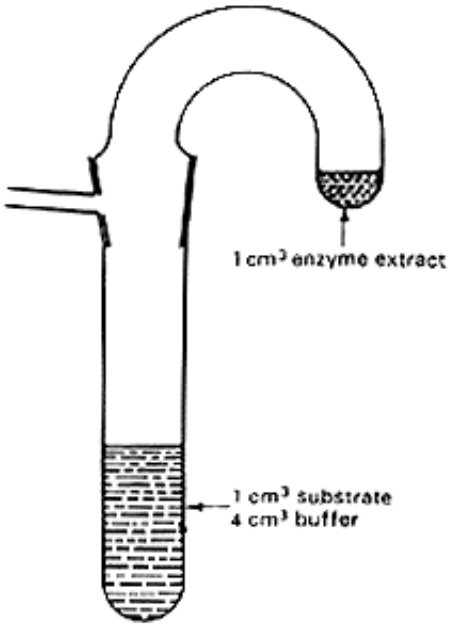

Figure 1

FOLLOWING THE COURSE OF THE REACTION USING A COLORIMETER

The enzyme-controlled stage of the reactions may be followed with both substrates. For class practicals however, there may be advantages in using catechol since it is much cheaper than dopamine and in the reaction mixtures suggested, the coloured intermediate (the product of the enzyme-controlled stage) is more slowly oxidized to melanin. The enzyme

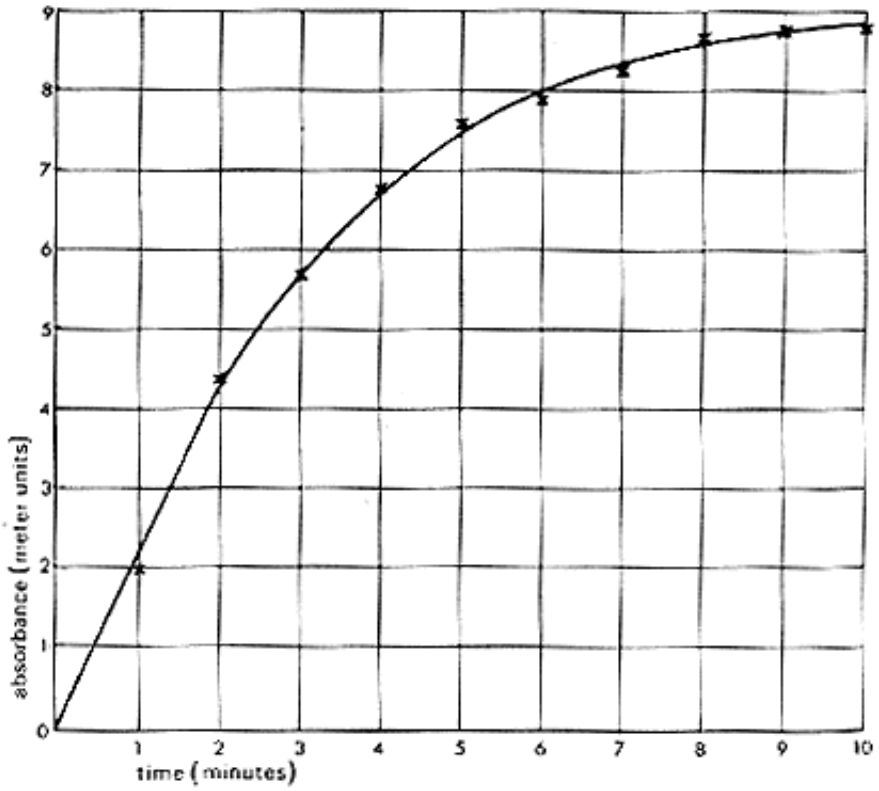

Figure 2

extract should be diluted for use. The concentration of enzyme used depends on the sensitivity of the particular colorimeter. For the instruments most frequently used in school an initial trial with a 1 in 10 dilution is suggested. A tube containing 1 cm^3 of extract (diluted 1:100), 1 cm^3 of 2 per cent catechol solution and 4 cm^3 of phosphate/citrate buffer pH7 placed in an EEL colorimeter fitted with a blue filter (Ilford 303) produced the results shown in Figure 2.

This activity curve could be used to generate a number of hypotheses about potential limiting factors. In fact the situation is complex because apart from substrate and oxygen limitation which occurs, the enzyme is also inhibited by the products of the reaction [5]. All these possibilities may be investigated by addition of more enzyme, substrate or oxygen to mixtures in which the reaction has ceased, which is shown by no increase in absorbance. Further work on oxygen concentration is well worth doing.

OXYGEN DEPLETION DURING THE REACTION

This is determined using an oxygen electrode and provides an alternative method to the colorimetric one for following the course of the reaction. It is unlikely that schools will possess enough oxygen electrodes and meters to run this as a class practical but the method seems attractive as the basis of an individual project. Some experimenting is necessary to find the volumes and concentrations of the reactants which are appropriate for different electrodes. 5 cm^3 of diluted extract (1:100), 10 cm^3 of 2 per cent catechol solution and 90 cm^3 of phosphate/citrate buffer (pH7) placed in a corked reagent bottle containing the electrode produced the results shown in Figure 3.

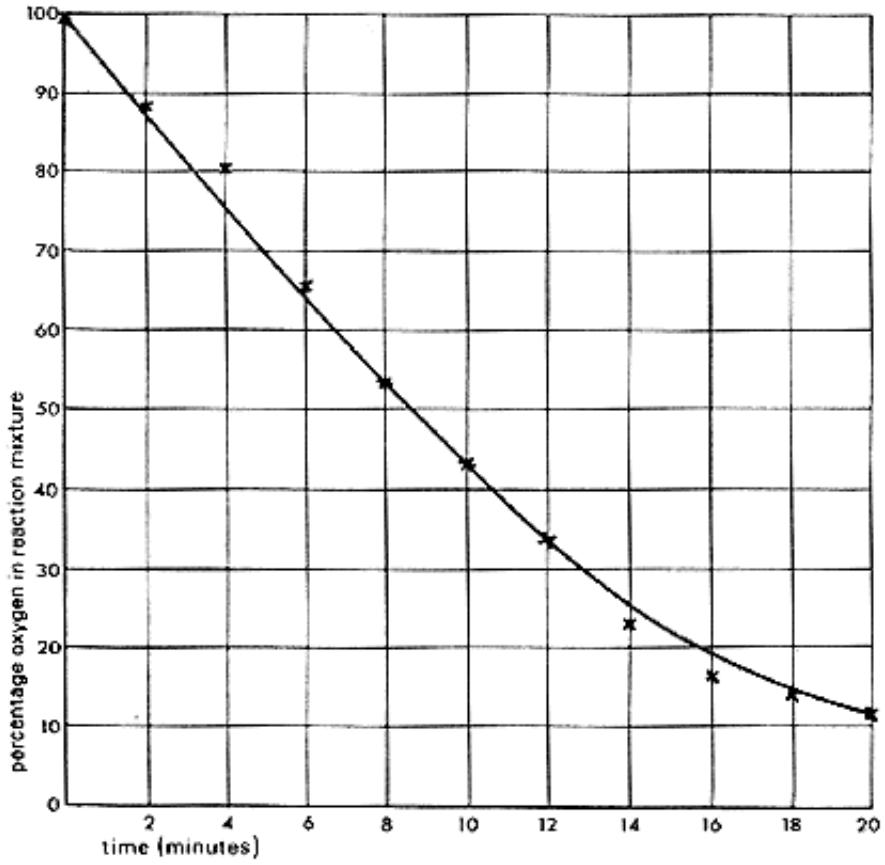

Figure 3

The rate of enzyme activity at different oxygen concentrations can also be investigated. Different volumes of 10 per cent sodium sulphite solution are added to the buffered substrate and the oxygen absorption is monitored until a constant value is obtained.

Different oxygen concentrations are then selected, e.g., 75, 50, 25 per cent of the original oxygen content.

The concentration of the substrate in each solution is held constant by adjusting the volume of buffer used. An appropriate volume of enzyme extract is added to samples of the buffered substrate and the reaction followed in the colorimeter. The colorimeter tubes should be corked to exclude atmospheric oxygen. Absorbance is plotted against time at the different oxygen concentrations.

FURTHER CHARACTERIZATION OF THE ENZYME

Experiments to determine the effect on the rate of reaction of varying substrate concentration, enzyme concentration, temperature and pH can all be performed with this material. In each case, results are obtained after several minutes, obviating the need for lengthy incubation. Specimen results are shown in Figure 4 where buffers ranging from pH4 to pH8 have been used in reaction mixtures containing 1 cm^3 of extract (diluted appropriately, here 1:100), 1 cm^3 of 2 per cent catechol solution and 4 cm^3 of buffer with the experiment run at room temperature.

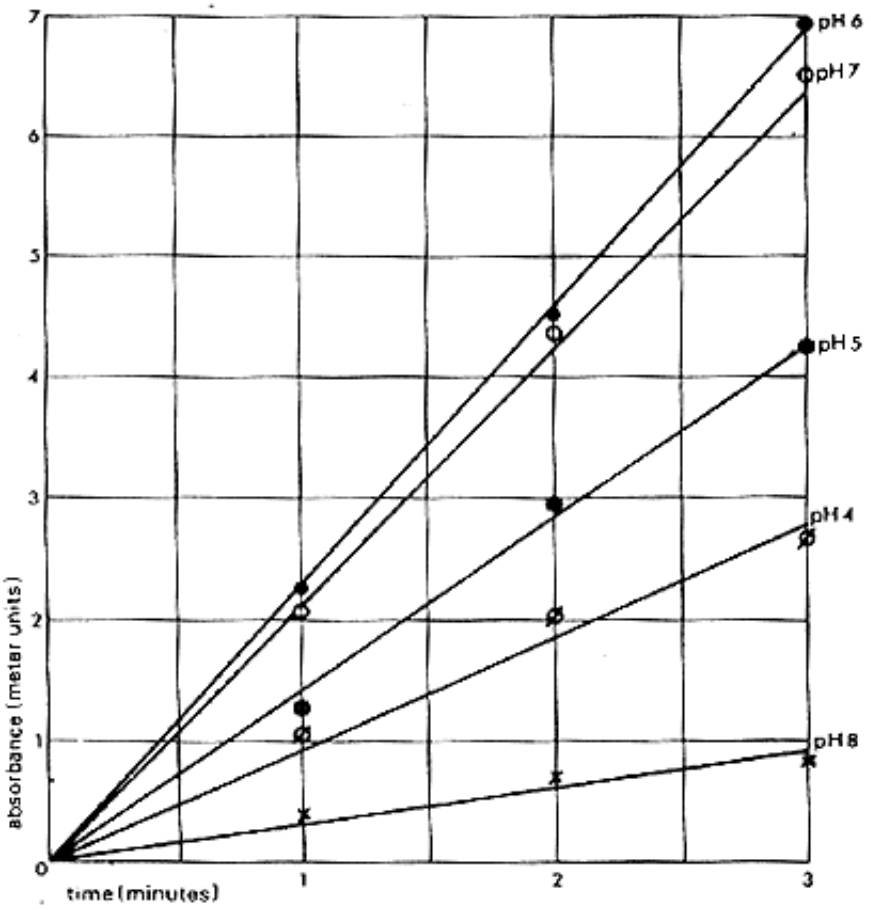

Figure 4

Since the increase in absorbance is linear over the first few minutes, the gradients can be plotted against pH to produce the usual pH/activity curve (Figure 5).

ADDITIONAL WORK

The importance of preventing browning in the commercial production of fruits and vegetables could be pursued and further work on enzyme inhibition attempted [6]. Studies on enzyme specificity could be undertaken by using phenol and a triphenolic substance

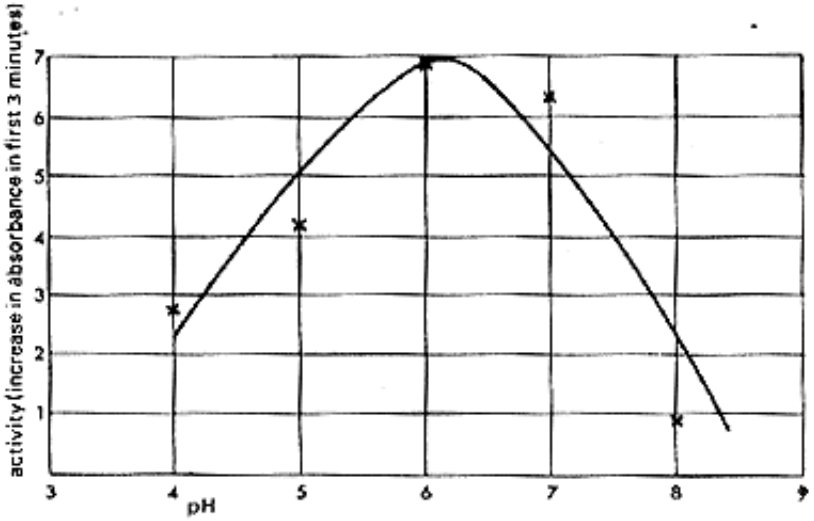

Figure 5. The effect of pH on enzyme activity

such as pyrogallol as alternative substrates. The degree of variation in PPO activity in bananas of different ripeness and countries of origin ('small' bananas versus 'large' ones) could also be examined.

ACKNOWLEDGEMENTS

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