# THE POLYPHENOLOXIDASE OF PEAR FRUIT*

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Following a study of the enzymic browning of apples (Walker 1964) a similar study of pear polyphenoloxidase (PPO) was initiated.

A soluble PPO preparation was prepared by disintegrating 100 g pear flesh (cv. Winter Cole) in 200 ml ice-cold 0·2M $Na_2CO_3$ containing 0·001M cysteine hydrochloride. The filtrate from this homogenate was treated with 2 volumes of cold (−18°C) acetone and the resultant precipitate resuspended in 20 ml water. Unwanted pectic material was removed by precipitation with 10 ml cold acetone, then addition of a further 40 ml of cold acetone yielded an enzymically active precipitate. This

Table 1

RELATIVE RATES OF OXIDATION OF PHENOLIC SUBSTRATES BY PEAR POLYPHENOLOXIDASE

Warburg flasks contained 10 μmoles substrate, 0·2 ml enzyme, 0·5 ml 1% gelatin, 0·1M phosphate–citrate buffer (pH 5·0) in 3 ml solution at 30°C; the gas phase was air

| Substrate | Rate of Oxygen Absorption (μl/min) | Relative Rates of Oxidation | |
|---|---|---|---|
| | | Pear Enzyme | Apple Enzyme |
| Chlorogenic acid | 23·3 | 100 | 100 |
| Catechol | 20·0 | 86 | 60 |
| D-Catechin | 14·0 | 60 | 60 |
| Caffeic acid | 10·0 | 43 | 50 |
| L-3-(3,4-dihydroxyphenyl)-alanine | 3·9 | 17 | 18 |
| 3,4-Dihydroxybenzoic acid | 1·4 | 6 | 18 |
| *p*-Cresol | 1·1 | 5 | 13 |

final precipitate was dissolved in 10 ml water and the golden-yellow solution applied to a column (2 by 15 cm) of Sephadex G-100 previously equilibrated with 0·01M $Na_2CO_3$ containing 0·001M cysteine hydrochloride. Elution of this column with the same carbonate–cysteine solution resolved the original extract into two fractions designated C and Y. Fraction C, which lacked colour, was not adsorbed and contained all the original PPO activity, whereas fraction Y was inactive and had a golden-yellow colour but became colourless when its pH was adjusted to 5·0.

Fraction C was adjusted to pH 5·0 by the addition of solid $KH_2PO_4$ and its ability to oxidize a variety of phenolic substrates was tested by conventional Warburg techniques. The relative rates of oxygen absorption, listed in Table 1, were similar to those previously obtained for apple PPO (Walker 1964). Likewise cysteine,

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glutathione, and other thiol compounds were found to inhibit colour production from phenolics by pear PPO and to lower the final oxygen absorption only when the thiol/phenolic ratio exceeded unity.

An alternative approach, similar to that used by Siegelman (1955), was also used to study the relative contributions to browning of the various phenolic constituents of pear fruit. Fruit tissue was disintegrated in 80% (v/v) ethanol to extract phenolic compounds and the filtrate concentrated *in vacuo*. This concentrated extract was applied to paper chromatograms which were subsequently developed with n-butanol–acetic acid–water (4 : 1 : 2·2 v/v) or with 5% (v/v) acetic acid. When the dried chromatograms were sprayed with PPO (fraction C) brown spots slowly appeared in the positions corresponding to chlorogenic acid. It was therefore concluded that this compound was the main substrate involved in the enzymic browning of pears.

Pear PPO was soluble at pH levels above 5·0 whereas apple PPO was soluble only at pH levels above 9·0, but apart from this important difference the PPO of these two pome fruits were closely similar, particularly in substrate specificity.

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