# Characterisation of Apricot Polyphenoloxidase During Fruit Development

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*"It seems to me that all sciences are vain and full of error that are not born of experience, mother of all certainty, and are not tested by experience, that is to say, that do not at the origin, middle or end pass through any of the five senses."*

Leonardo da Vinci 1452-1519

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# Abstract

This study was aimed at determining the expression and activity of polyphenoloxidase (PPO) during apricot fruit development together with the biochemical characteristics of the enzyme extract at different development stages. Biochemical factors considered include substrate, pH, NaCl level, inhibitor type, high temperature inactivation and sulphur dioxide level.

Changes in apricot (Prunus armeniaca L., cv. 'Moorpark') polyphenoloxidase (PPO) were measured during fruit development from a few days after full bloom until over-ripe at 92 days after full bloom. Cold ground samples in McIllvaine's buffer were analysed for PPO activity over a range of pH (5.0, 6.0, 6.8 and 7.2); for response to intact fruit sample pre-heating (25, 35, 45, 55 and 65 $^{o}$C); for sulphite and NaCl inhibition (0.2, 0.5, 2 and 5mM) and other inhibitors (SHAM 0.2mM, cinnamic acid 2.5mM and tropolone 0.5mM). PPO activity was measured at 25$^{o}$C using a Clark-type oxygen electrode with 4-methyl catechol (20mM) as substrate.

As fruit ripened PPO activity increased under all conditions tested. The increase in activity was not even with fruit development. Three common peaks of PPO activity occurred at ages 22-29 days, 57 days and for fully-ripe fruit at 85-92 days.

Optimum pH was found to be 6.8 with a wide range for all ages of fruit. PPO activity tended to be higher for more mature fruit at a higher pH of 7.2 to 8.0, whereas activity tended to be higher in less developed fruit at the lower pH of 6.0.

Catechol and chlorogenic acid showed reduced PPO activity compared with 4-methyl catechol over all development ages, however, there was a different pattern of response. Both catechol and chlorogenic acid showed greater PPO activity in the fully mature, day 92 fruit and less in the very young day 8 fruit, relative to the control 4-methyl catechol substrate. L-DOPA, as a substrate, showed a reaction lag as previously reported, and quite depressed PPO activity with no particular variation with development age compared to the control.

Pre-heating of fruit samples in air for 30 minutes resulted in increased inactivation with holding temperature (35$^{o}$C - 31%, 45$^{o}$C - 82%, 55$^{o}$C - 97%, 65$^{o}$C - 99%). Sulphite and NaCl acted as inhibitors with increasing effect as concentration increased. Added sulphite depressed PPO activity by about 30% at the level (2mM) used. This was less than the literature would suggest and it appeared that fully-ripe fruit were less inhibited than mature, non-ripe fruit. NaCl has a greater inhibitory effect on apricot PPO activity at the lower pH 5.0 tested. As NaCl added increases PPO activity decreases after an initial small rise. Again, less sensitivity to NaCl inhibition is shown by fruit of greater development age. Sensitivity to inhibition by SHAM, cinnamic acid and tropolone decreased with development age. Tropolone was the most effective inhibitor of apricot PPO.

The pattern of change in PPO activity, was consistent with physiological and biochemical changes reported by other workers as fruit develop from hard, green to soft, ripe. Regarding the existence of different PPO isozymes during development, no evidence of a isozyme based differential response with age was found within the constraints of the parameters tested.

# Declaration

This thesis contains no material which has been accepted for an award of any degree or diploma in any University and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

I give my consent to this copy of my thesis, when deposited in the University library, being available for loan and photocopying.

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R. B. Barrett, December, 2002

I, Andreas Klieber, consider that this thesis is, prima facia, worth examining for the award of Master of Applied Science (Agriculture).

Dr. A. Klieber ..... Dated .....

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# Abbreviations

| | |
|---|---|
| ABA | Abscisic acid |
| $A_w$ | Available water |
| bp | base pairs |
| $^{o}$Brix | degrees Brix (a refractive index measure of g solute/100 g solution) |
| $^{o}$C | degrees Celsius |
| CA | controlled atmosphere |
| ca. | circa (around a given date) |
| cDNA | complementary DNA to an RNA |
| conc. | concentration |
| Cu | copper |
| cv | cultivar |
| $C0_2$ | carbon dioxide |
| DNA | deoxyribonucleic acid |
| e.g. | exempli gratia (for example) |
| *et al.* | Et alii (and others) |
| g | gram |
| GC | gas chromatograph |
| $H_2S$ | Hydrogen sulphide |
| HPLC | high performance liquid chromatography |
| hr. | hour |
| k | first-order reaction constant for enzyme activity |
| KCl | Potassium chloride |

| | |
|---|---|
| kg | kilogram |
| kPa | kilo Pascal |
| $K_m$ | equilibrium constant for enzyme activity |
| L | litre |
| L. | Linnaeus |
| µg | microgram |
| mg | milligram |
| min. | minute |
| µl | microlitre |
| µm | micromole |
| ml | millilitre |
| M | molar |
| mM | Millimolar |
| mRNA | messenger RNA |
| M wt. | molecular weight |
| $N_2$ | nitrogen |
| NaCl | sodium chloride |
| nm | nanometre |
| NMR | nuclear magnetic resonance |
| $O_2$ | oxygen |
| OD | optical density |
| PAGE | polyacrylamide gel electrophoresis |
| PCR | polymerase chain reaction |
| pers. comm. | personal communication |

| | |
|---|---|
| pH | negative log of hydrogen ion concentration |
| Pi | isoelectric point of enzyme or protein |
| PPO | polyphenoloxidase |
| ppm | part per million |
| PVP | polyvinylpyrolidone |
| RH | relative humidity |
| RNA | ribonucleic acid |
| s | seconds |
| SDS | sodium dodecyl sulphate |
| SHAM | salicylhydroxamic acid |
| sp. | Species |
| spp. | a number of species |
| syn | synonym |
| T | temperature |
| TA | Titratable acidity |
| TCA | trichloroacetic acid |
| Tween | polyoxyethylene-sorbitan monolaurate detergent |
| UV | ultraviolet (UV-vis for untraviolet and visible wavelengths) |
| vol | volume |
| $V_{max}$ | maximum velocity of enzyme-catalysed reaction (substrate saturated) |
| V/V | volume/volume |
| WWW | world wide web |

# Tables & Figures

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