This Week's Citation Classic

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Anderson J W. Extraction of enzymes and subcellular organelles from plant tissues. *Phytochemistry* **7**:1973-88, 1968. [Botany Department, University College London, England]

The extraction of enzymes and organelles from plants is confounded by phenolic oxidation products formed during extraction. The theory and methods for countering inactivation of enzymes and organelles by phenol oxidation products during extraction are reviewed. [The *SCI*® indicates that this paper has been cited over 110 times since 1968.]

John W. Anderson
Department of Botany
La Trobe University
Bundoora, Victoria 3083
Australia

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"The harsh extracting methods required to break the tough cell walls of plants for the preparation of cell-free extracts liberates O_2:phenoloxidases and phenolic substrates into the extracting medium. This results in the formation of phenolic oxidation products (POP) causing browning of extracts. When I was a PhD student at the University of Melbourne (1962-5) my supervisor, Kingsley Rowan, whom I respect so much, impressed on me that POP decreased the recovery of active enzymes by protein precipitation and/or enzyme inactivation. However, little was generally known about countering this problem.

"My PhD involved a study of some enzymes in senescing tobacco leaf. My initial extracts were brown and essentially inactive, but extracts prepared in media containing thiols and certain reducing agents were considerably more active and essentially colourless; the higher activity was attributable to decreased production of POP.[1] In 1966 when I moved to University College London (UCL), several authors reported in the fine print of their papers that thiols and copper complexing agents made the difference between success and failure in detecting several enzymes. After finding similar observations in some much older reports I was encouraged by Leslie Fowden and the itinerant family of scholars always associated with his laboratory at UCL to draw attention to these and other developments. The review which ensued details the experiences of many authors in countering the deleterious effects of POP on the extraction of enzymes and organelles from plants. Some of the examples date back to 1947. It seems that our tardiness in applying these countermeasures is because they didn't form the main theme of the papers in which they were reported, a matter which presents obvious problems when reviewing the subject. After all, would the method of Lowry, *et al.*[2] for estimating protein be so widely used today if the procedure had been tucked away in the methods section of a paper entitled 'Succinate dehydrogenase of *Thiobacillus'* and published in a bacteriological journal? "A more recent account of the POP problem is that of Van Sumere, *et al*.[3] Nowadays dithiothreitol is commonly employed, but it is sobering to reflect that keepers of fish and chip shops have long had the problem beaten; they treat their freshly cut potatoes with a reducing agent to prevent browning! "

1. **Anderson J W & Rowan K S.** Extraction of soluble leaf enzymes with thiols and other reducing agents. *Phytochemistry* **6**:1047-56, 1967.
2. **Lowry O H, Rosebrough N J, Farr A L. & Randall R J.** Protein measurement with the I olin phenol reagent. *J. Biol. Chem.* **193**:265-75, 1951.
3. **Van Sumere C F, Albrecht J, Dcdoncr A, De Pooter H & Pé I.** Plant proteins and phenolics. (Harborne .J B & Van Sumere C F, eds.) *The chemistry and biochemistry of plant proteins.* London: Academic Press, 1975. p. 211-64.