

POINTS OF CAUTION IN STUDYING HEAT INACTIVATION OF ENZYMES, EXEMPLIFIED BY THE POLYPHENOLOXIDASE FROM THE DEGLET-NOUR DATE (*PHOENIX DACTYLIFERA*).

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A better knowledge of the thermolability of the date polyphenoloxidase activity, involved in the browning process, is required for concerns about the fruit quality after processing. Enzyme heat stability is often reported as "optimal temperature". This optimum is subject to changes with enzyme assay conditions and it obliterates the duration of the heat-treatment. Another usual way is to measure the residual activity for a given time of treatment at different temperatures. A better approach is to study the deactivation kinetics at different temperatures, leading to a collection of rate constants-their variation with temperature allows the determination of the activation energy. In this kinetic approach, rate constants are often derived from a logarithmic graphical procedure rather than through direct curvilinear regression. Our results show that false conclusions may be drawn from inadequate data processing. Polyphenoloxidase activity of *Deglet- Nour* date extracts is decreased after heat treatment in the 25 - 70 °C range. When kinetic studies are undertaken (initial velocity evaluating enzyme activity) at different temperatures, the linear regression after logarithmic transformation of data erroneously leads to the appearance of a simple "two steps-one state" first order process. In non-linear regression analysis, perfect fits were obtained with a simple 3-parameters-biexponential expression, shown as a common kinetic equation for consecutive reactions, competitive reactions and heterogeneous systems with iso-enzymes. The apparent first-order rate constants collected at different temperatures were analyzed using the Arrhenius equation. The low values of apparent activation energies signed a high sensitivity to temperature (polyphenoloxidases are recognized as quite thermolabile enzymes) and indicated that the loss of activity results from a complex mechanism, rather than from two concomitant simple reactions as in the situation with isoenzymes.