# THERMAL INACTIVATION KINETICS OF POLYPHENOLOXIDASE EXTRACTED FROM WHITE GRAPES

GABRIELA RÂPEANU, MIRCEA BULANCEA

*"Dunarea de Jos", University of Galati, Romania, Faculty of Food Science and Engineering, 111 Domnească Street, Galaţi 800201, Romania, e-mail* Gabriela.Rapeanu@ugal.ro

**Abstract:** Polyphenol oxidase (PPO) was isolated from Victoria grapes (*Vitis Vinifera ssp. Sativa*) grown in South Africa and its activity, thermal stability and thermal inactivation were studied on a kinetic basis. Kinetic studies showed that the thermal inactivation of Victoria grape PPO followed first-order kinetics in the temperatures range 55-65$^{o}$C, with a $z_T$ =9.66 ± 0.63 $^{o}$C and an activation energy of $E_a$ = 225.43 ± 13.47 kJ/mol.

**Keywords:** polyphenoloxidase, enzymatic browning, enzyme stability, thermal inactivation kinetics

## INTRODUCTION

Enzymatic browning of damaged fruits and vegetables is a major problem in the food industry and is believed to be the one of the main causes of quality deterioration during post-harvest handling, storage and processing.
Because of this decrease in market value and the concomitant economic losses, control of enzymatic browning is very important to food manufacturing industries.
Polyphenoloxidase (PPO) is a copper containing oxidoreductase which catalyzes two distinct reactions involving phenolic compounds and molecular oxygen, namely a) the o-hydroxylation of monophenols to o-diphenols, or cresolase activity (monophenol, mono-oxigenase, EC 1.14.18.1); and b) the subsequent oxidation of o-diphenols to o-quinones, or catecholase activity (diphenol oxygen oxidoreductase, EC 1.10.3.1). These quinones are highly reactive, electrophilic molecules that covalently modify one cross-link to a variety of cellular constituents.
The main step in enzymatic browning is the oxidation of phenolic compounds to corresponding quinones intermediates that polymerize to form undesirable pigments, by PPO in the presence of oxygen (Weemaes *et al*., 1998). Browning reactions in grape products such as fresh fruits, juices and

wines during processing and storage are well known and are an economic problem for producers and consumers.
The aim of this work was to investigate the grape polyphenoloxidase thermal inactivation on a kinetic basis.

## MATERIALS AND METHODS

### Enzyme Sources

White grapes (Victoria variety, South of Africa) were purchased at commercial maturity from a local store. Catechol, insoluble PVP were obtained from Sigma Aldrich (USA). Triton X-100 was purchased from Fluka Chemicals Co. (Switzerland). All others chemicals were of analytical grade.

### Extraction and partial purification procedure

Enzyme extraction from grapes was carried out using a modification of the method of Valero *et al*. 1988. Grapes (250 g) were suspended in 125 mL McIlvaine buffer (pH = 5.0) and mixed for 15 s in a Waring commercial blender (New Hartford, U.S.A.). The resulting homogenate was filtered through four layers of cheese cloth, the filtrate centrifuged for 15 min (4 °C; 4.000 x *g*) in a Beckman J2-HS centrifuge. The resulting precipitate was extracted for 1 hour/4°C with 1.5% Triton-X-100 and 2.0% insoluble PVP in 100 mL McIlvaine buffer (pH = 5) and centrifuged for 1 hour (4°C; 15.000 x g). The resulting supernatant was subjected to ammonium sulphate (UCB) precipitation. The fraction precipitating between 30 and 90% saturation was redissolved in the same buffer and dialysed for 24 hours (cellulose membrane, Medicell International Ltd., 6-27/32) to remove excess ammonium sulphate ions. The dialyzed samples were kept in tubes at -80 °C and were used as the PPO enzyme source in further experiments.

### PPO Activity Assay

PPO activity was assayed by a spectrophotometric procedure. The increase of absorbance at 400 nm at 25°C was recorded automatically for 30 min (Ultrospec 2100 pro, UV-visible spectrophotometer). The sample cuvette

contained 1.0 ml substrate catechol 10 mM in McIlvaine buffer (pH = 5.0) and 100 µL undiluted enzyme extract.

The blank sample contained only 1.0 ml substrate solution in McIlvaine buffer (pH = 5.0). Enzyme activity was calculated from the linear portion of the curve, which was $OD_{400}$ nm/min due to the oxidation of substrate. All activity analyses were done in triplicate and the relative standard deviations were less than ± 1%.

**Isothermal treatment**

Thermal stability of grape PPO extract was investigated at pH 5.0 at various constant temperatures from 25°C to 90°C using an incubation time of 10 minutes, whereas detailed thermal inactivation kinetics of Victoria grape PPO extract at pH 5 was determined in a temperature range from 55°C to 65°C.

The samples were filled in capillaries 200 µL (Blaubrand, Germany), thermally treated, cooled in ice water and the residual activity was measured within 60 min of storage at 0°C.

**Kinetic data analysis**

In general for the thermal inactivation as well as for the denaturation and formation, the reaction rate *v* can be described by equation (1):

$$v = \frac{dA}{dt} = -k_{obs} A^n \quad (1)$$

where A is the activity of the compound at treatment time t , $k_{obs}$ is the reaction rate constant at the temperature studied and n the reaction order. For constant extrinsic/intrinsic factors, in case of first order reaction, the kinetics can be described by equation (2) or (3):

$$\log A = \log A_0 - \frac{t}{D} \quad (2) \text{ or } \ln A = \ln A_0 - k \cdot t \quad (3)$$

where $A_0$ is the initial activity or concentration, A the activity at time t, and D the decimal reduction time needed to reduce the initial activity by one log unit at a constant temperature.

At each temperature, the decimal reduction time D and the rate constant k were estimated using linear regression analysis on respectively equation (2) and (3). The temperature dependence of the D-value is characterized by the

$z_T$ value, which is the temperature increase necessary to induce a 10-fold reduction in D.

$$\log D = \log D_0 - \frac{T - T_0}{z_T} \quad (4)$$

The $z_T$ value was estimated using linear regression analysis on equation (5). The temperature dependence of the inactivation rate constants can be estimated using the Arrhenius model (5)

$$\ln(k) = \ln(k_0) + \left[ \frac{E_a}{R} \cdot \left( \frac{1}{T_0} - \frac{1}{T} \right) \right] \quad (5)$$

Where T and $T_0$ are the absolute and the reference temperature (K), $k_0$ is the rate constant at $T_0$, $E_a$ is the activation energy ($kJmol^{-1}$) and R is the universal gas constant (8.314 $Jmol^{-1}K^{-1}$). The activation energy was estimated using linear regression analysis on equation (5).

## RESULTS AND DISCUSSION

### Thermal stability of PPO

Thermal grape PPO inactivation was screened at different temperatures in the range between 25°C and 90°C for 10 min (Figure 1).

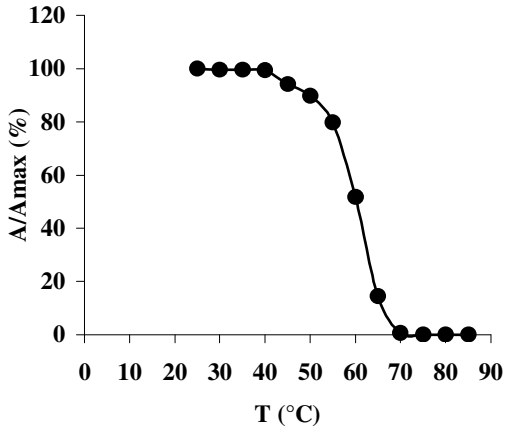


Figure 1. Thermal stability of grape PPO extract

Ten minutes heating at 60°C reduces the PPO activity in Victoria grape extract by 50%. The enzyme is almost completely inactivated at 70°C after 10 min of thermal treatment.

(Lamikanra *et al,*. 1992) reported a loss about 70% of PPO activity for Welder and Noble grapes at 60°C for 30 min, while Wissemann *et al.* 1981 found a 50% reduction of PPO activity after 15 min at 68.1°C for Ravat and 76.1°C for Niagara grapes.

**Thermal inactivation kinetics**

Detailed inactivation kinetics of Victoria grape PPO was studied at temperatures between 55 and 65°C. Linear curves were obtained when Victoria grape PPO activity retention was plotted versus time on a loglinear scale (Figure 2).

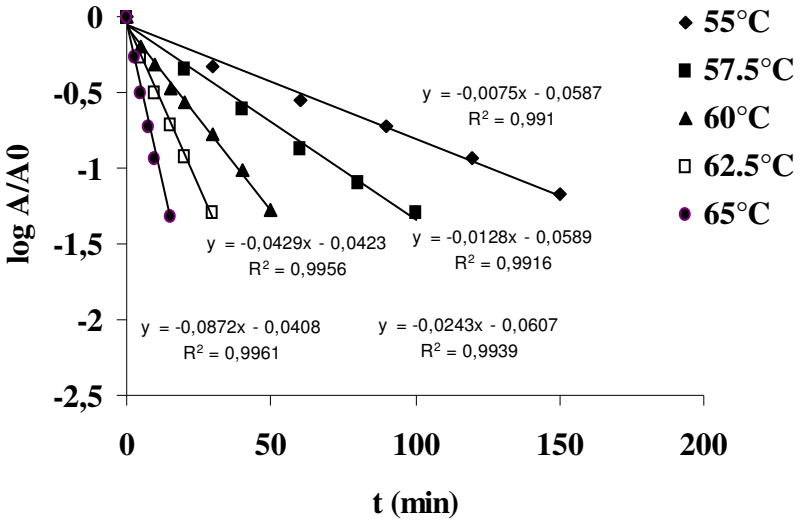


Figure 2. Thermal inactivation of grape PPO extract

From the loglinear plots of residual Victoria grape PPO activity versus inactivation time at constant temperature it can be concluded that the thermal inactivation of Victoria grape PPO can be adequately described by a first order reaction.

D-values and rate constants for thermal inactivation of Victoria grape PPO extract were calculated using equation (1) and are presented in Table 1. As expected the decimal reduction time decreases with temperature increase. At $65^0$C the D-value is almost 12 min.

**Table 1**. D, k, $z_T$ and Ea values of thermal inactivation of Victoria grape PPO enzyme extract

| T ($^0$C) | D-values (min) | k ($min^{-1}$) |
|---|---|---|
| 55 | 133.33 ± 2.66[a] | 0.0172 ± 0.0021 |
| 57.5 | 78.13 ± 3.72 | 0.0294± 0.0350 |
| 60 | 41.16 ± 0.61 | 0.0559 ± 0.0125 |
| 62.5 | 23.31 ± 0.39 | 0.0987 ± 0.0176 |
| 65 | 11.47 ± 0.19 | 0.200 ± 0.0239 |
| | $z_T$ (°C) | Ea (kJ/mol) |
| | 9.66 ± 0.63 | 225.43 ± 13.47 |

[a] standard error of regression

**Determination of $z_T$ and $E_a$**

In Figure 3 is presented the relation between decimal reduction time and temperature, where the slope of the curve represents $-1/z_T$. The estimated value was $z_T = 9.66 \pm 0.63$.

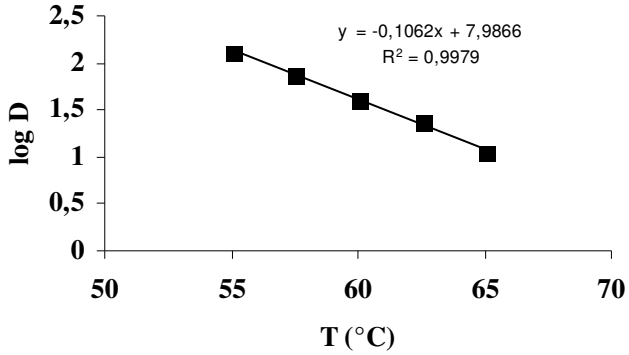


Figure 3. Temperature dependence of decimal reduction time

The temperature dependence of rate constants for thermal inactivation of PPO in the grape extract is depicted in Figure 4.

Figure 4 shows no obvious deviation from linearity ($R^2$ = 0.9972), with an activation energy of 225.43 kJ/mol. This value is in the same range (219-276 kJ/mol) found for DeChaunac and Ravat 51 grape PPO (Lee *et al.* 1983, Wisemann *et al.* 1981), but higher than the one found by (Wisemann *et al.* 1981) for Niagara grape PPO. The larger value of ($E_a$) indicates a greater influence of temperature on thermal inactivation rate (Weemaes *et al.* 1998).

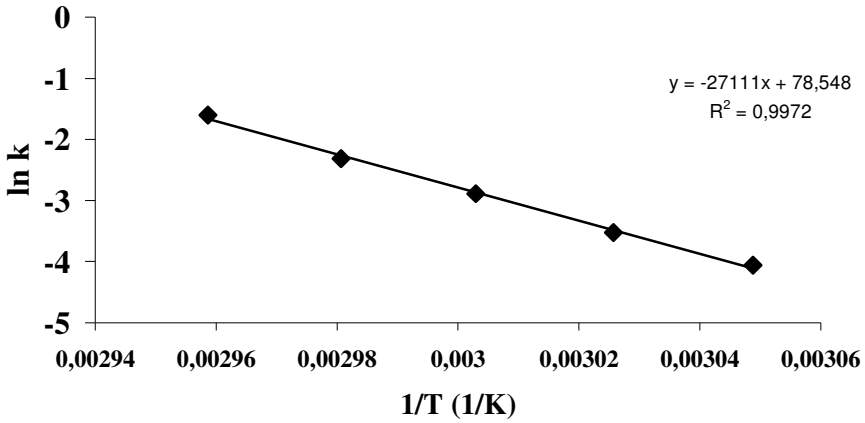


Figure 4. Temperature dependence of rate constants for thermal inactivation of PPO

## CONCLUSIONS

Polyphenoloxidase was extracted from white grapes and partially purified by using ammonium sulphate precipitation.
The thermal inactivation of grape PPO can be adequately described by a first order model in the temperature range from 55°C to 65°C with a $z_T$ =9.66 ± 0.63 $^oC$ and an activation energy of $E_a$ = 225.43 ± 13.47 kJ/mol.

## ACKNOWLEDGMENT

The authors wish to thank the European Commission (QLK1-CT-2000-60014) and the Fund for Scientific Research – Flandres (FWO) for their financial support.

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