INACTIVATION OF PPO BY PULSED LIGHT

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INTRODUCTION

Pulsed light (PL) technology is a non-thermal method of food processing based in the application of one or more pulses of high-intensity wide-spectrum light encompassing from UV to infrared (Gómez-López et al., 2007). Polyphenol oxidase (PPO, E.C. 1.14.18.1) is one of the most important enzymes in food technology. It catalyzes the oxidation of phenols to quinones, which undergo a series of reactions leading to browning (Pellicer and Gómez-López, 2017).

OBJECTIVES

In the present work, it was studied the emissive properties of commercial PPO, focusing on structural changes promoted by PL treatment. To this, intrinsic fluorescence, the parameter A, the phase diagram method, fluorescence quenching experiments using iodide and absorption spectra were the analytical tools used.

MATERIALS AND METHODS

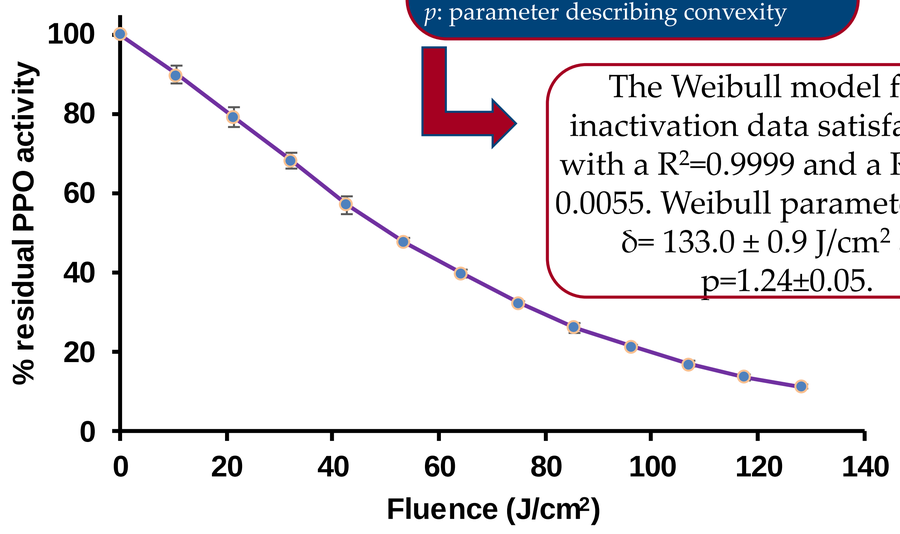
- A Petri dish without cover with 20 mL of PPO solution was placed on the stirrer below the centre of the lamp. PPO activity was measured at 400 nm in a standard reaction mixture containing 100 mM sodium phosphate buffer (pH 6.5), enzyme sample and 2.5 mM of 4-tert-butyl catechol.
- Intrinsic Trp fluorescence was used to characterize potential changes in the tertiary structure of the enzymes.
- Phase diagrams were constructed using intrinsic fluorescence spectra data to probe if the structural transformation of the PPO from native to inactive form follows an all-or-none or a multi-step process.
- Fluorescence changes were also characterized in terms of parameter A .

RESULTS

WEIBULL MODEL

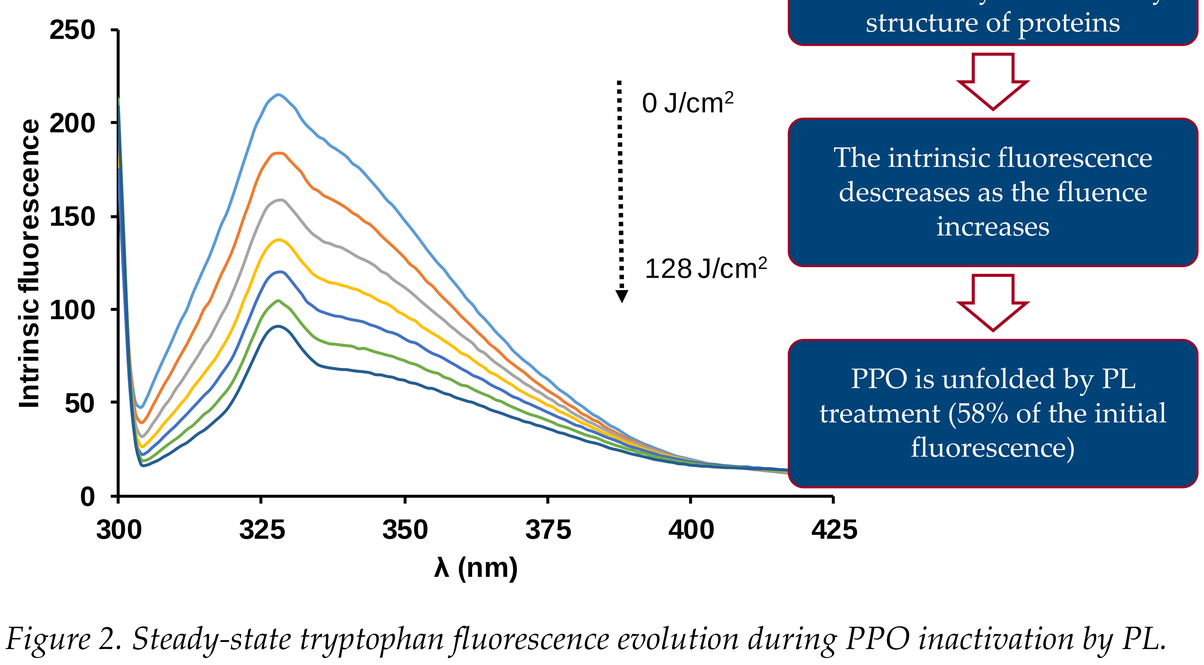
$$\log(U_F) = \log U_0 - \left(F/\delta\right)^p$$

U_F: enzymatic activity after a treatment
U_0: enzymatic activity wihout treatment
F: (J/cm²) is the incident fluence.
δ: F for the first log-reduction
p: parameter describing convexity



The Weibull model fitted inactivation data satisfactorily, with a R^2=0.9999 and a RMSE of 0.0055. Weibull parameters were δ= 133.0 ± 0.9 J/cm² and p=1.24±0.05.

Figure. 1. Inactivation of PPO by PL.



Trp fluorescence is used as an internal fluorescence probe in order to analyze the tertiary structure of proteins

The intrinsic fluorescence descreases as the fluence increases

PPO is unfolded by PL treatment (58% of the initial fluorescence)

Figure 2. Steady-state tryptophan fluorescence evolution during PPO inactivation by PL.

Parameter A is a quantitative characteristic of the fluorescence spectrum of protein Trp residues.

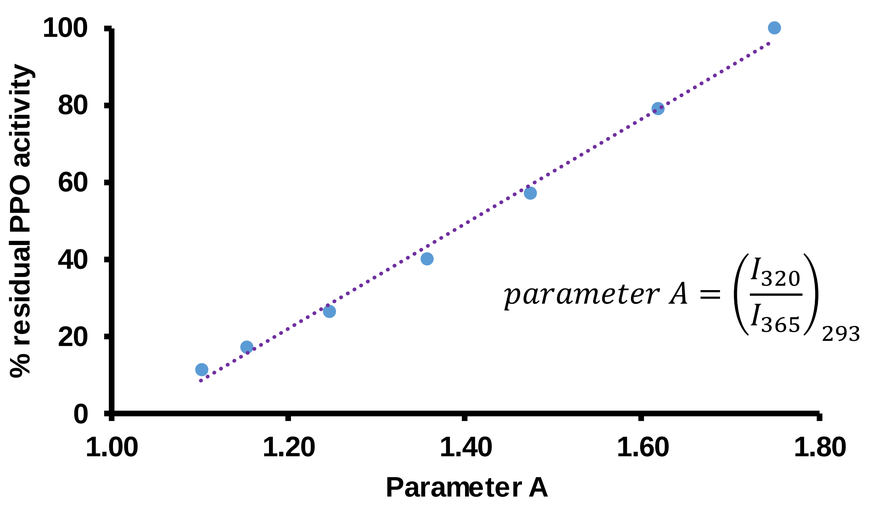


Figure 3. Parameter A evolution during PPO inactivation by PL.

High correlation between parameter A and PPO inactivation

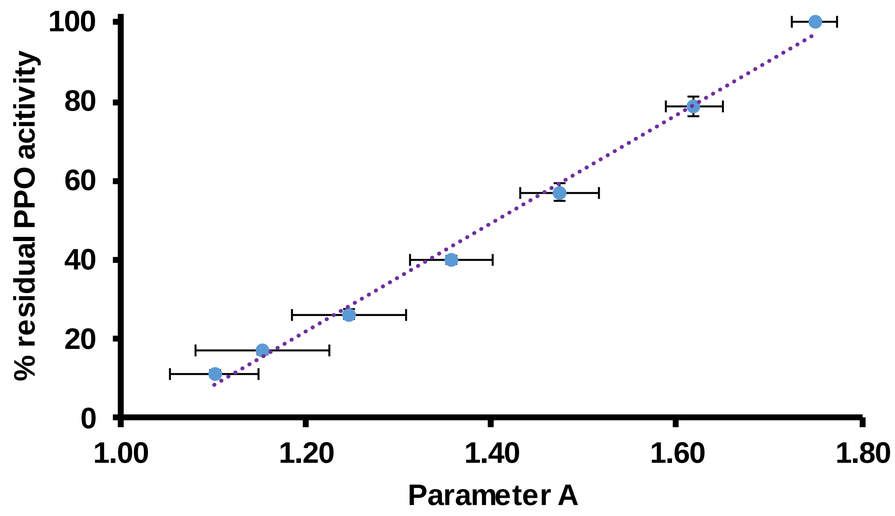
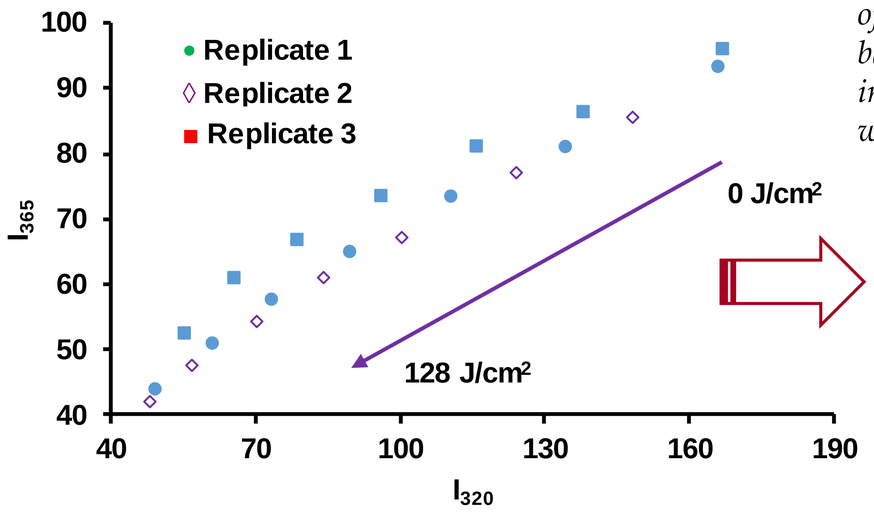


Figure 4. Correlation between PPO inactivation and parameter A.

Figure 5. Phase diagram analysis of PL-induced changes of PPO based on intrinsic fluorescence intensity values measured on wavelengths 320 and 365 nm.



It can be seen that the diagram is linear and the ratio I_{365}/I_{320} continuously decreased with increasing fluence. This finding shows that the inactivation of PPO by PL is an all-or-none process.

Parameter A decreases in a way directly proportional to the progress of the treatment, which indicates that the changes in the tertiary structure of the PPO during PL treatment are proportional to the amount of photons impinging the protein.

CONCLUSIONS

PPO inactivation was explained by the Weibull model

The loss of PPO activity was correlated to protein unfolding according to Trp fluorescence

The inactivation of PPO by PL is an all-or-none process

ACKNOWLEDGEMENTS

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