INACTIVATION OF POLYGALACTURONASE BY PULSED LIGHT, A KINETIC AND STRUCTURAL STUDY

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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). Polygalacturonase (PG) is an enzyme related to the quality of vegetables, fruits and their products, it catalyzes the degradation of pectin.

OBJECTIVES

The aim of this study was to investigate the capability of PL to inactivate PG, its kinetic and associated structural changes measured by chemical and fluorometric methods.

MATERIALS AND METHODS

- A Petri dish without cover with 20 mL of PG solution was placed on the stirrer below the center of the lamp. The PG activity assay was based on the release of reducing groups produced by PG and measured by spectrophotometry at 276 nm.
- Free sulfhydryl content was determined by the method of Ellman ((1959).
- Intrinsic Trp fluorescence was used to characterize potential changes in the tertiary structure of the enzymes.
- Fluorescence changes were also characterized in terms of parameter A .
- 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.

RESULTS

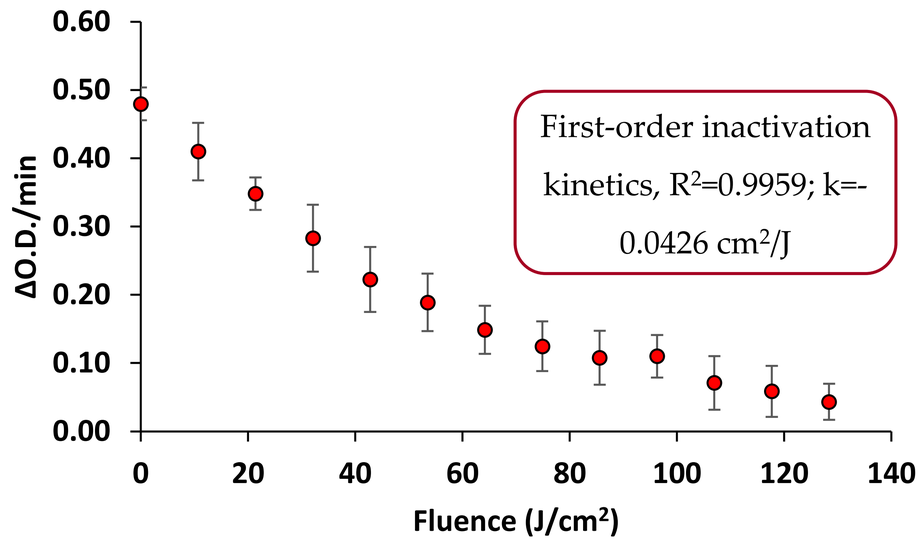

Figure. 1. Inactivation of PG by PL.

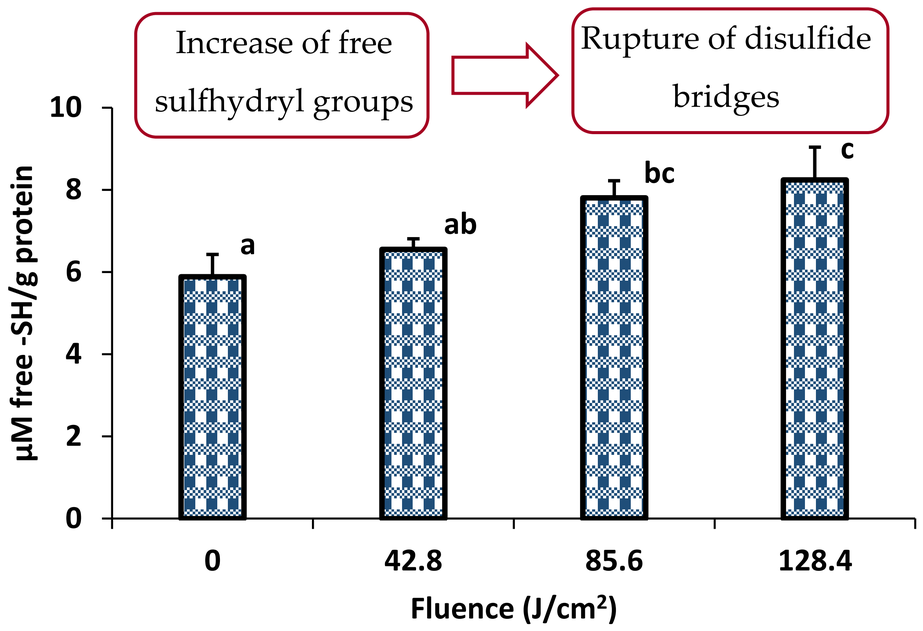

Figure 2. Free sulfhydryl evolution during PG inactivation by PL.

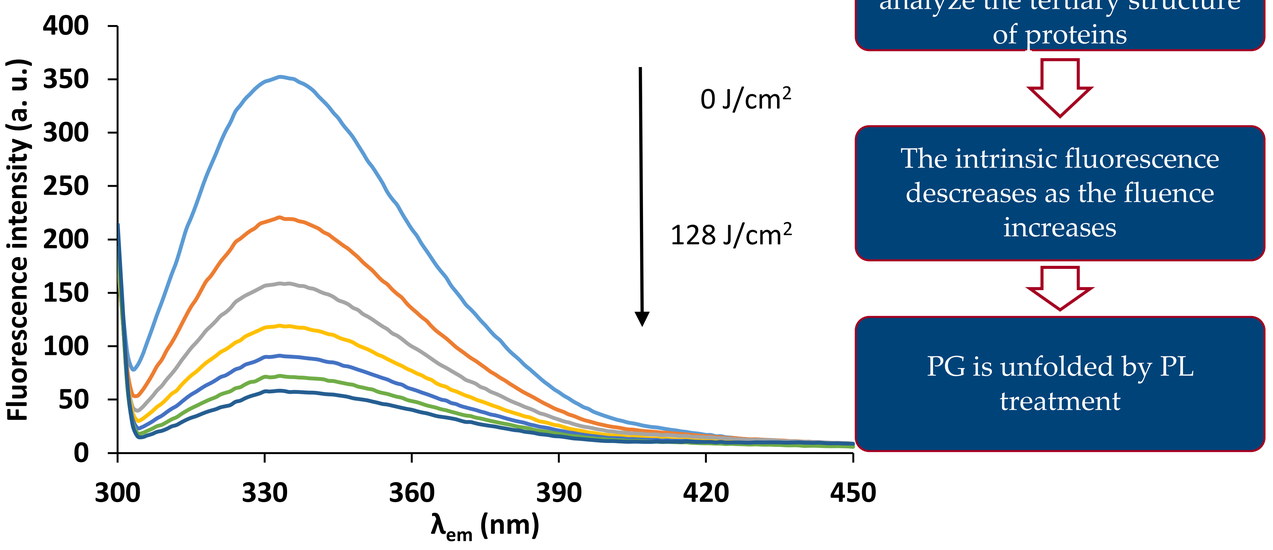

Figure 3. Steady-state tryptophan fluorescence evolution during PG inactivation by PL.

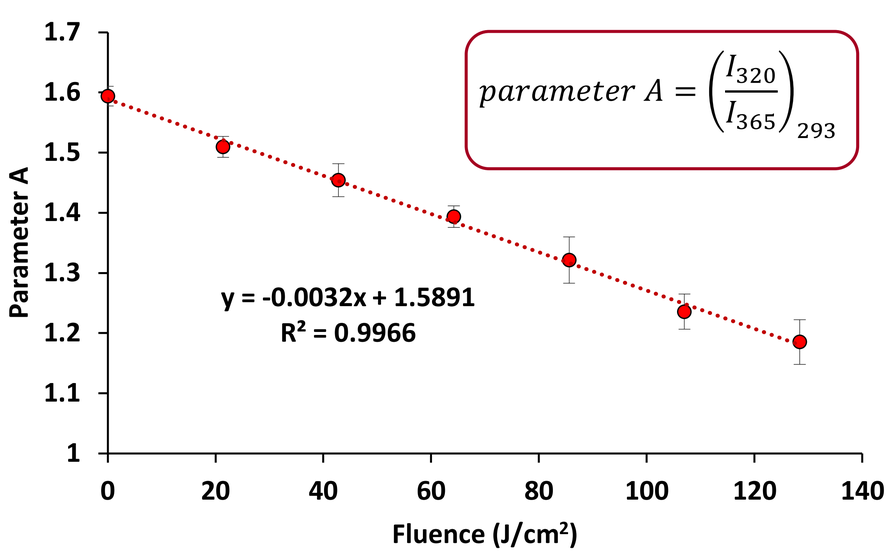

Figure 4. Parameter A evolution during PG inactivation by PL.

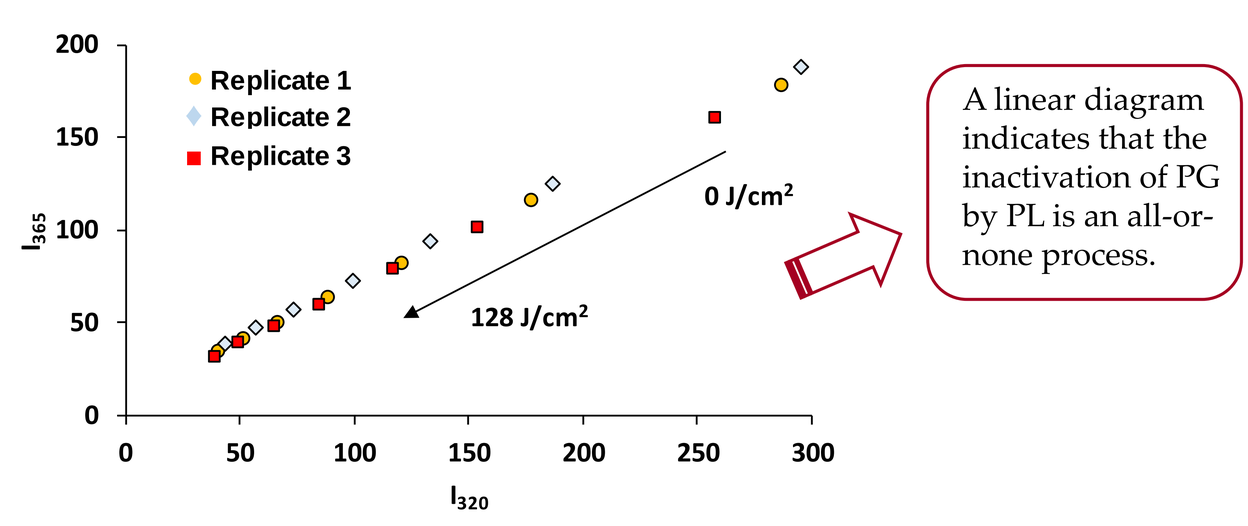

Figure 5. Phase diagram analysis of PL-induced changes of PG.

CONCLUSIONS

- PPO inactivation was log-linear
- The loss of PPO activity was related to disulfide bridges rupture and protein unfolding
- The inactivation of PG by PL is an all-or-none process

ACKNOWLEDGEMENTS

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REFERENCES

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