**INTRODUCTION**

Consumption of cloudy apple juice seems to be more beneficial than clear apple juice. However, one of the main problems with cloudy apple juice is the color and cloud stability. Two enzymes are responsible for this quality loss: **PPO** and **PME**.

**High Pressure Carbon Dioxide (HPCD)** is an alternative to the traditional thermal treatments to inactivate those enzymes. Typical CO₂ operating pressures do not exceed 50 MPa and temperatures (20-55°C) are lower than the conventional thermal treatments. In this work, two commercial PPO and PME will be used to study the inactivation mechanism without the interferences of other species present in the juice.

**EXPERIMENTAL RESULTS**

**HPCD Mechanism of Action**

- pH decrease
- CO₂ solubilization
- Molecular effects of CO₂
- Depressurization
- Conformational changes in the active site of enzymes
- Modifies the structure of the enzyme

**EXPERIMENTAL CONDITIONS**

- **TEMPERATURE**: (25-45°C): high temperatures increase the inactivation rate
- **PRESSURE**: (60-200 bar): pressure promotes the inactivation of PPO
- **TIME**: (2-15 min): very fast inactivation. After 5 minutes 90% of the total activity loss is achieved

**CONCLUSIONS**

- **PPO** and **PME** activity is significantly affected by HPCD, but in different ways: PPO is affected by both pressure and temperature, while PME is only affected by temperature.
- It is critical the ratio CO₂/amount of enzyme loaded in the reactor. Using three times more CO₂ than enzyme the maximum inactivation of both enzymes is guaranteed at a given pressure and temperature.
- PPO and PME have completely different inactivation kinetics. PPO presents a biexponential kinetic while PME exhibits a first order kinetic.
- The analysis of the kinetic parameters reveals a higher stability of PME to the HPCD treatment.

**References**


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