High Pressure Carbon Dioxide Technology as cold pasteurization in cloudy apple juice

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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 CO_2 operating pressures do not exceed 50 MPa and temperatures (20-55ºC) 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.

HPCD Mechanism of Action

✓ **pH decrease**
CO_2 solubilization

✓ **Molecular effects of CO_2**
Conformational changes in the active site of enzymes

✓ **Depressurization**
Modifies the structure of the enzyme

EXPERIMENTAL RESULTS

Oxidation of phenolic compounds
Browning effect

PPO

Polyphenol Oxidase
(EC 1.14.18.1)

Demethylation of pectin present in the juice
Clarification
Crosslinking with polyvalent cations (Ca^{2+})
Insoluble precipitates

PME

Pectinmethyl Esterase
(EC 3.1.1.11)

EXPERIMENTAL CONDITIONS

PPO:

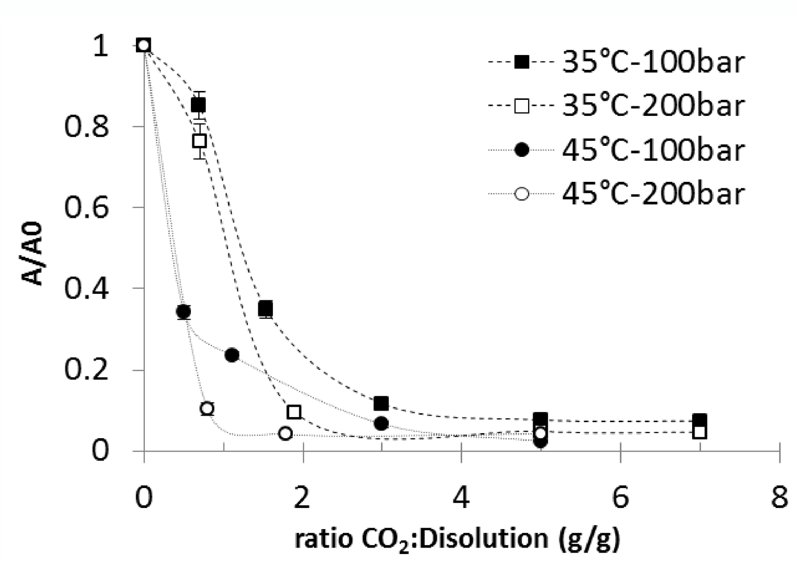
- **TEMPERATURE (25-45ºC)**: high temperatures increase the inactivation rate
- **PRESSURE (60-200bar)**: pressure promotes the inactivation of PPO
- **TIME (2-15min)**: very fast inactivation. After 5 minutes 90% of the total activity loss is achieved

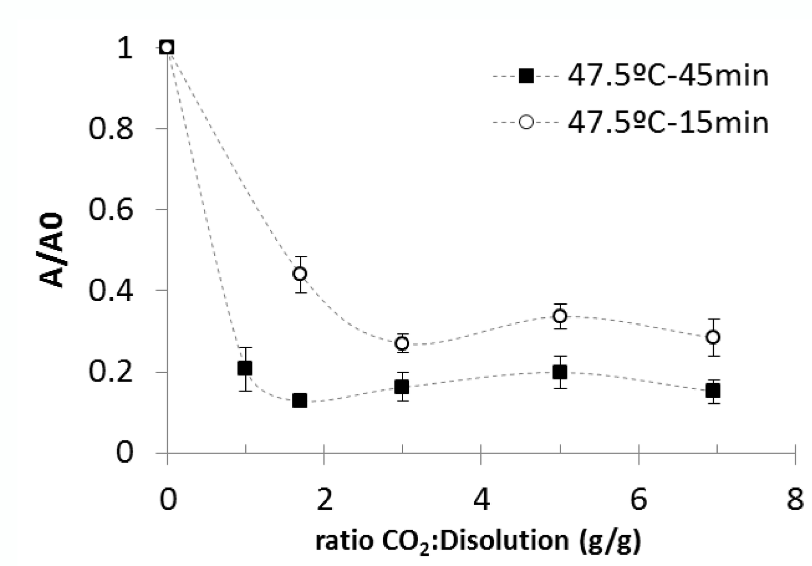
PME:

- **TEMPERATURE (40-55ºC)**: Higher thermal stability than PPO; higher temperatures needed
- **PRESSURE (60-180bar)**: no significant effect
- **TIME (5-45min)**: linear decrease in residual activity; longer times required

CO_2/ENZYME RATIO (g/g)

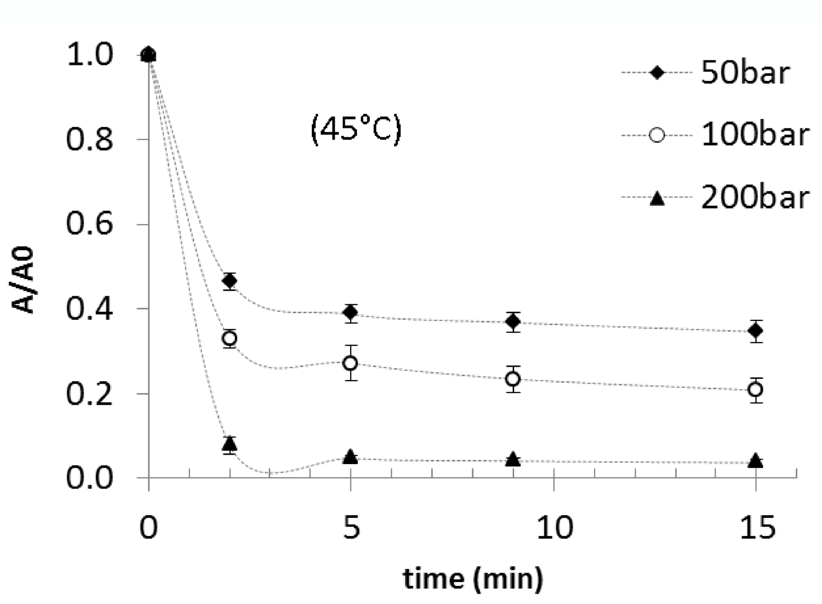
Critical parameter; at 200bar ratios > 3 guarantee a complete inactivation of the enzyme, regardless the temperature





Critical parameter. Experimental times can be reduced by increasing the amount of CO_2 used

KINETIC MODEL



TWO FRACTION

- Two isozymes: labile and stable
- Sharp initial activity decrease followed by slowed activity decay

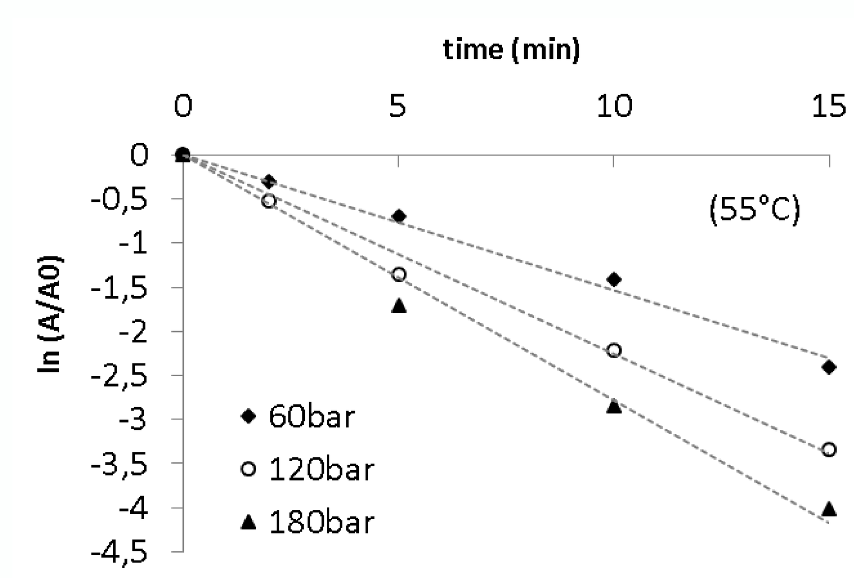
Kinetic Parameters

	PPO	PME
ZP (bar)	70-78	300-450
ZT (ºC)	27-40	7-9
E_a (kJ/mol)	25-16*	230
$-V_a$ (cm³/mol)	94-69*	135-200

** Results shown for the labile fraction*

FIRST ORDER

- Linear decrease of the activity throughout the time exposed to the HPCD.



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 CO_2/amount of enzyme** loaded in the reactor. Using three times more CO_2 than enzyme the maximum inactivation of both enzymes in 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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