

#### HOW ENZYME DIFFUSION IN PECTIN AND PECTIN/CELLULOSE COMPOSITE SYSTEMS IS INFLUENCED BY THEIR MECHANICAL PROPERTIES

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## HOW ENZYME DIFFUSION IN PECTIN AND PECTIN/CELLULOSE COMPOSITE SYSTEMS IS INFLUENCED BY THEIR MECHANICAL PROPERTIES

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# Diffusion of Enzyme in Biopolymer Systems

Enzymes used in food and non food products to modify their properties :

Protease, amylase, cellulase, xylanase, lipase :

Milk, bread, brewery, fruit industries Paper and detergent industries

Pepsin in digestion process

Is their diffusion affected by the structure or the texture of the system?





# I. Pectins: General points

- Plant cell wall polysaccharide:
  - -> Cell shape and mechanical properties
  - -> Cell growth & development
  - -> Defence against phytopathogens



http://www.ccrc.uga.edu/~mao/intro/ouline.htm

- Functionnal ingredients for food and drug industries:
  - -> Gelling agent
  - -> Stabilising agent
  - -> Coating agent

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## I. Pectins: Structure



Scheller et al., Physiologia Plantarum, 2007.

Very complex structure

Polyelectrolyte character

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# **I. Pectins:** Homogalacturonans



O'Neill, Darvill, & Albersheim, 2001. Pectic Substances.

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# II. Cellulose

#### Cellulose microfibril

Pectin

Hemicellulose

Proteins



Carpita & Gibeaut The Plant Journal (1993)



Very abondant polymer

 $\beta$ -(1 $\rightarrow$  4) linked glucose units



#### Insoluble





# III. Pectin methylesterases (PME)

- Plant or Fungal enzymes
- Decrease of DM

#### **HM pectin**





# III. Pectin methylesterases (PME)

- Plant or Fungal enzymes
- Decrease of DM



Micheli, Trends in Plant Science, 2001.

Modification of gelation properties with calcium Solution Gel

# **III. PME & Gelation Properties**





Global Scale or Macroscopic scale Influence of de esterification type Diffusion coefficient ~  $100 \ \mu m^2/s$ 

#### Videcoq et al., Soft Matter, 2013





 Characterisation of PME diffusion in pectin solution, pectin gel in formation, composite systems at different concentrations



Mesoscopic scale



Fluorescent Recovery After Photobleaching (FRAP)

- Characterisation of the rheological properties
- Compare results of experiments with those obtained by modelling



# V. Material

- Pectin : Orange, DM 46%, Cargill Texturizing Solutions, 0.25-1.5 wt %
- PME (0,68 mg/ml, covalently labelled by FITC):

- Aa-PME Aspergillus aculeatus, Novozyme

- Pectic environment:
  - Pectin solution : pectin / MES (50 mM, pH 6)/ 30°C/Aa-PME
  - Pectin gel during formation: Pectin/ Calcium 5 mM /30°C /Aa-PME
- Cellulose: Micro-fibrillated, from sugar beet (Agoda-Tandjawa, 2009)



## VI. Results – Mesoscopic scale



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## VI. Results – In macromolecular solutions



Slight decrease of D along time, without changes in the physical state Decrease of D with increasing pectin concentrations

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#### **VI. Results** – During pectin gel formation

1% Pectin + 5 mM Ca<sup>2+</sup>



Strong increase of G' upon demethylation

#### **Delayed gelation kinetics**

RFSII & AR2000, TA Instruments

(%) MQ 20 10 0 L 0 10 15 20 25 5 Time (h)

14

30

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## **VI. Results** – During pectin gel formation

#### 1% Pectin + PME + 5 mM Ca<sup>2+</sup>



Higher D values obtained during the very first steps of gelation Higher values than those found in solution At longer times, D values are in the same range



#### **VI. Results** – During pectin gel formation



Higher D values obtained for low storage moduli

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## VI. Results – During pectin gel formation (1.5-0.25%)



For all concentrations, the evolution of D with the storage modulus is the same

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## VI. Results – During pectin gel formation (1.5-0.25%)



Higher D values obtained during the first steps of gelation In agreement with a rapid decrease of the DM at the beginning of the reaction

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#### Molecular dynamics simulations

Polymers and enzymes evolve due to forces acting on them  $\rightarrow$  structure and dynamics



## **VI. Results** – Modelisation of gel formation





## VI. Results – Modelisation of gel formation

Diffusion coefficient of the enzymes





### **VI. Results** – Gelation in presence of cellulose



Agoda-Tandjawa, 2009

Slighly higher D values obtained in the first steps of reaction compared to pectic gels alone at the same concentation



Steric effect increases the local pectin concentration leading to increased viscoelastic properties

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Strong decrease of D during gelation for all the pectin gels

In the first steps of gelation, D increased with polymer concentration

At longer times (higher G' values): diffusion coefficients are in the same range (< 5  $\mu$ m2/s)

In agreement with the demethylation profile of PME and modelling

Diffusion and activity are they linked?

**Experiments with inactivated PME** 

Determine the fine morphological parameters of gels (size of pores, correlation length) and microrheology experiments



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## V. Results – During pectin gel formation (1.5-0.25%)



Higher D values obtained during the first steps of gelation

