INSTITUT DES SCIENCES ET INDUSTRIES DU VIVANT ET DE L'ENVIRONNEMENT
PARIS INSTITUTE OF TECHNOLOGY FOR LIFE, FOOD AND ENVIRONMENTAL SCIENCES

*EuroFoodWater, 22-23 March, Reims, France*

# Water in freeze-dried lactic acid bacteria: Friend or Foe

**Stéphanie Passot**, Stéphanie Cenard, Inès Douania, Cristian Tréléa, Fernanda Fonseca

Joint Research Unit of **Microbiology and food process engineering**
Thiverval-Grignon, France

ALIMENTATION
AGRICULTURE
ENVIRONNEMENT

# Outline

1. Context and objective of the work
2. Experimental methods
3. Main results
4. Conclusions

# Context

- ✓ Concentrates of lactic acid bacteria are used as starters for cheese, fermented milk…
- ✓ Freeze-drying is widely used to improved the shelf life of these concentrates.

Excipients
(sugars, polymers…)

Active ingredient
(Bacteria)

Freeze-drying

Freezing

Primary Drying

Secondary Drying

Product

Storage

Quality criteria

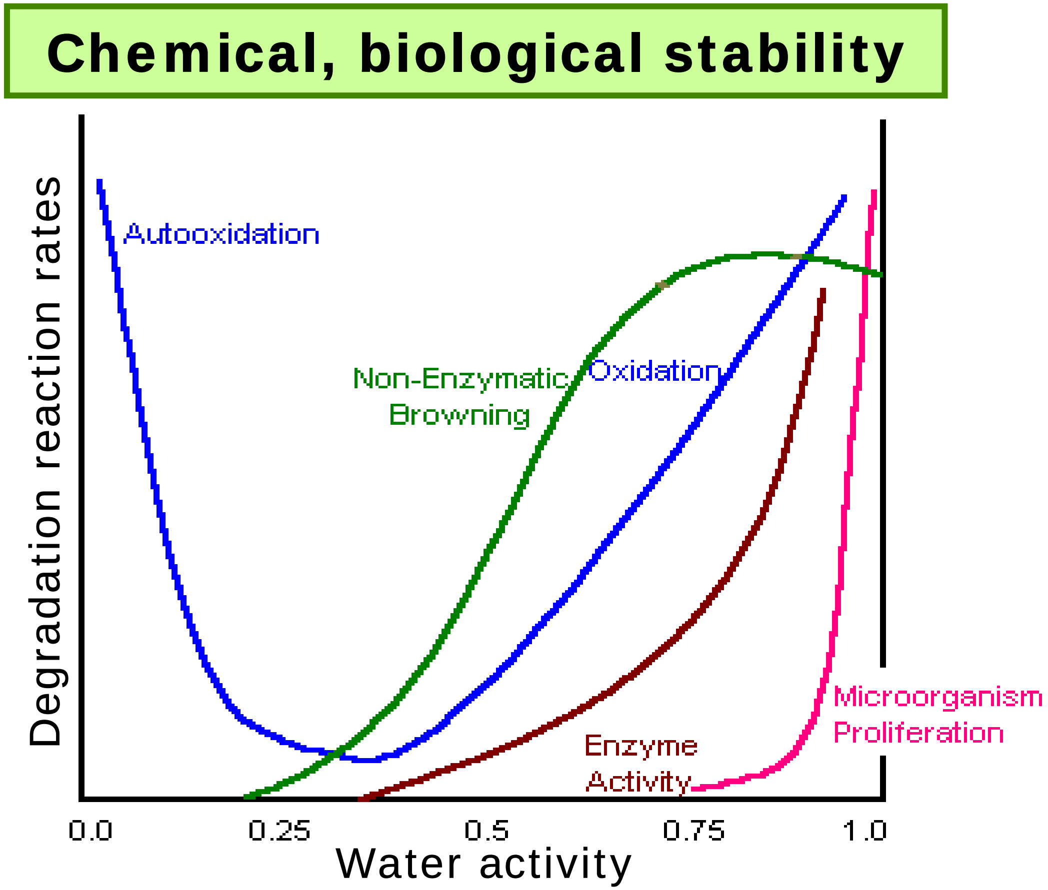
Water content (<3%)

Activity

Structure

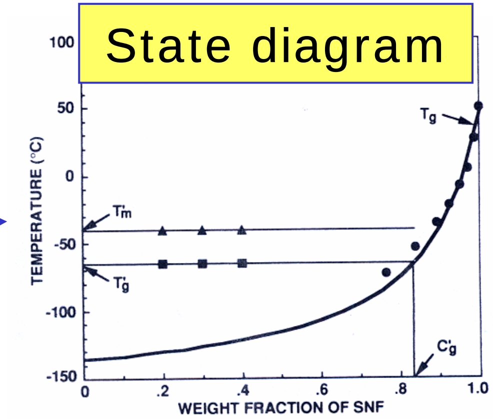
"The dryer, the better ?"

# Context


Chemical, biological stability
Degradation reaction rates
Autooxidation
Non-Enzymatic
Browning
Oxidation
Enzyme
Activity
Microorganism
Proliferation
0.0
0.25
0.5
0.75
1.0
Water activity

# Context

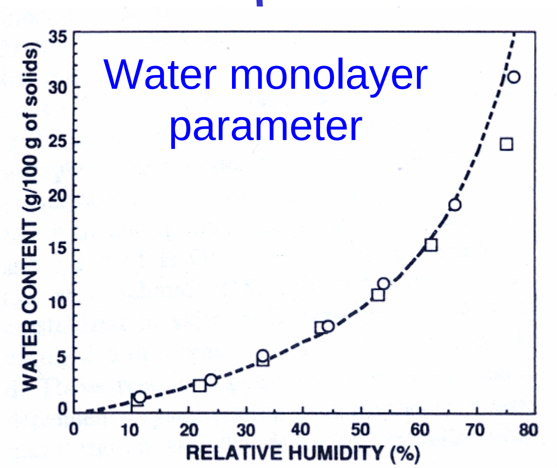
State diagram

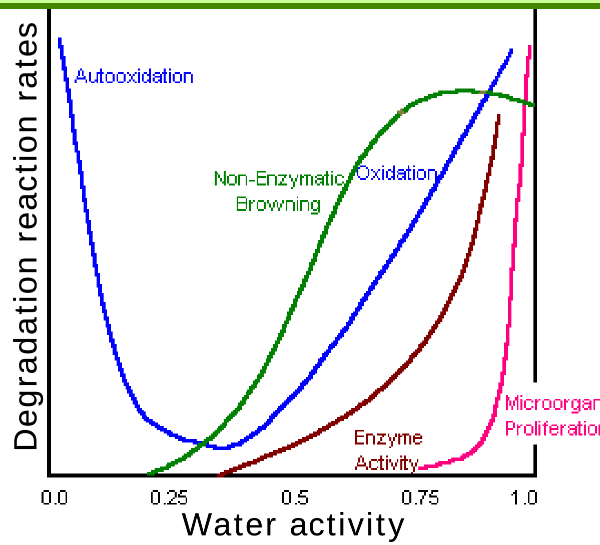


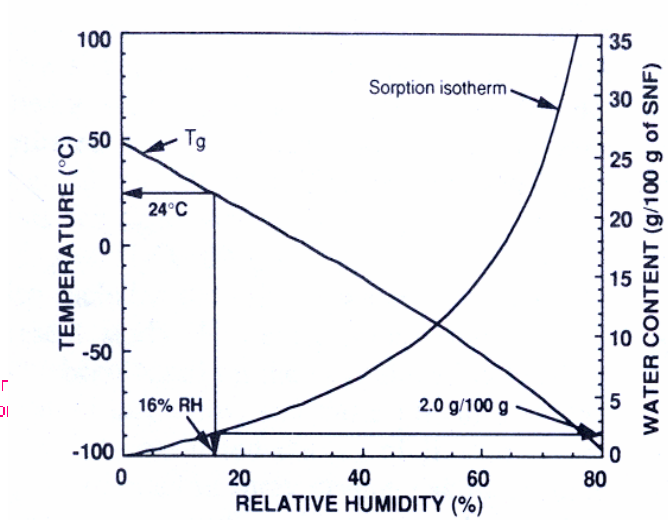

**Glass transition temperature**

**Chemical, biological stability**

**Water content**




***Roos 1993***

Sorption isotherm

**Water activity**

# Objective

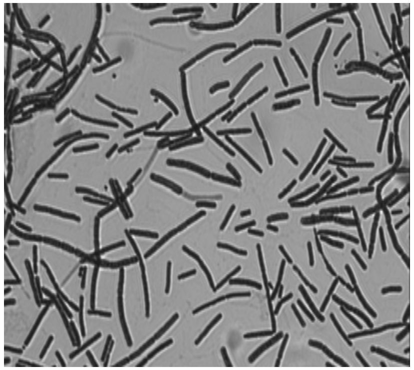
Our objective was to investigate the effect of water activity on:

- ✓ The physical properties of freeze-dried amorphous matrix: Tg, water content
- ✓ The lactic acid bacteria stability

# Outline

1. Context and objective of the work
2. Experimental methods
3. Main results
4. Conclusions

# Experimental



✓ **Lactic acid bacteria :**

***Lactobacillus delbrueckii subsp. bulgaricus* CFL1**

**Fermentation**
**Concentration**

**Protective medium**
**20% of sucrose**

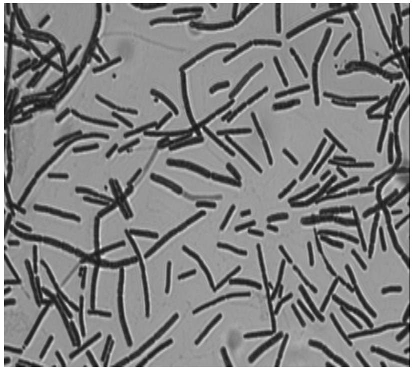
**Freeze-drying**

**Equilibration under various HR (25°C)**

**Storage (25°C)**

| Salt | HR (%) |
|---|---|
| $P_2O_5$ | 0 |
| LiBr | 0.06 |
| LiCl | 0.11 |
| $CH_3COOK$ | 0.23 |
| $MgCl_2$ | 0.33 |
| $K_2CO_3$ | 0.44 |
| $Mg(NO_3)_2.6H_2O$ | 0.53 |
| NaCl | 0.75 |
| KCl | 0.84 |

# Experimental



✓ **Lactic acid bacteria :**

***Lactobacillus delbrueckii subsp. bulgaricus* CFL1**

**Fermentation**
**Concentration**

**Protective medium**
**20% of sucrose**

**Freeze-drying**

**Equilibration under various HR (25°C)**

**Storage (25°C)**

**Physical properties**

✓ **Glass transition temperature: DSC Pyris 1, Perkin Elmer**

✓ **Water content: Karl Fisher titration (Metrohom)**

✓ **Water activity: Aw-meter (Novasina)**

**Biological properties**

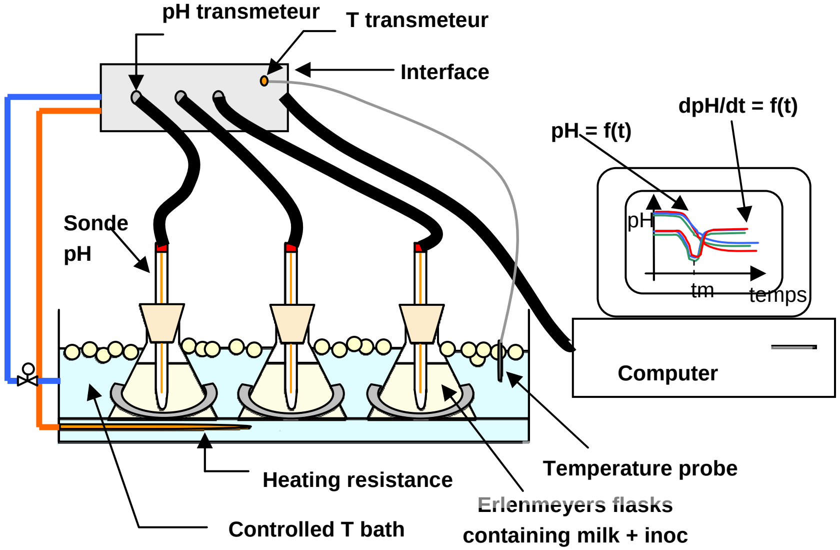
✓ **Acidification activity (CINAC system)**

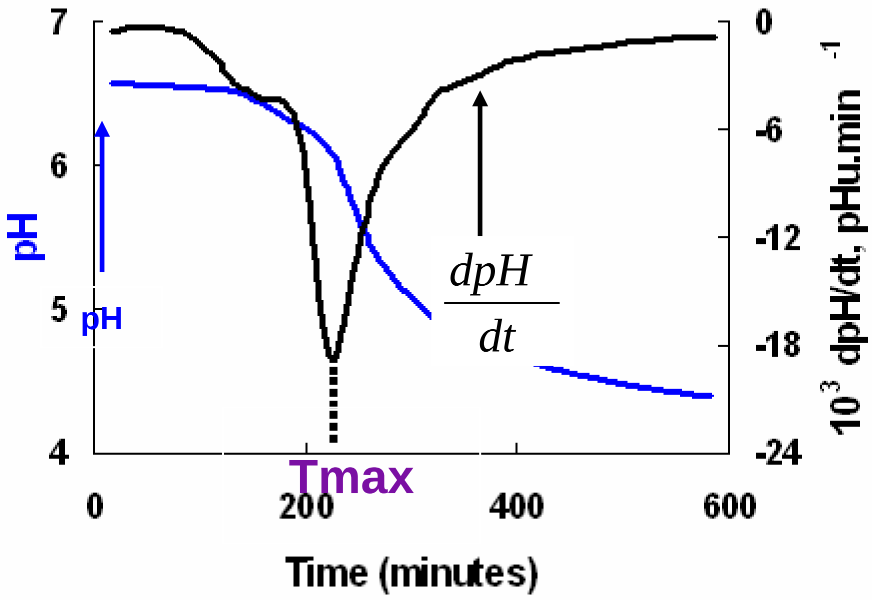
✓ **Viability**

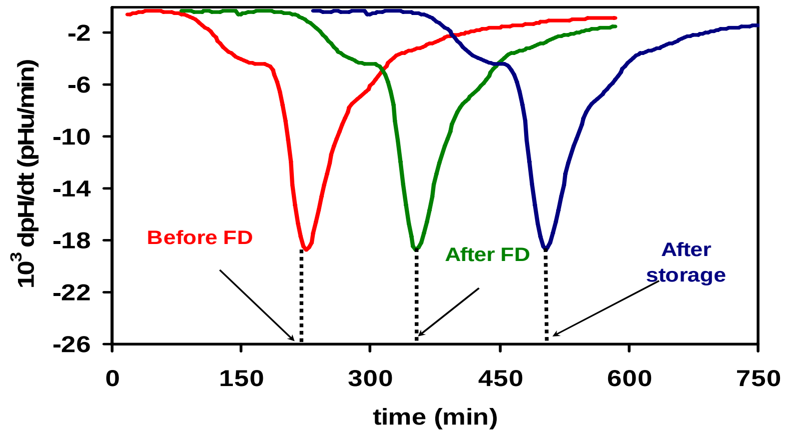
**After equilibration and storage at 25°C**

# Acidification activity

## Cinac System




The higher the Tmax value,

The lower the acidification activity

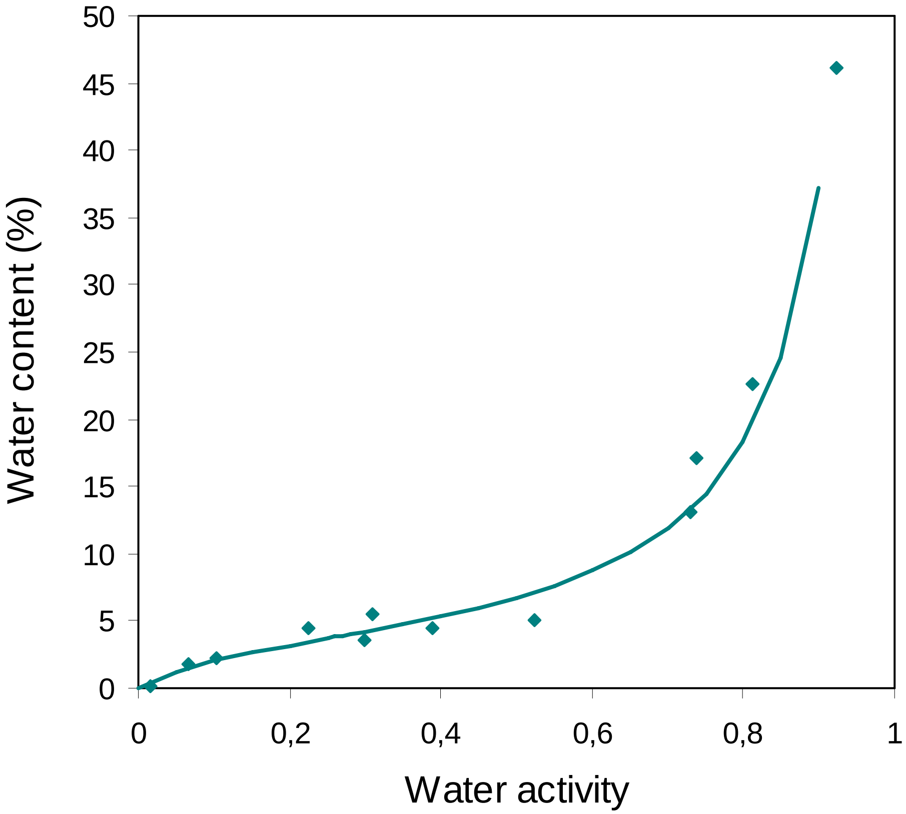
FD: Freeze-drying

# Outline

1. Context and objective of the work
2. Experimental methods
3. Main results
4. Conclusions

# Results: Sorption isotherm

**➢Determination of the water monolayer (Mo)**




**BET equation**

$$m = \frac{M_0 C A_w}{(1 - A_w)(1 - A_w + C A_w)}$$

| *Parameter* | *Mo (%)* | *C* |
|---|---|---|
| **C200+LAB** | **3.77** | **8.39** |

Tg = 22°C

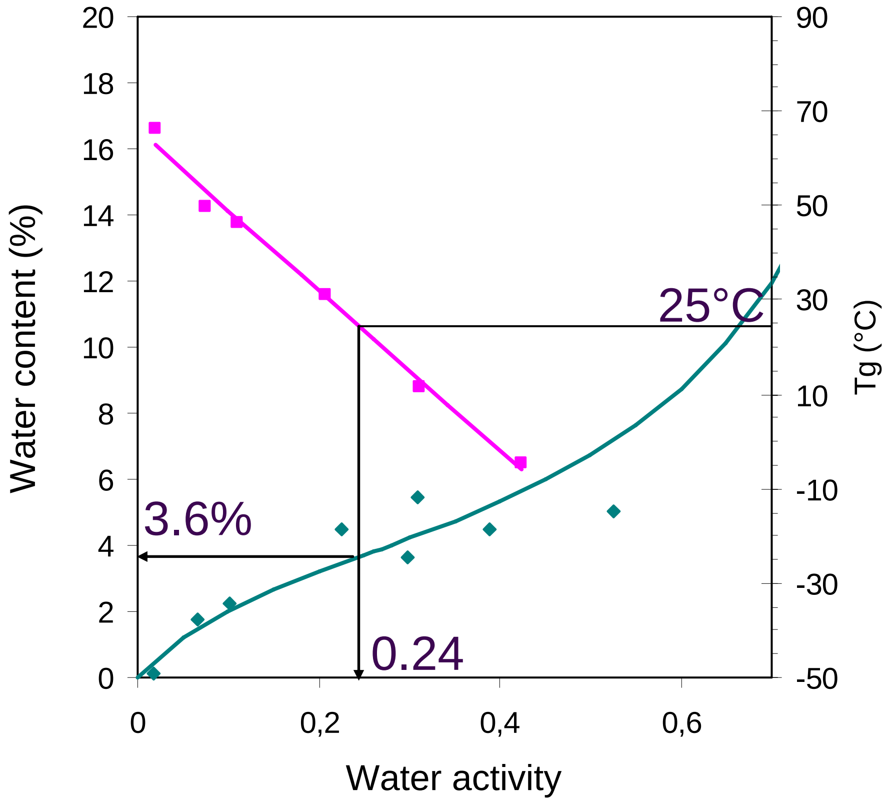
Aw = 0.257

BET:

Mo: water monolayer parameter

# Results: Sorption isotherm

➢**Determination of the water monolayer (Mo)**




**BET equation**

$$m = \frac{M_0 C A_w}{(1 - A_w)(1 - A_w + C A_w)}$$

| *Parameter* | *Mo (%)* | *C* |
|---|---|---|
| **C200+LAB** | **3.77** | **8.39** |

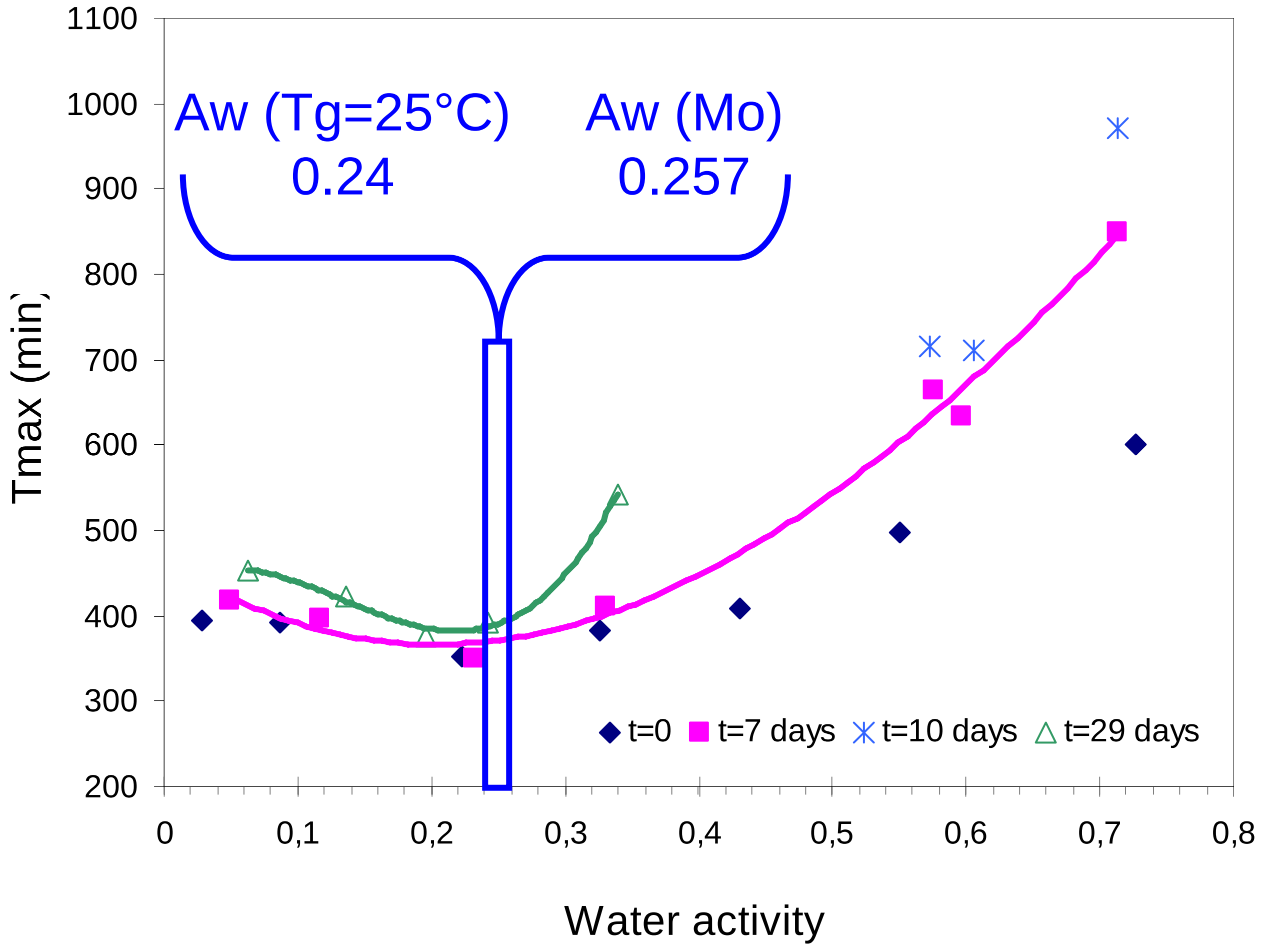
Tg = 22°C

Aw = 0.257

BET:

Mo: water monolayer parameter

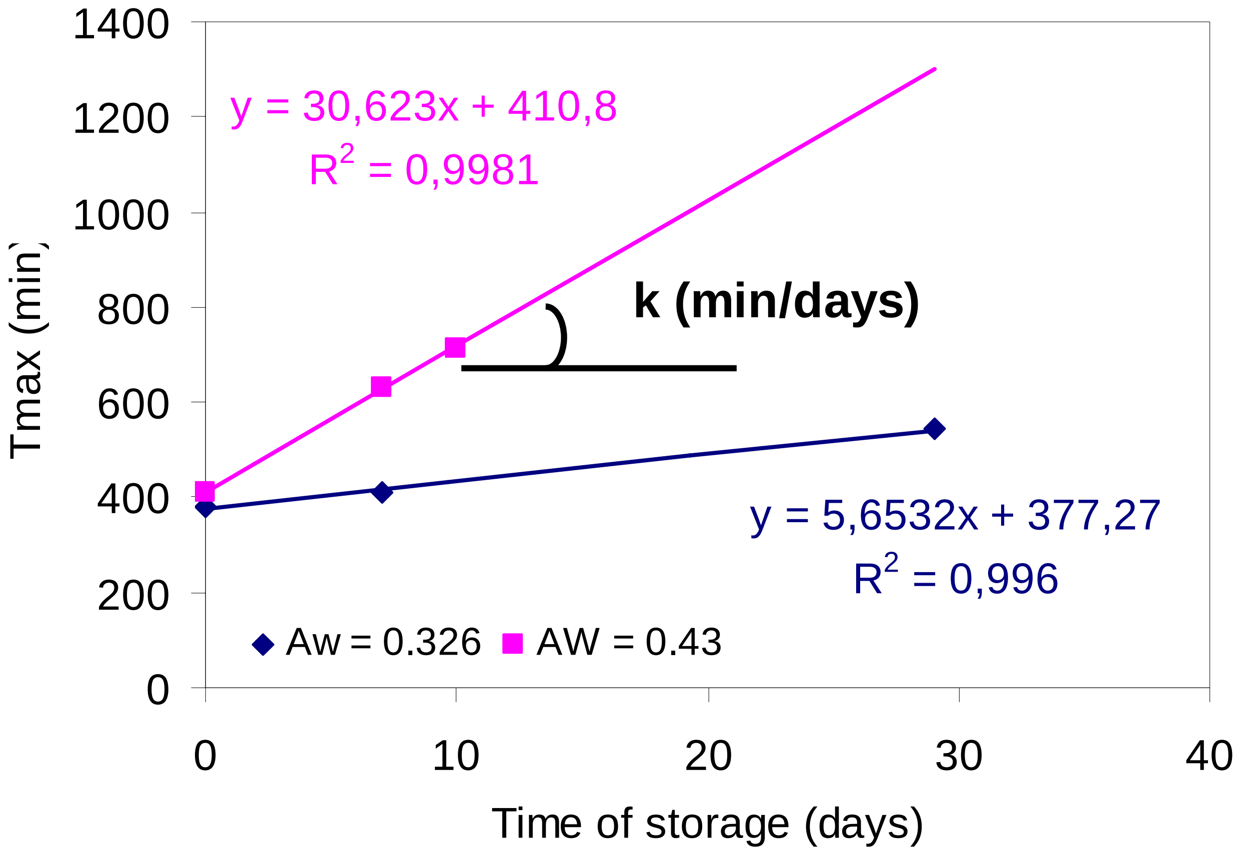
# Results: Acidification activity




The higher the Tmax value, The lower the acidification activity

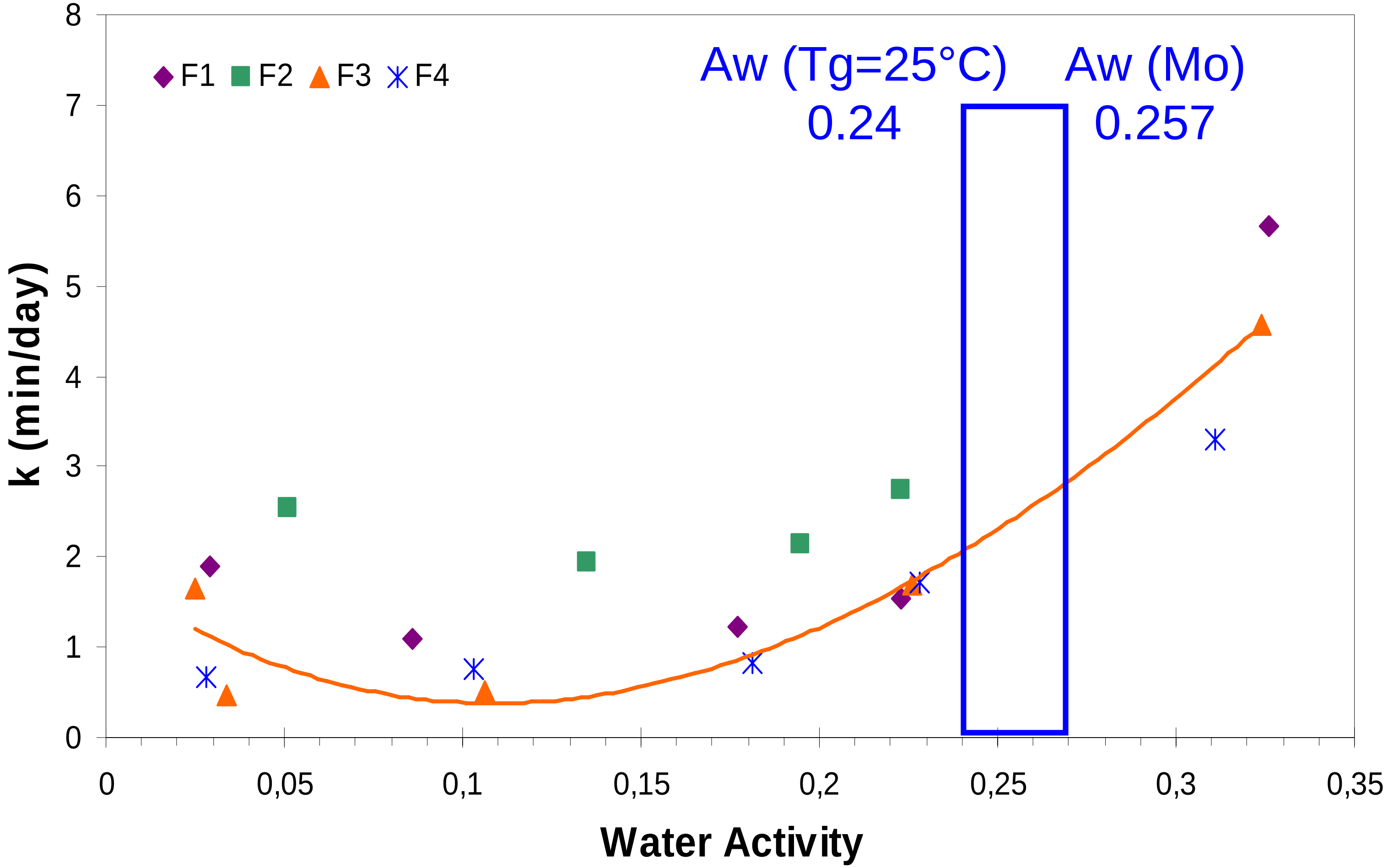
# Results: Acidification activity

Quantification of the rate of loss of acidification activity during storage



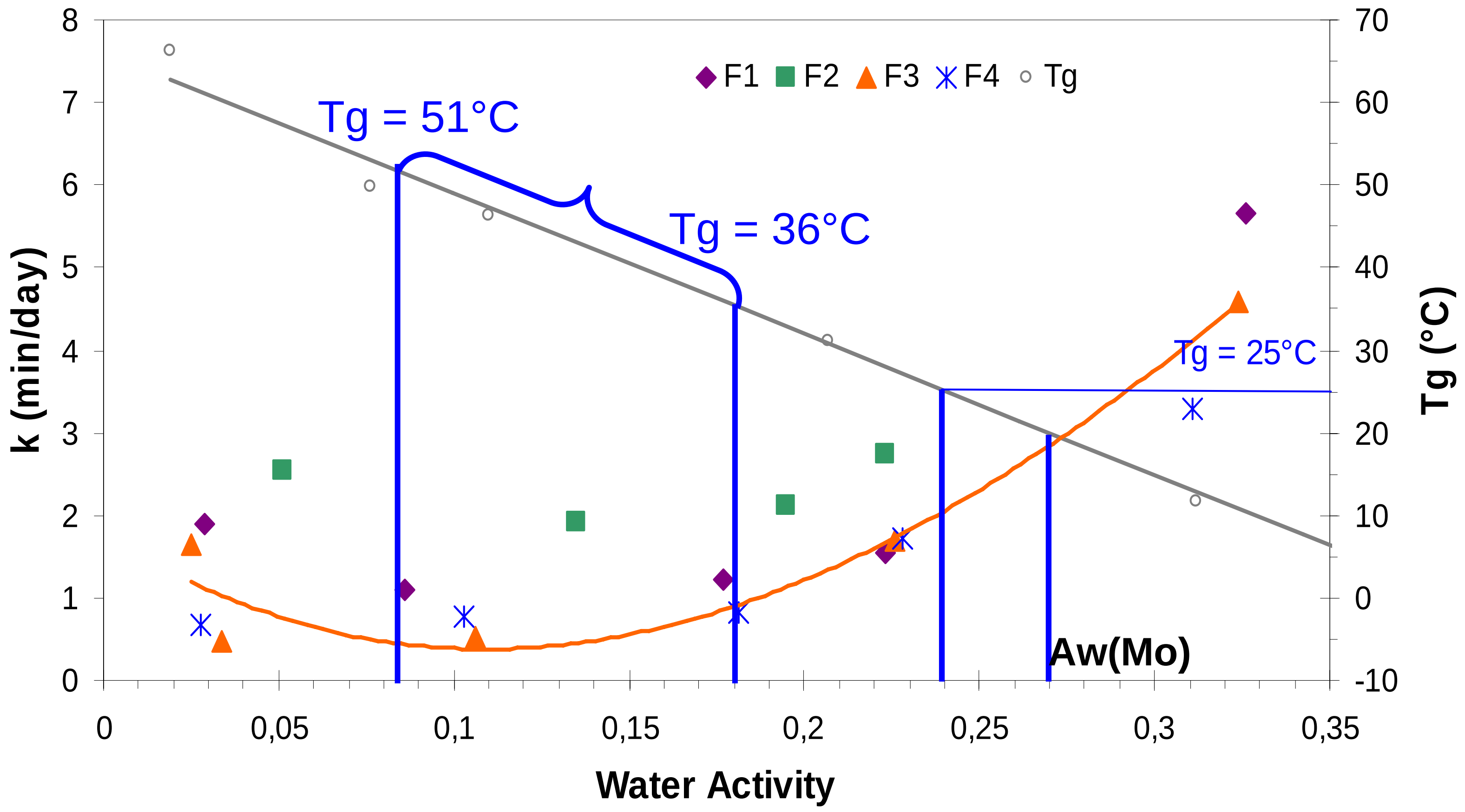
# Results: Acidification activity

4 fermentations ⇨Various physiological states of bacteria



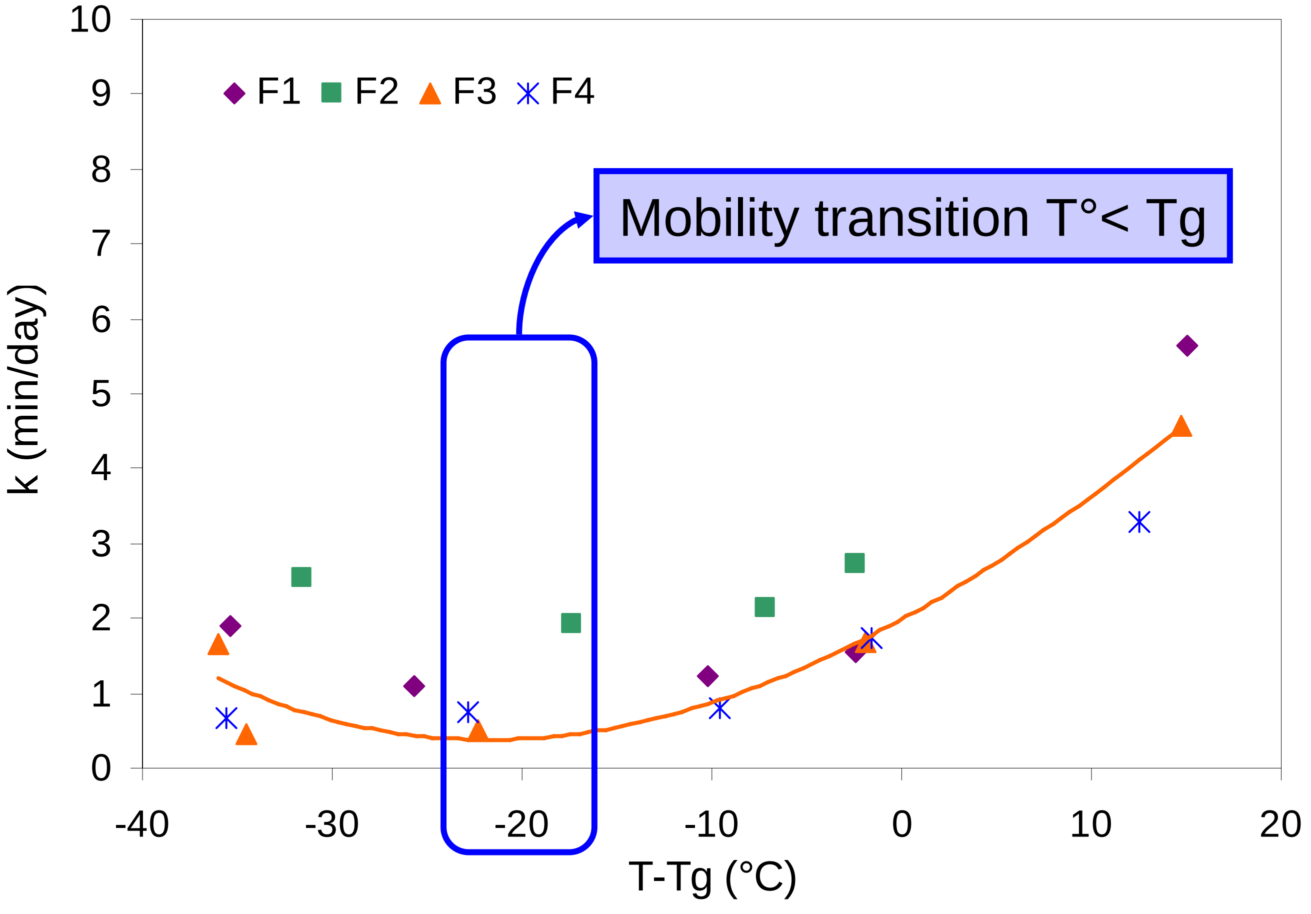
# Results: Acidification activity

4 fermentations ⇨Various physiological states of bacteria



# Results: Acidification activity

4 fermentations ⇨Various physiological states of bacteria



# Conclusion

“The dryer, the better” is not always true !

- Concepts of critical water activity (hydration limit) and critical temperature (mobility transition T° < Tg): interesting approach for predicting stability of freeze-dried lactic acid bacteria.

→ Further works are needed to better investigate the water mobility within the glassy matrix: Various storage T° and protective excipients.

- Relating glass transition temperature and bacteria activity to water activity appears as a useful tool to optimize :
  - ✓ Freeze-dried lactic acid bacteria stability during processing and storage.
  - ✓ Freeze-drying cycle, especially secondary drying

# Thank you for your attention

**This work was financially supported by the European Community:**

**CAFÉ Project: Computer-Aided Food processes for control Engineering**

Joint Research unit AgroParisTech INRA

**Microbiology and food process engineering**

Grignon, FRANCE

# Titre (Arial taille 44)

## Titre secondaire (Arial taille 32)

**> texte avec puce (Arial ou** Arial narrow **)**

**Texte courant (Arial ou Arial narrow taille 20)**