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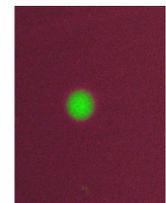
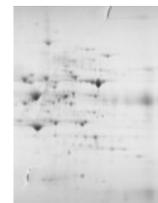
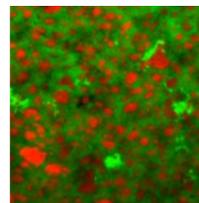
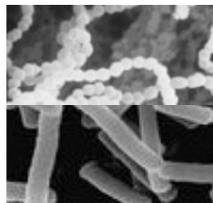
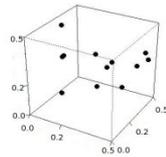
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“Image analysis of cheese microstructure and diffusion properties of solutes: An important link to understand cheese ripening mechanisms”



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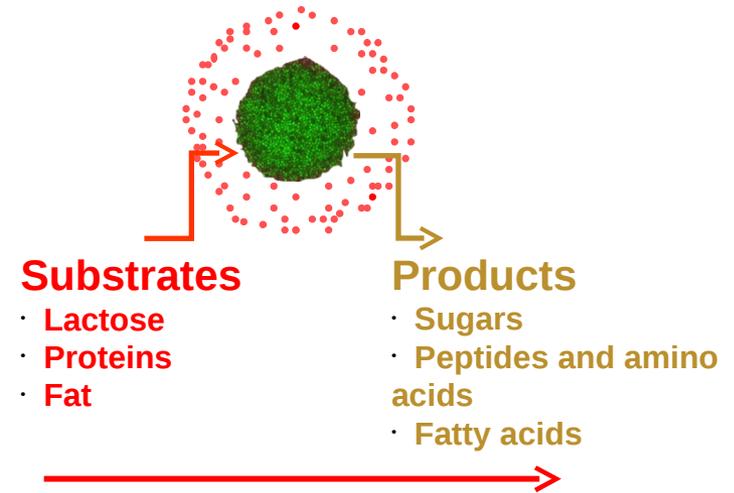
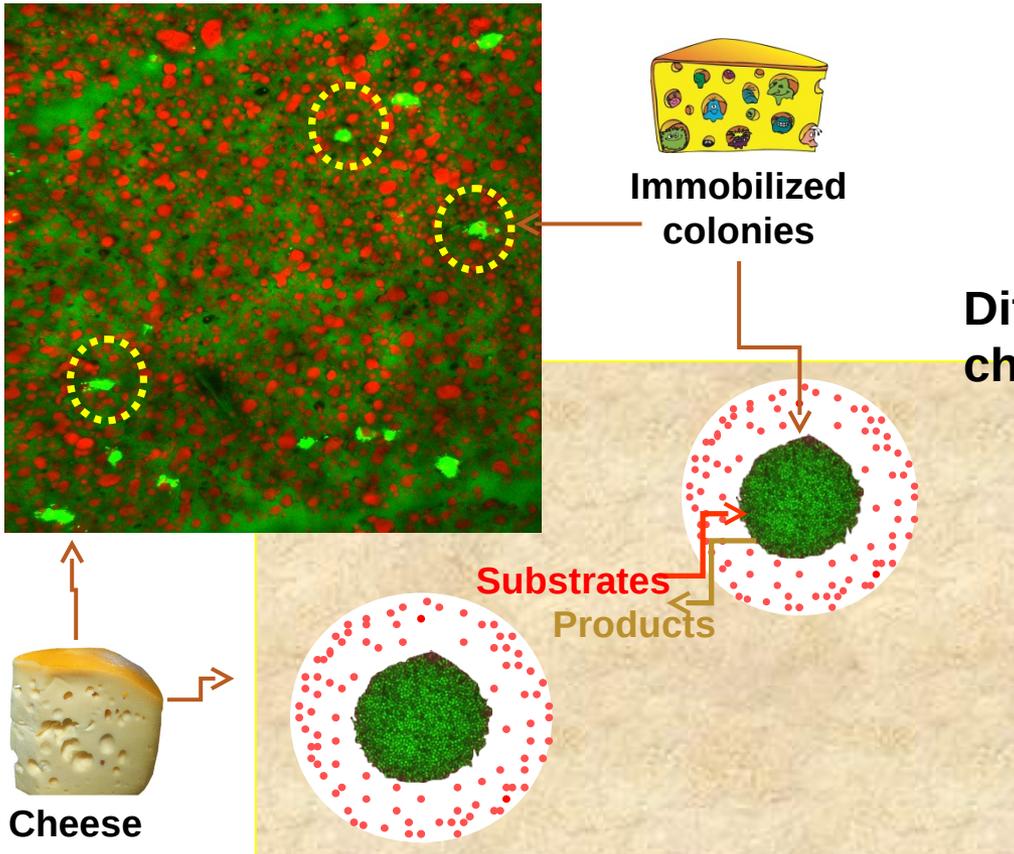
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Context

Cheese

- Key step of cheese making technology
- Complex and specific of each variety of cheese



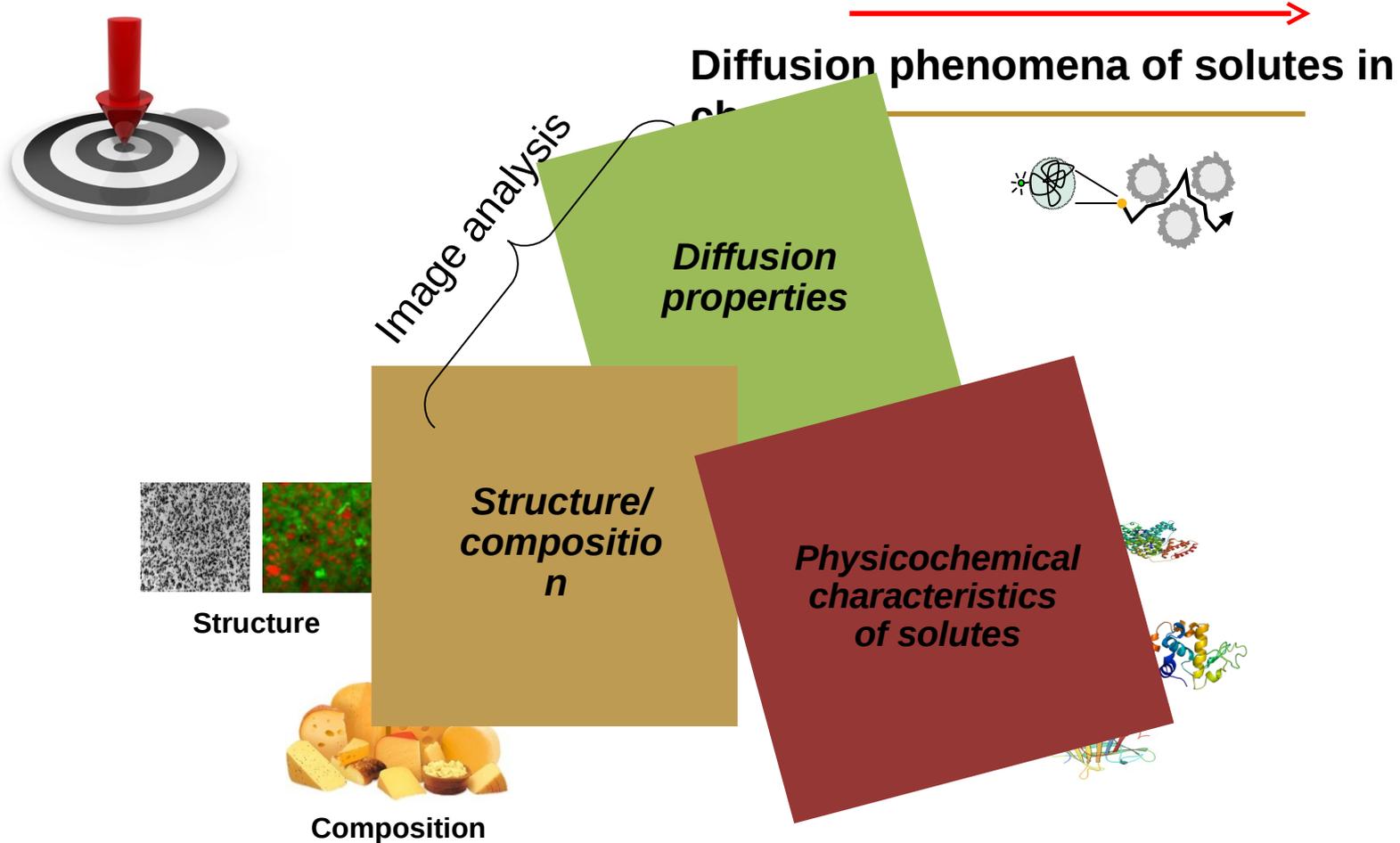
Diffusion phenomena of solutes in cheese

- Microbial and enzyme activities
- Development of flavor and texture

Final quality of cheese



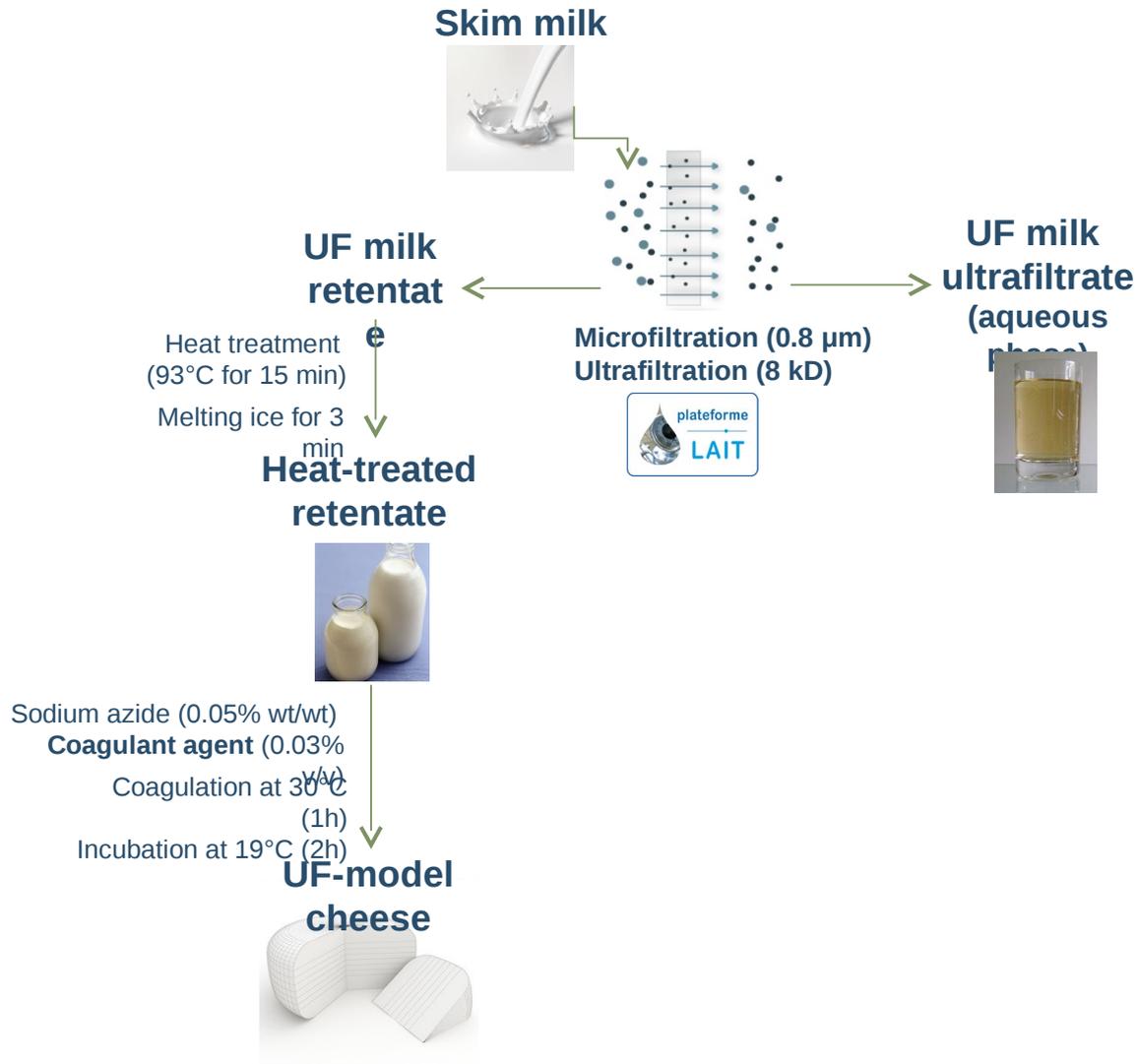
Objective



To enable a better understanding of the **ripening mechanisms...**

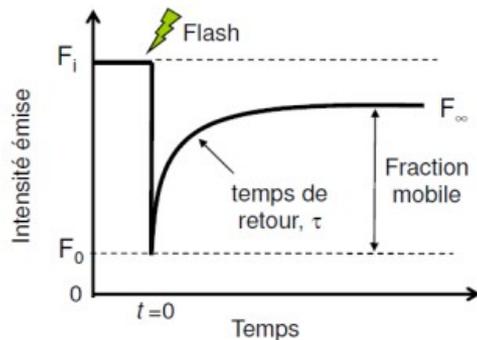
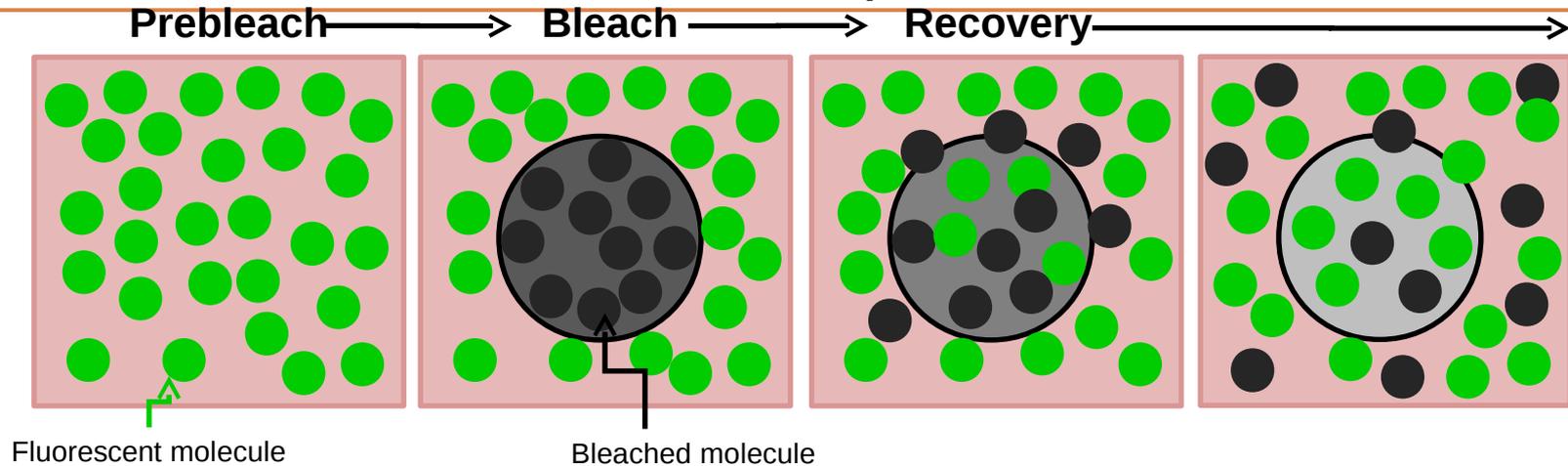
Materials and Methods

UF-model cheese manufacture



Materials and Methods

Diffusion of solutes through the UF-model cheese and in the ultrafiltrate using the FRAP technique



Typical recovery curve of fluorescence in function of time

Def: effectif diffusion coefficient in the UF-model cheese ($\mu\text{m}^2/\text{s}$)

Daq: diffusion coefficient in the aqueous phase (ultrafiltrate)

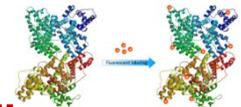
wM: profile width in the radial direction (m)

τ : characteristic time of diffusion

Fluorescent solutes:

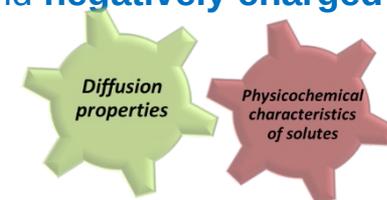
FITC-dextrans (4 kDa to 2 MDa)

- **Flexible and neutral solutes**



FITC-labeled dairy proteins (α -LA, β -LG and BSA)

- **Rigid and negatively charged solutes**



Materials and Methods

Study of the **microstructure** of the **UF-model cheese** using **TEM**

Freeze-substitution and low-temperature embedding protocol:

- Protects the structure of dairy constituents



Leica EM PACT

Cryo-fixation



High-pressure freezing:

- Samples are frozen in liquid nitrogen (-196°C) at high-pressure (2100 bars)
- Advantage: at 2 100 bars the melting point of water decreases because the water becomes 1 500 times more viscous. The increase in viscosity reduces the ice crystal growth rate.



Leica EM AFS2

Freeze-substitution



- The sample is immersed in a **solvent**
- Substitution of **ice** by the **solvent**

Embedding



The **solvent** is replaced with a low-temperature **resin**:

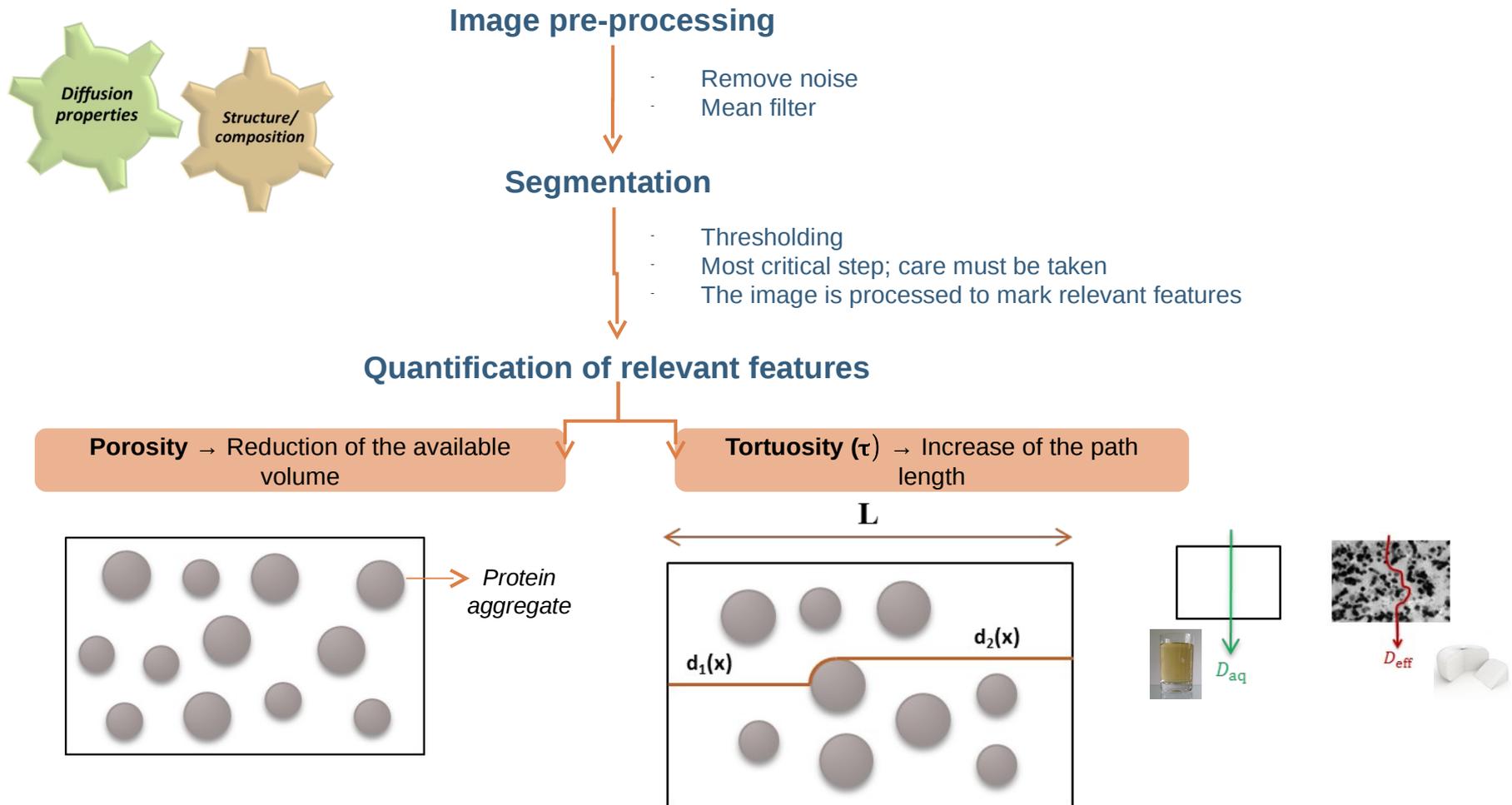
- Ethanol 3:1 resin
- Ethanol 1:1 resin
- Ethanol 1:3 resin
- 100% resin

Sectioning

Thin sections (**90nm thick**) of embedded material are cut with a diamond knife using a Reichert ultramicrotome

Materials and Methods

Quantitative analysis of the microstructure of the UF-Model Cheese



Shortest path containing a point x in the matrix, connecting two opposite edges of the image (Delarue et al. , 2003).

Results and Discussion

Influence of different physicochemical characteristics of solutes on diffusion in the UF-model cheese

> Influence of the solute size...

FITC-dextrans (4 kDa to 2 MDa)
- Flexible and neutral solutes

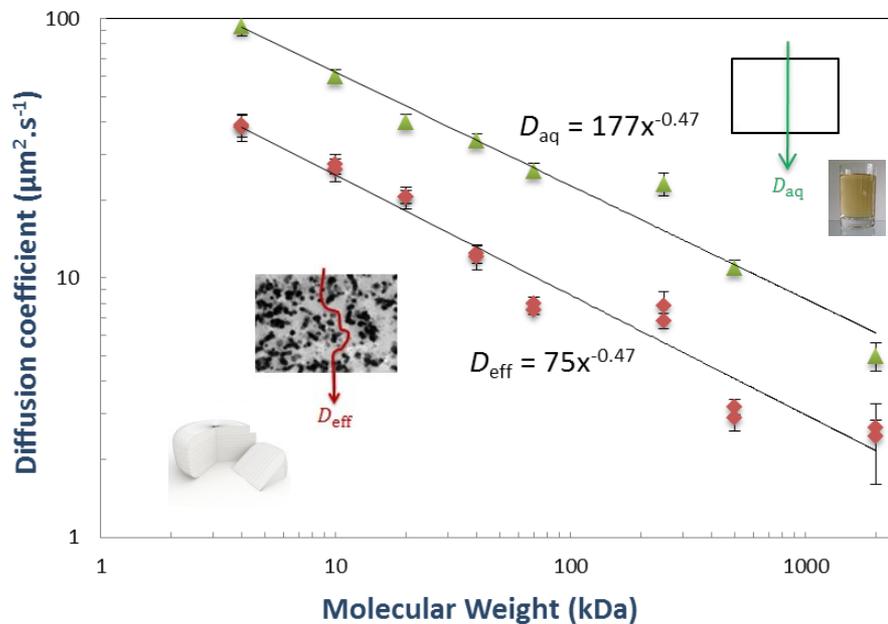


Figure 1. Logarithmic plots of experimental diffusion coefficients of FITC-dextrans in the UF-model cheese (□) and in the ultrafiltrate (□) as a function of their molecular weight.

- The **larger FITC-dextrans** were **no more hindered** by the structure of the UF-model cheese when compared to the smaller ones.
- The mobility of FITC-dextrans in the UF-model cheese was reduced by a **constant obstruction factor** ($k = 0.42$).
- The **pore sizes** of this model cheese were believed to be sufficiently large when compared to the **size of the solute molecules**.

Results and Discussion

Influence of different physicochemical characteristics of solutes on diffusion in the UF-model cheese

- > Influence of the **shape** and **charge** of solutes...

FITC-labeled dairy proteins (α -LA, β -LG and BSA) 
 - Rigid and negatively charged solutes

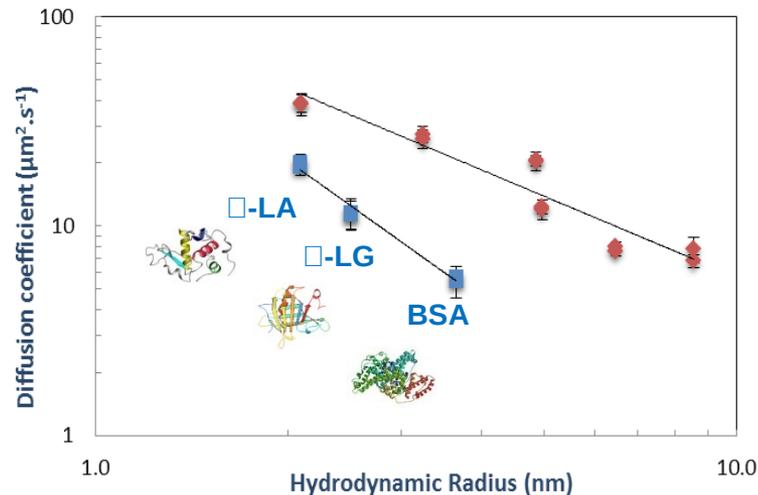
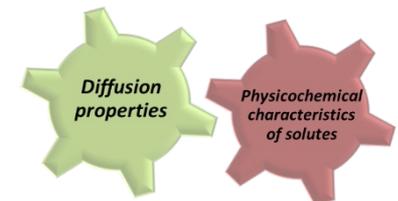


Figure 2. Logarithmic plots of experimental diffusion coefficients of FITC-dextran (\square) and FITC-dairy proteins (\square) in the UF-model cheese as a function of their hydrodynamic radius.

Rigid and negatively charged solutes were **hindered more** than the **flexible and neutral solutes** in the UF-model cheese

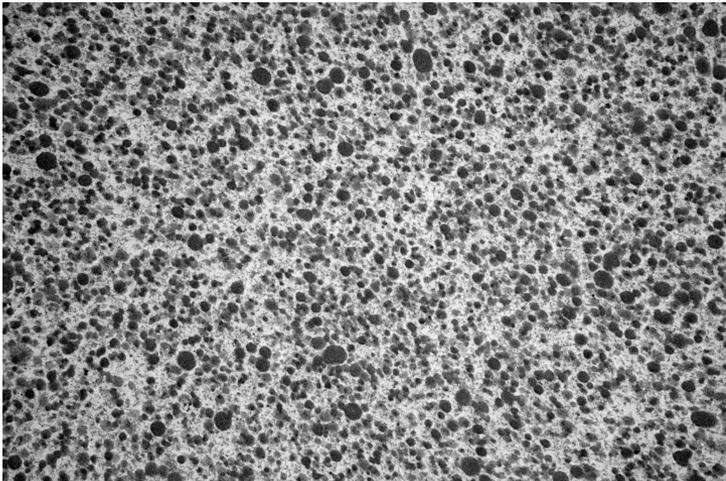
- The existence of steric and electrostatic interactions between the **protein matrix of the UF-model cheese** and the **FITC-dairy proteins** is suggested.
- The **diffusion through the protein aggregates** could play an important role in the overall diffusion of the **FITC-dairy proteins**, retarding their movement when they diffuse in the UF-model cheese.



Results and Discussion

Influence of the microstructure of the UF-model cheese on diffusion of solutes

Image Analysis of the UF-model cheese

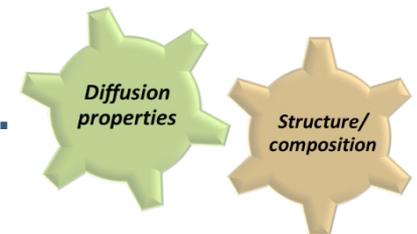


TEM micrograph of the UF-model cheese



Segmented (binary) image of the UF-model cheese

Quantification of relevant features...

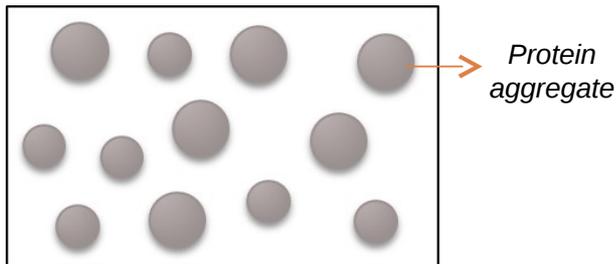


Results and Discussion

Influence of the microstructure of the UF-model cheese on diffusion of solutes

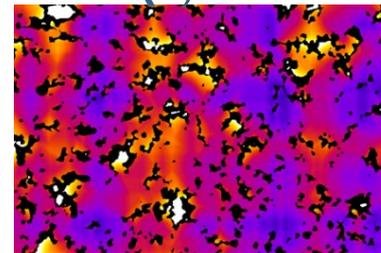
- Image Analysis of the UF-model cheese

Porosity (ϵ)



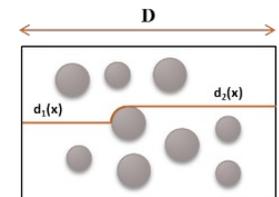
$$\epsilon = 0.638 \pm 0.007$$

Tortuosity (τ)



Tortuosity distribution in the UF-model cheese.
Blue zones: low tortuosity. Yellow/orange zones: high tortuosity

$$\tau = 1.085 \pm 0.008$$

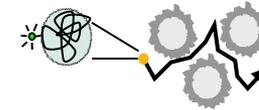


Under way...

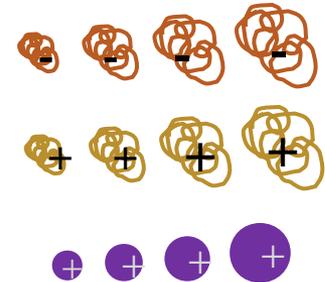
To establish the relationship between these **microstructural parameters** and **diffusion properties** by comparing results obtained in **model matrices** with **different microstructures**.

Conclusions and Perspectives

- ❖ The **physicochemical characteristics** of solutes influence their diffusion in the UF-model cheese



- ❖ The **diffusion of other solutes** (flexible and negatively charged, rigid or flexible positively charged) may be studied to elucidate the **solute-matrix interactions**



- ❖ Studies of the **diffusion** and **structure** in **other model cheese matrices** are required to the development of **predictive models** of diffusion of solutes



- ❖ A better understanding of the influence of the **matrix microstructure** on the **diffusion of solutes** should allow a more generic view of **cheese ripening kinetics** for future innovations



Acknowledgements

My research team

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- Sylvie Lortal
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- Sophie Jeanson
- Valérie Gagnaire
- Chantal Cauty



- Platform MRic



- David Legland





Thank you for your attention!

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