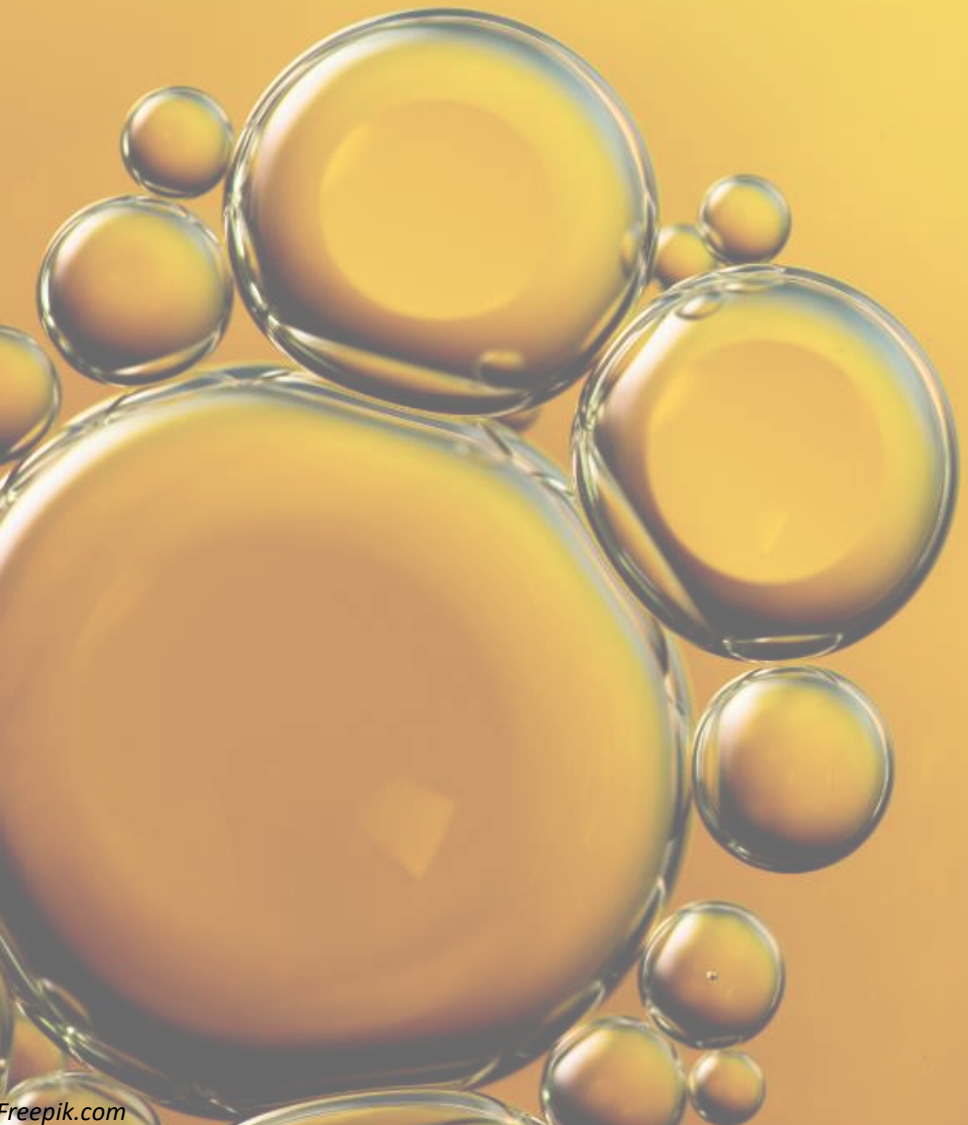
Endogenous lipids: disregarded co-passengers shaping the emulsifying and interfacial properties of pea and lupin protein ingredients

Eléna Keuleyan, Jeanne Kergomard, Adeline Boire, Elisabeth David-Briand, Veronique Vie, Anne Meynier, Alain Riaublanc, Claire Berton-Carabin



Freepik.com

2024 AOCS Annual Meeting & Expo

April 29th, 2024

Endogenous lipids: disregarded co-passengers shaping the emulsifying and interfacial properties of pea and lupin protein ingredients

Eléna Keuleyan[a], Jeanne Kergomard[a], Adeline Boire[a], Elisabeth David-Briand[a], Véronique Vié[b,c], Anne Meynier[a], Alain Riaublanc[a], Claire Berton-Carabin[a,d]

[a] *INRAE, UR BIA, Nantes, France*
[b] *Université de Rennes, CNRS, Rennes, France*
[c] *Univ Rennes, CNRS, ScanMAT - UMS 2001, Rennes, France*
[d] *Wageningen University & Research, Laboratory of Food Process Engineering, The Netherlands*

Protein transition

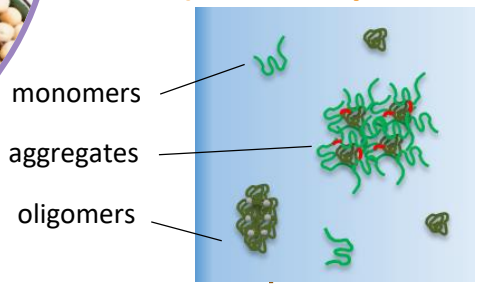
Protein fractionation

Vogelsang-O'Dwyer, *et al.* (2021) *Trends in Food Science and Technology*, 110(01)

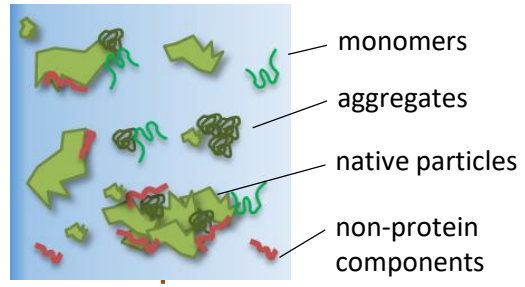
Schmitt, *et al.* (2021) *Current Opinion in Colloid and Interface Science*, 56

Schutyser and van der Goot (2011) *Trends in Food Science and Technology*, 22(4)

Protein isolates
(> 80 wt.% proteins)



Protein concentrates
(> 50 wt.% proteins)



Emulsion

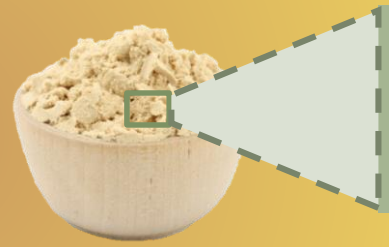
Pea

Seed composition

14 - 40% (d.m.) proteins

1 - 4% (d.m.) lipids

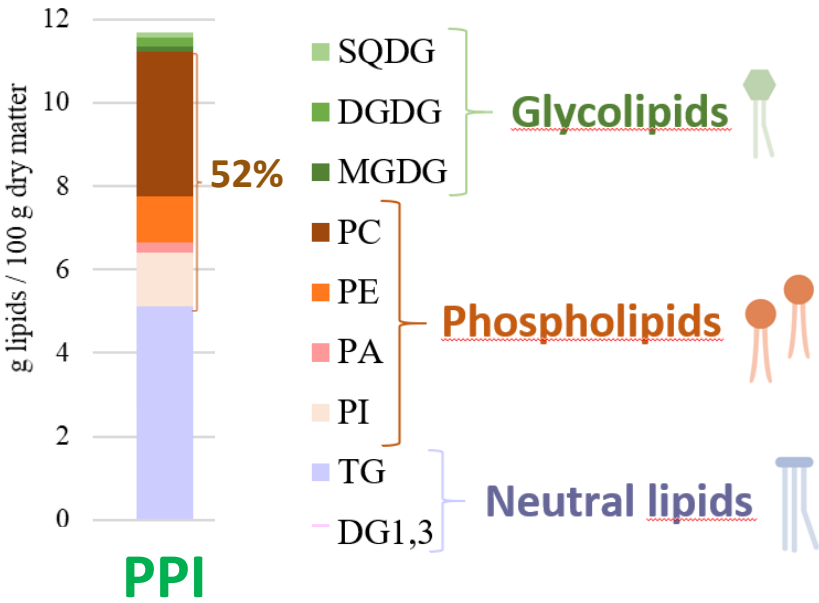
To characterize the fine chemical composition
of commercial ingredients



Proteins
In their colloidal state: native, aggregates…

Non-proteinaceous compounds
Focus on endogenous lipids

Chloroform ($CHCl_3$) / methanol (MeOH) extraction adapted for protein powders



Surface-active properties

ELSEVIER
Food Hydrocolloids
Volume 141, August 2023, 108671

Pea and lupin protein ingredients: New insights into endogenous lipids and the key effect of high-pressure homogenization on their aqueous suspensions

Eléna Keuleyan [a], Perrine Gélébart [a], Valérie Beaumal [a], Alice Kermarrec [a], Lucie Ribourg-Birault [a], Sophie Le Gall [a b], Anne Meynier [a], Alain Riaublanc [a], Claire Berton-Carabin [a c]

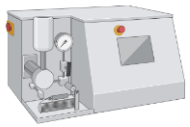
To understand the functionalities of the ingredients and how we can enhance them

High-pressure homogenization

Powders

pH 7 ; 90 mM NaCl ; 10 mM phosphate

Non-treated protein suspension

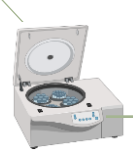


High-pressure homogenization

300 bars

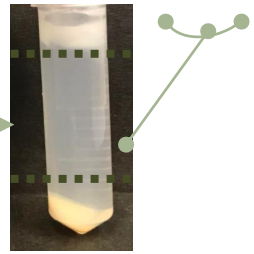
3 min

HPH-treated protein suspension

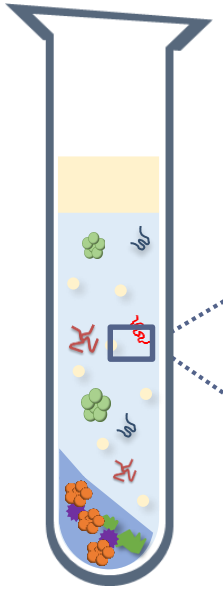


20 000g

30 min



PPI - HPH



HPH-treated soluble fraction

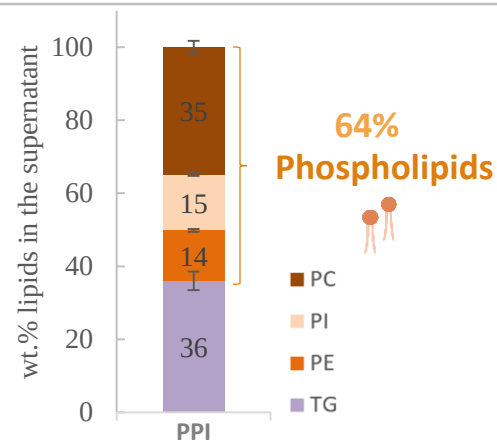
Protein solubility

Lipid content

Lipid extraction

0.86 ± 0.02 mg lipids / g supernatant

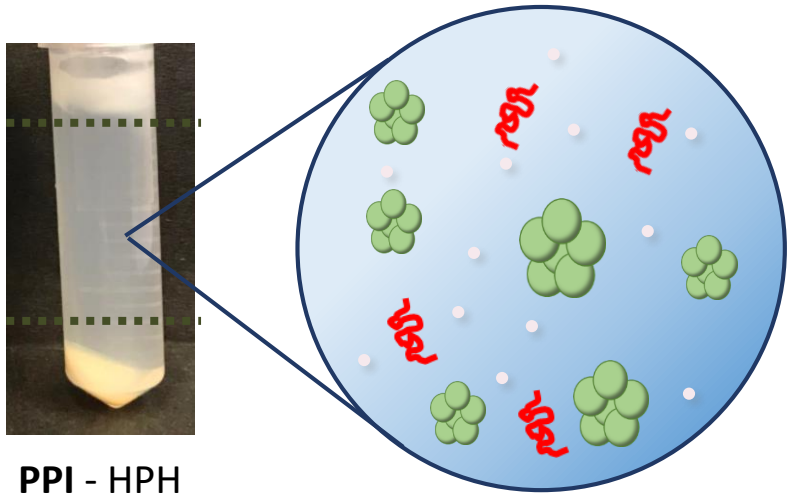
Protein-to-lipid ratio **6 : 1**



64% Phospholipids

HPH helps to release endogenous lipids from the grain powders to the "soluble" phase.

The lipid fraction is representative of the total lipid content



PPI - HPH

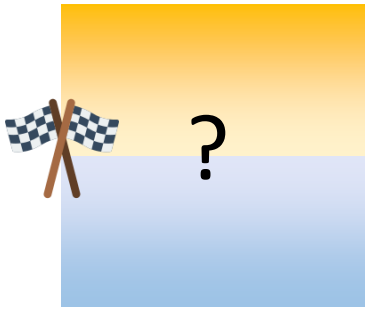
Protein aggregates

Native proteins

Lipid assemblies

+

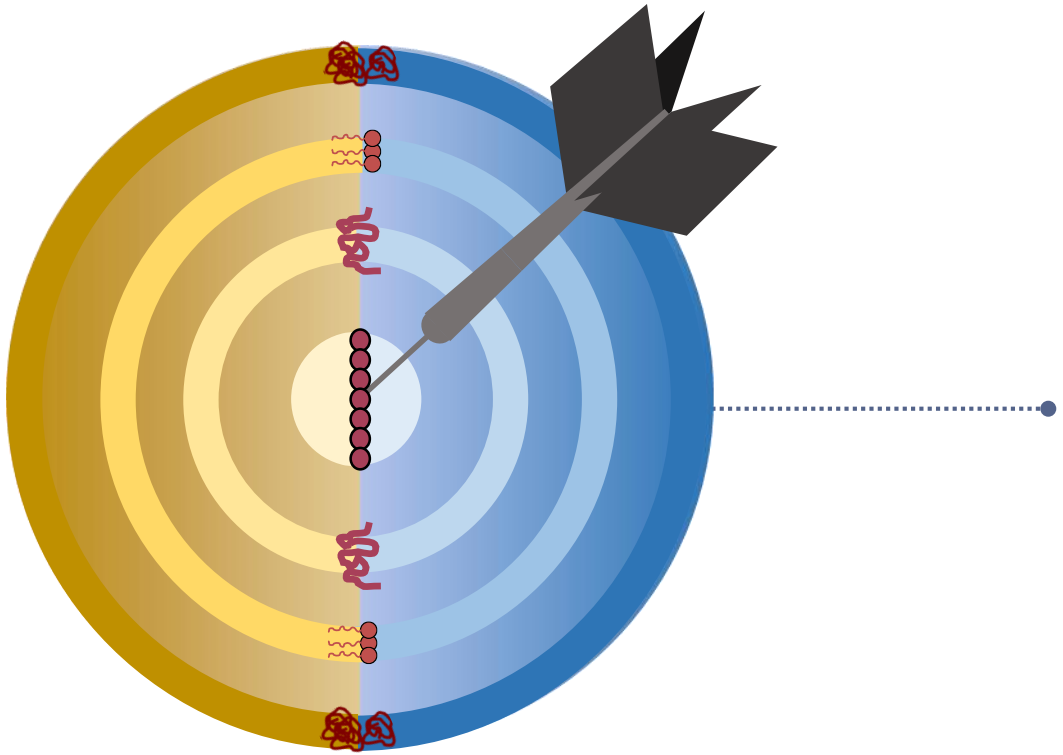
Freepik.com



Mackie, A. R. *et al*. (2000), *Langmuir*, 16

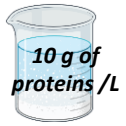
Bos, A. M. and van Vliet, T. (2001), *Advances in Colloid and Interface Science*, 91

Wilde, P. *et al.* (2004) , *Advances in Colloid and Interface Science*, 108–109



The aim of this work is to investigate the **possible competition** between endogenous **polar lipids** and **proteins** from a commercial pea protein isolate (PPI) for **interfacial adsorption**

PPI

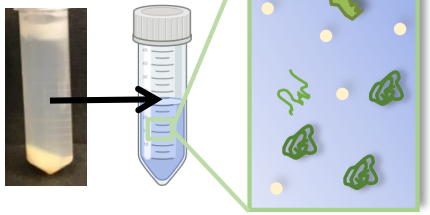


Non-treated protein suspension

300 bars
3 min

HPH-treated protein suspension

20 000g
30 min



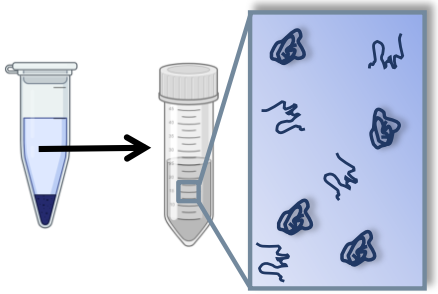
Protein to lipid mass ratio of ***6 : 1***

7S pea proteins

Purified pea vicilins and convicilins

Overnight hydration

20 000g
30 min



Lipids

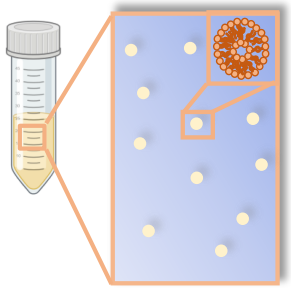
PPI soluble fraction

$CHCl_3$/MeOH extraction

Chloroformic lipid extract

Dispersion in phosphate buffer

Rotor stator
6 000 rpm
30 sec



Hyp. : Lipids self assemble in ***vesicles***

Protein : lipid mixes

1:6

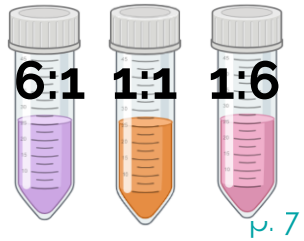
7S + lipids

1:1

7S + lipids

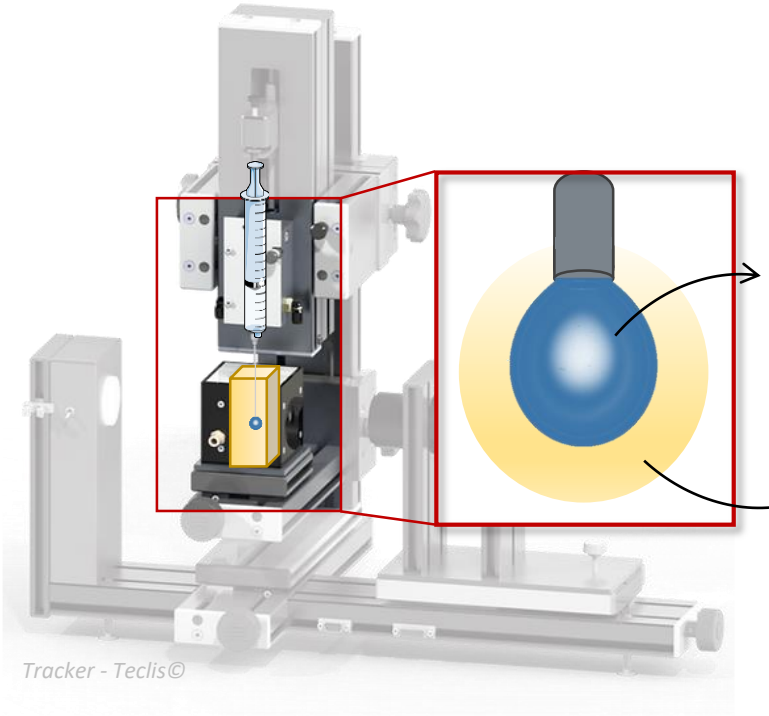
6:1

7S + lipids

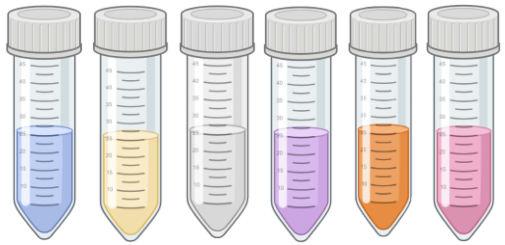


Drop tensiometry

Automated drop tensiometer



Aqueous phase
3 concentrations

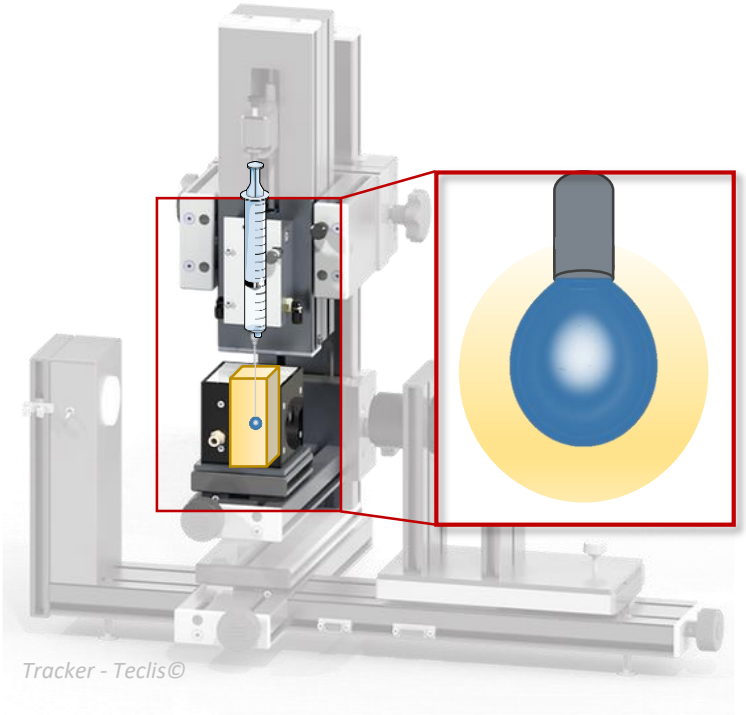


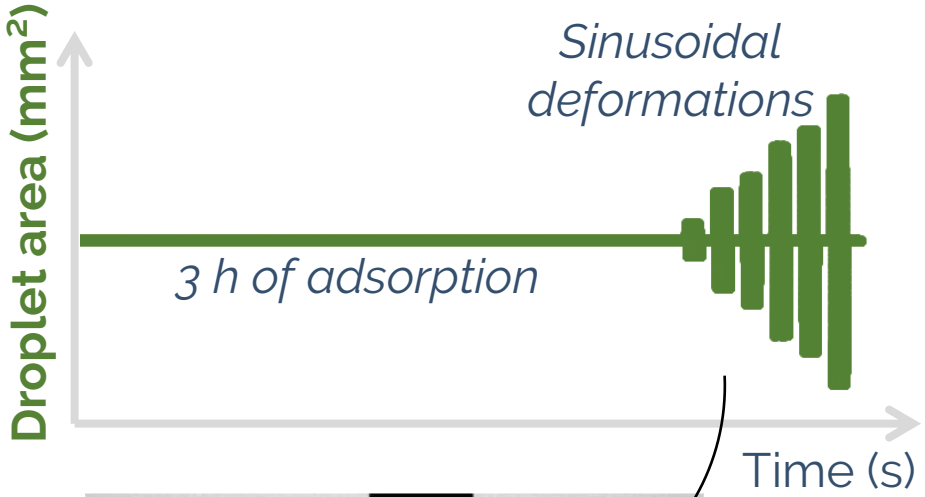
Stripped rapeseed oil

Drop tensiometry

Amplitude sweeps are performed : applying fluctuations of the interface area at a constant frequency (0.02 Hz)

Automated drop tensiometer

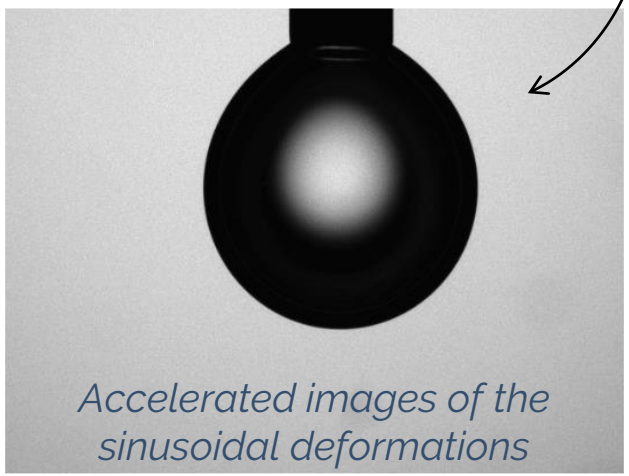




Monitoring of droplet area (mm²)

↓

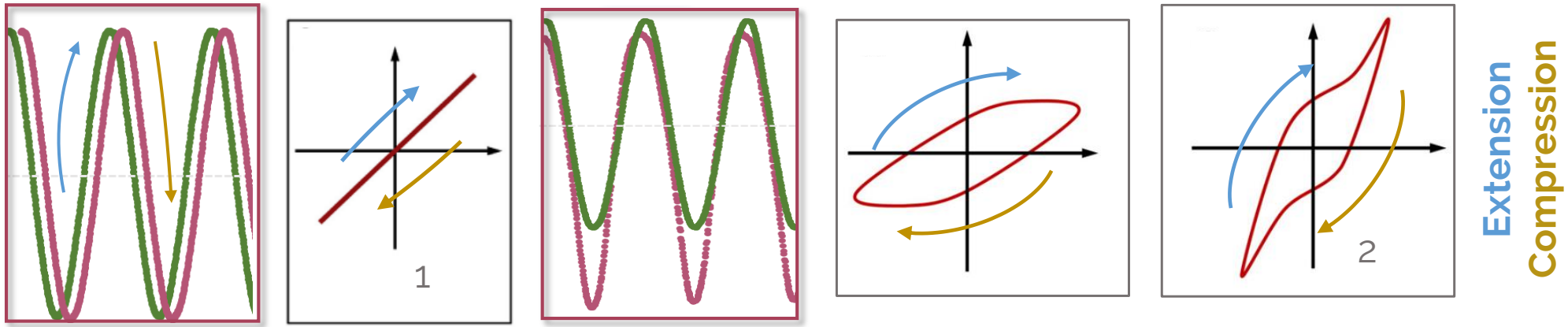
Recording of interfacial tension (mN/m)



Accelerated images of the sinusoidal deformations

Drop tensiometry

Lissajous plots represent **the amplitude of the interfacial response** $(\Delta\pi = \gamma - \gamma_0)$ as a function of **the deformation amplitude** $\Delta A = \frac{A - A_0}{A_0}$

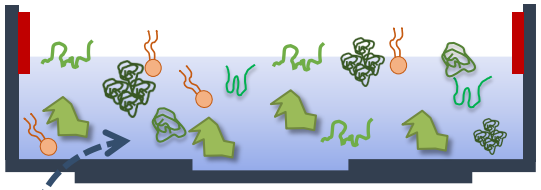


Linear regime | **Elastic** | Non linear regime | **Strain softening** | **Strain hardening**

1: Sagis *et al.*, 2014, *COCIS*, 19(6)
2: Sagis et al., 2014, *TFST*, 37(1)

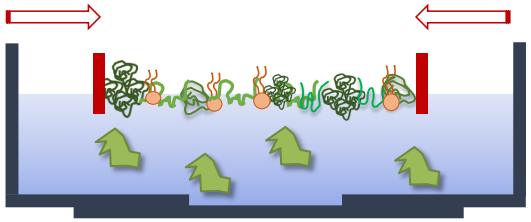
Atomic force microscopy (AFM)

1. The aqueous solutions are poured into the Wilhelmy balance

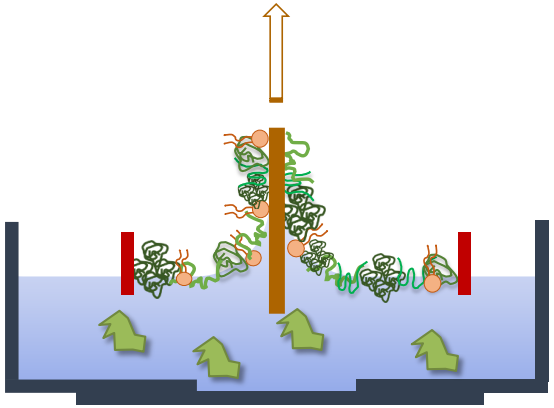


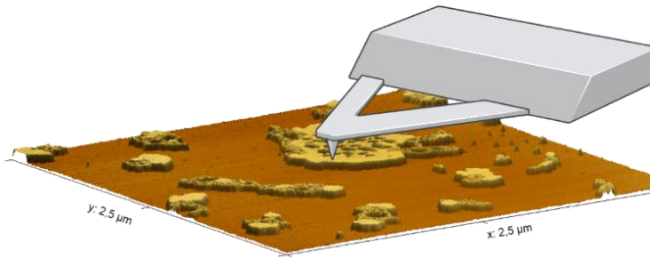
Aqueous phase

2. The barriers stabilize the film at the surface pressure reached after **3 h** of adsorption

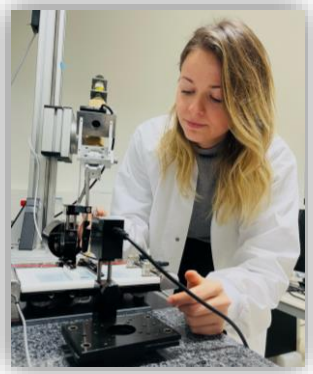


3. The air-water interfacial film is transfered on **a mica sheet** at constant surface pressure

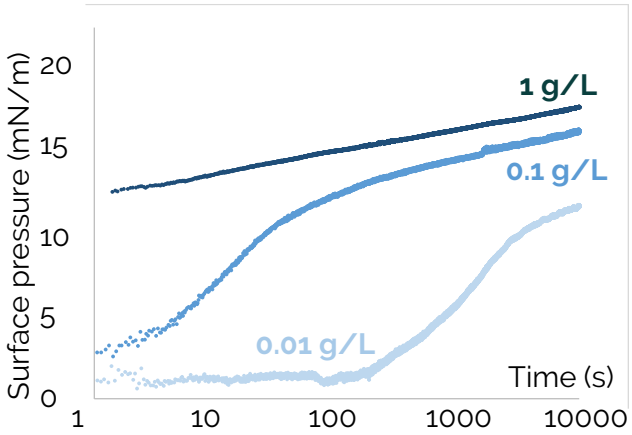




The **topography** of the air-water film is analyzed with **atomic force microscopy**



Experiments carried out by **Jeanne Kergomard** at the **Institute of Physics of Rennes**

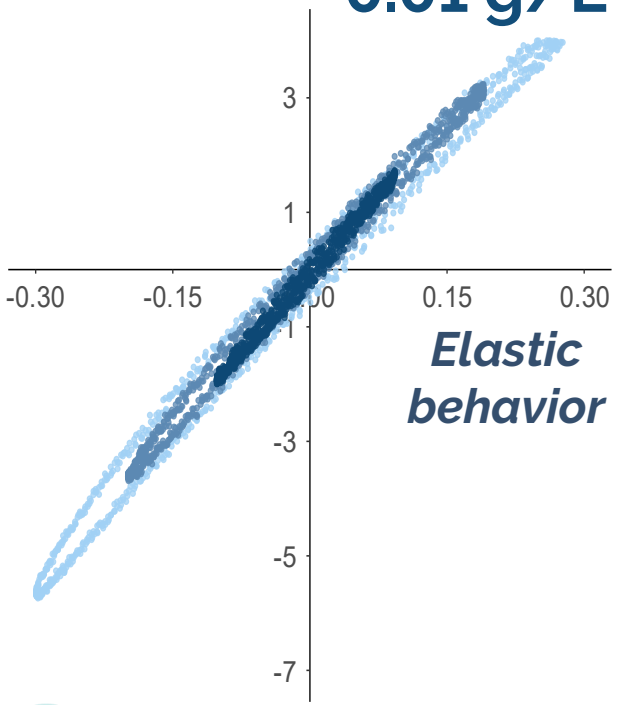


PPI | Purified components | Protein to lipid mixes | AFM

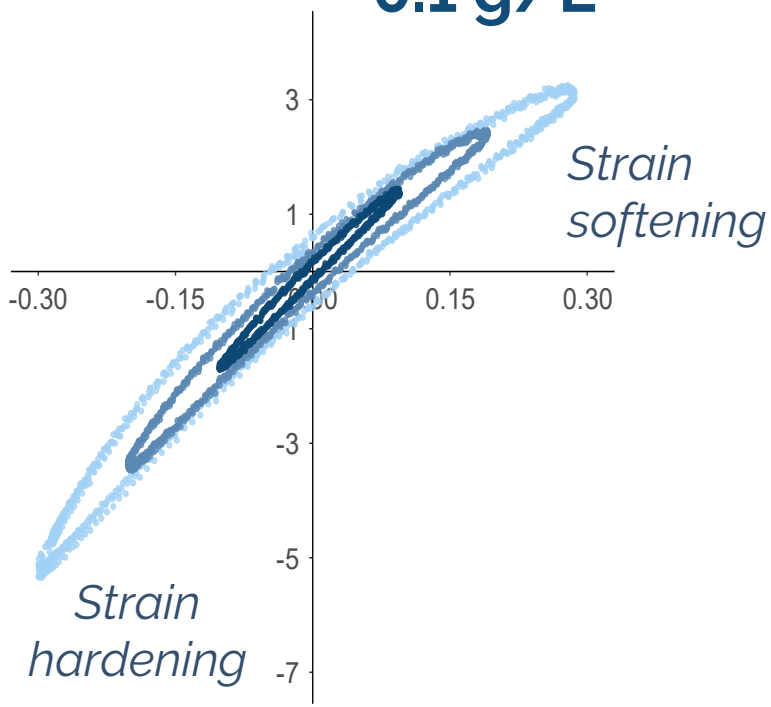
Observation of a **concentration dependency** of the rheological behavior, with an **increase in non-linearities**

Suggesting **different interfacial film composition**

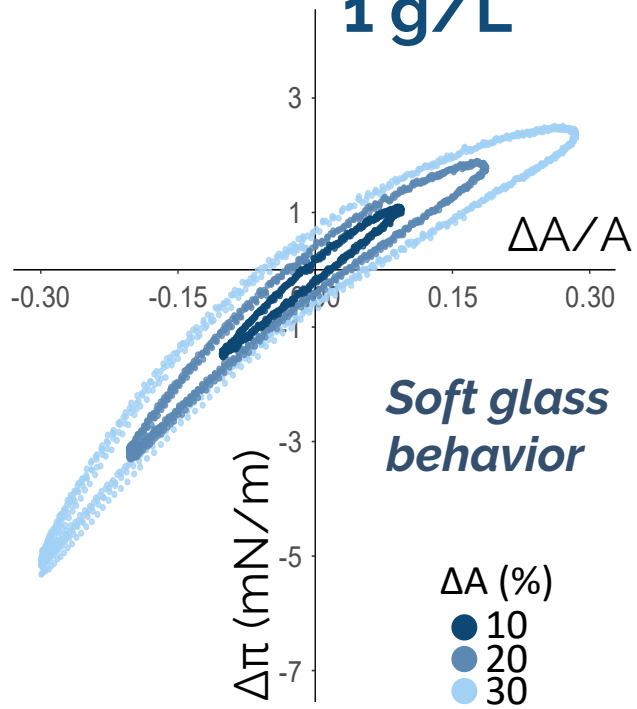
0.01 g/L



0.1 g/L

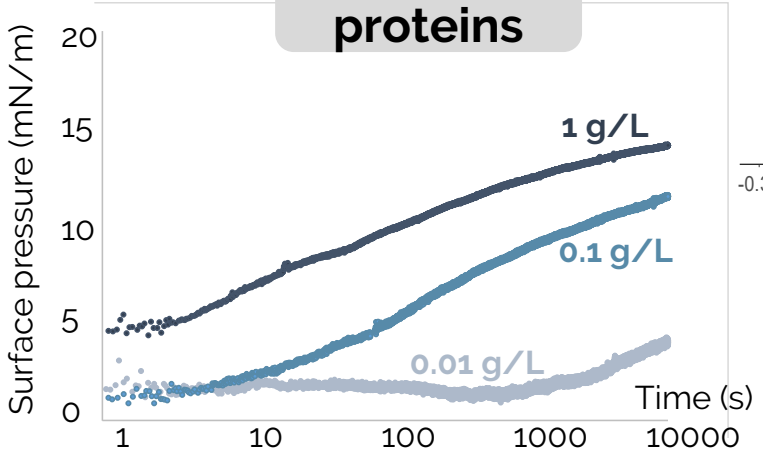


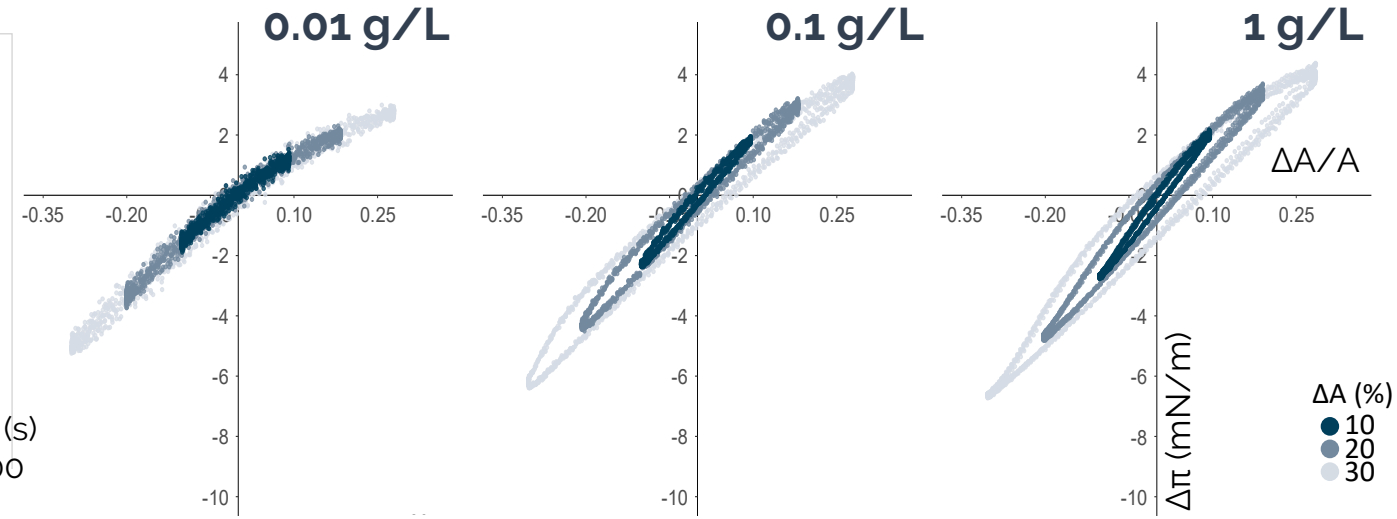
1 g/L



PPI | Purified components | Protein to lipid mixes | AFM

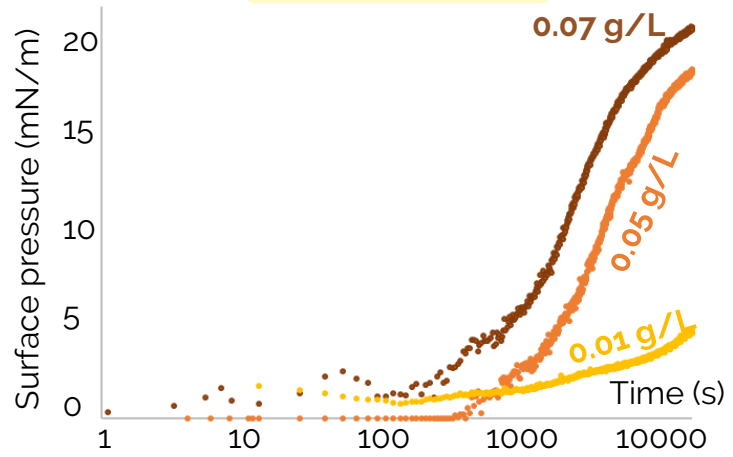
7S pea proteins

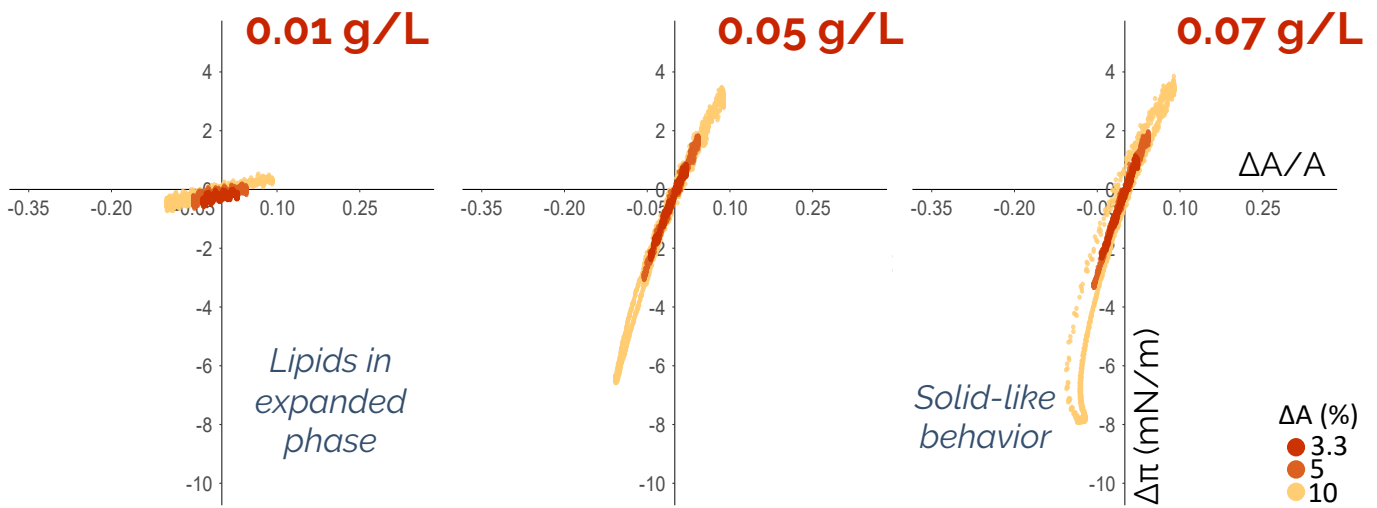




Limited asymmetric behavior: the applied deformation does not strongly affect the interfacial film.

Lipids

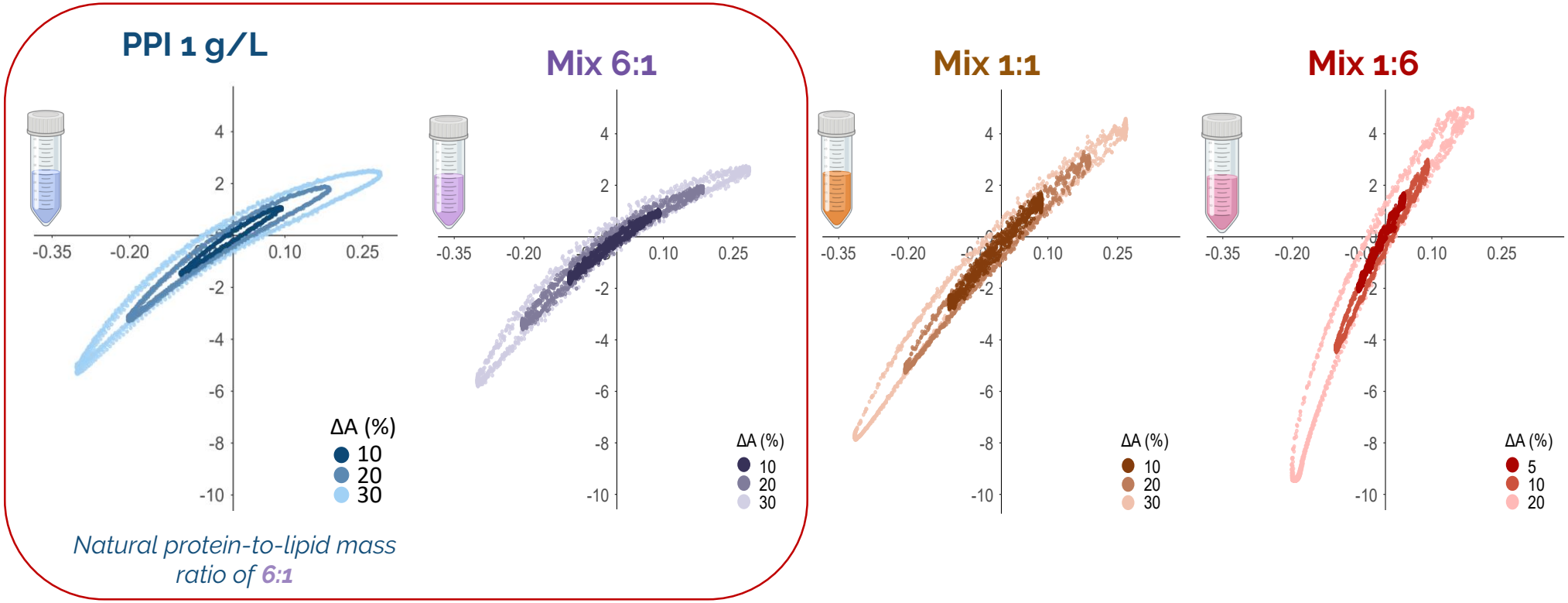




Interface extremely **stiff**, for very low concentrations.

Very strong resistance against the deformation.

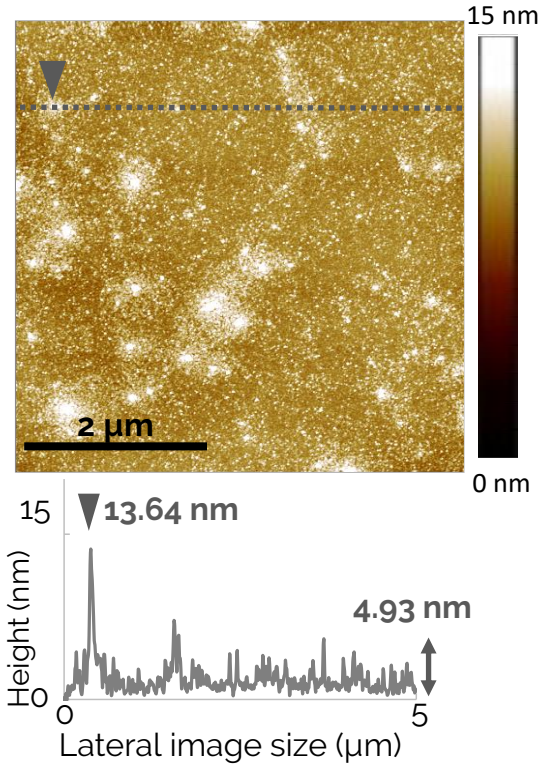
PPI | Purified components | Protein to lipid mixes | AFM



*Natural protein-to-lipid mass ratio of **6:1***

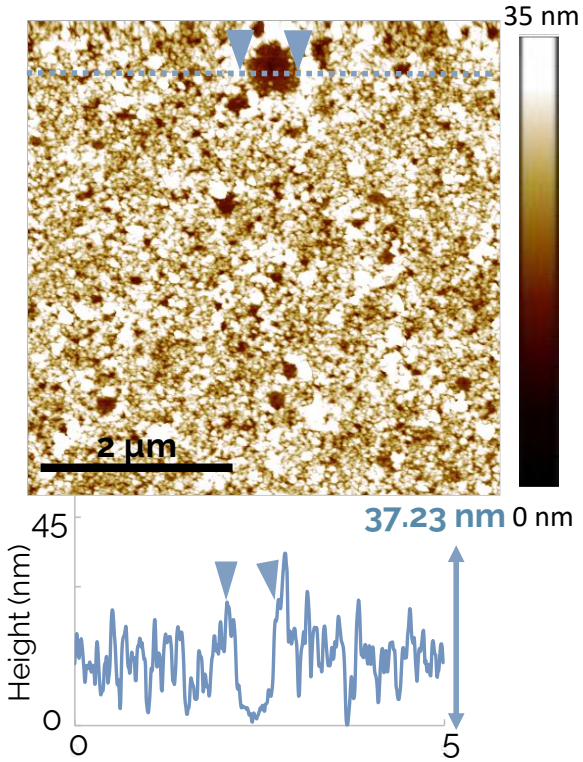
Close signatures between PPI (1 g/L) and the 6:1 system: the interfacial film of PPI **comprises both proteins and phospholipids, competing** for interfacial adsorption.

7S – 0.1 g/L



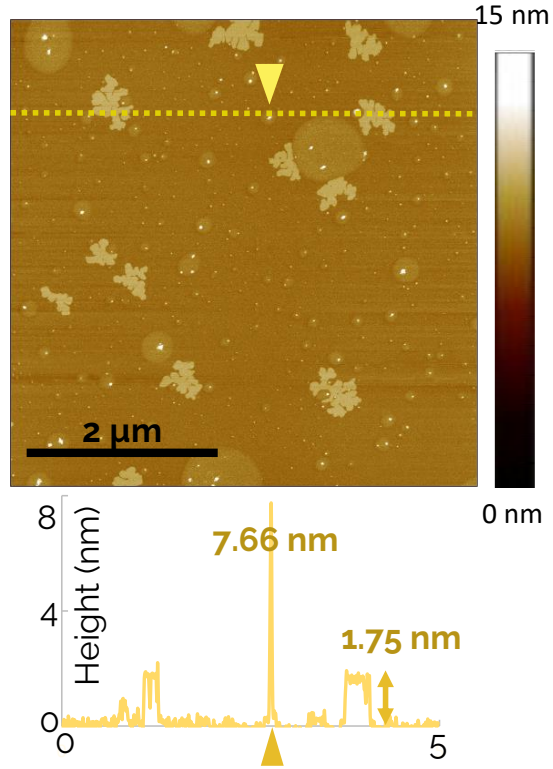
Homogeneous film, with **protein aggregates** all over the sample.

PPI – 1 g/L

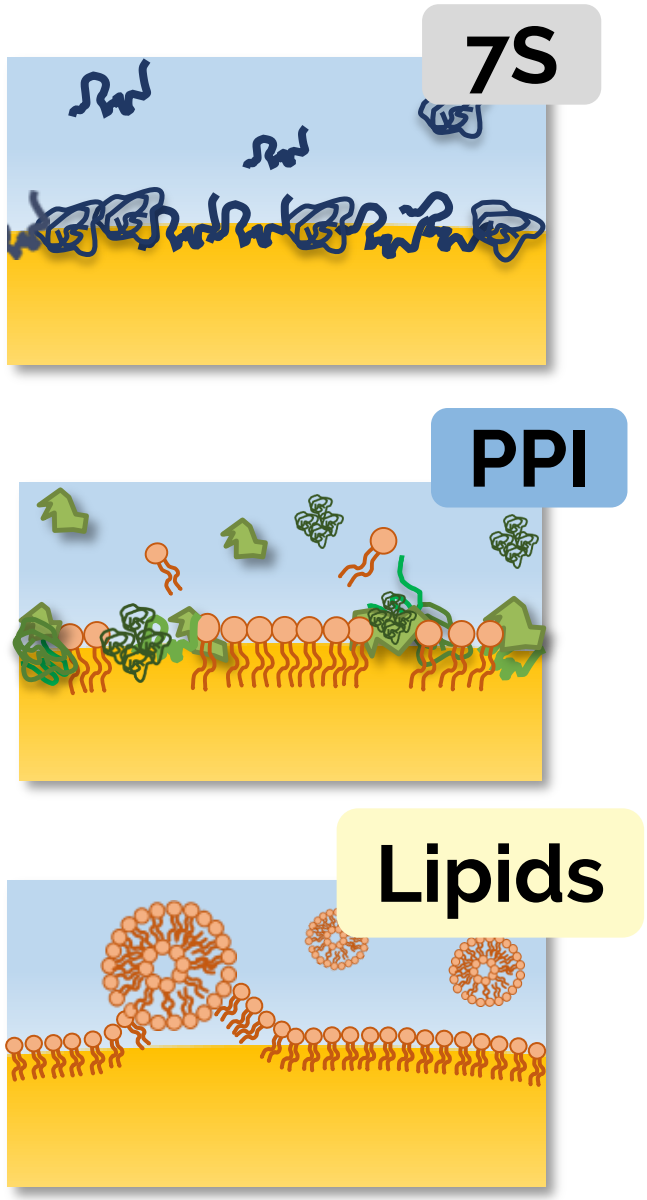


Very **high protein clusters (aggregates)** amongst which circular and low domains are forming « islands », very likely to be **lipid domains.**

Lipids – 0.07 g/L



Heterogeneous film, comprising a **fluid background**, **high clusters** (nuclei?) among **circular domains**, and **flower-shaped structures** (phospholipid monolayers?)



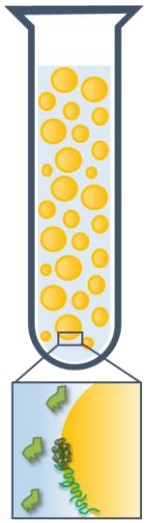
Proteins and **phospholipids** from a protein ingredient may adsorb and **co-exist** at fluid interfaces, forming complex and composite interfaces having **peculiar interfacial signatures**.

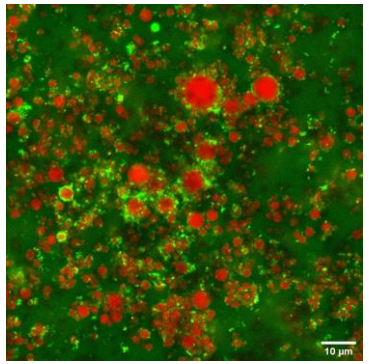
These results highlight that with a **rational use** of food processing combined with a deep compositional and functional characterization, emulsions' stabilization can be enhanced by benefiting from the **natural complexity** of plant protein ingredients.

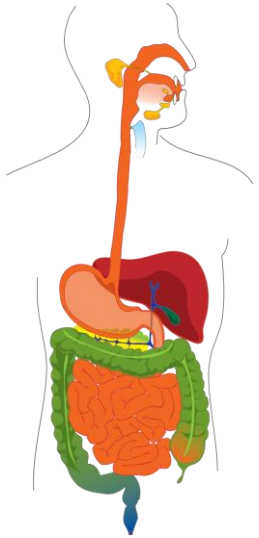
Top-down approach from the **formulation** to the **digestion** of food emulsions

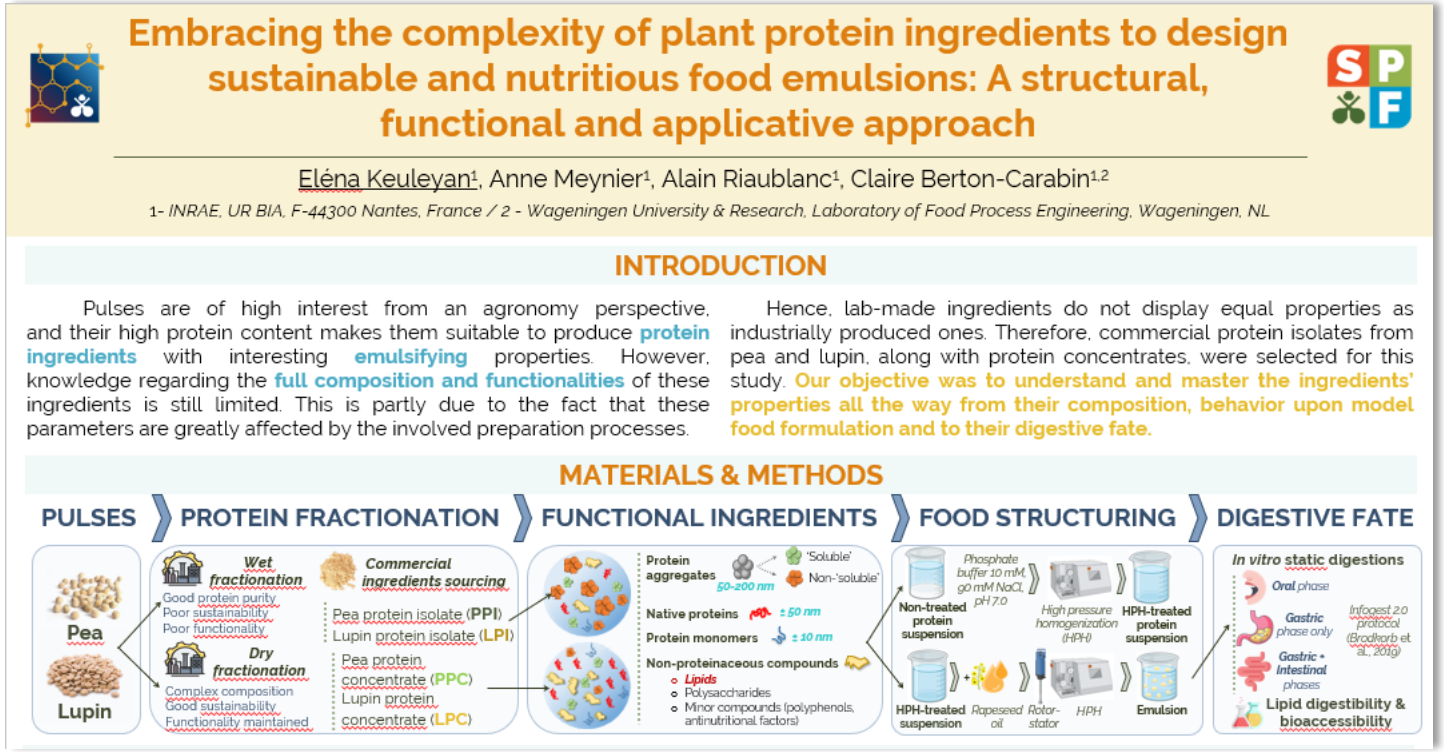
Pea

Lupin









Embracing the complexity of plant protein ingredients to design sustainable and nutritious food emulsions: A structural, functional and applicative approach

Eléna Keuleyan[1], Anne Meynier[1], Alain Riaublanc[1], Claire Berton-Carabin[1,2]

1- INRAE, UR BIA, F-44300 Nantes, France / 2 - Wageningen University & Research, Laboratory of Food Process Engineering, Wageningen, NL

INTRODUCTION

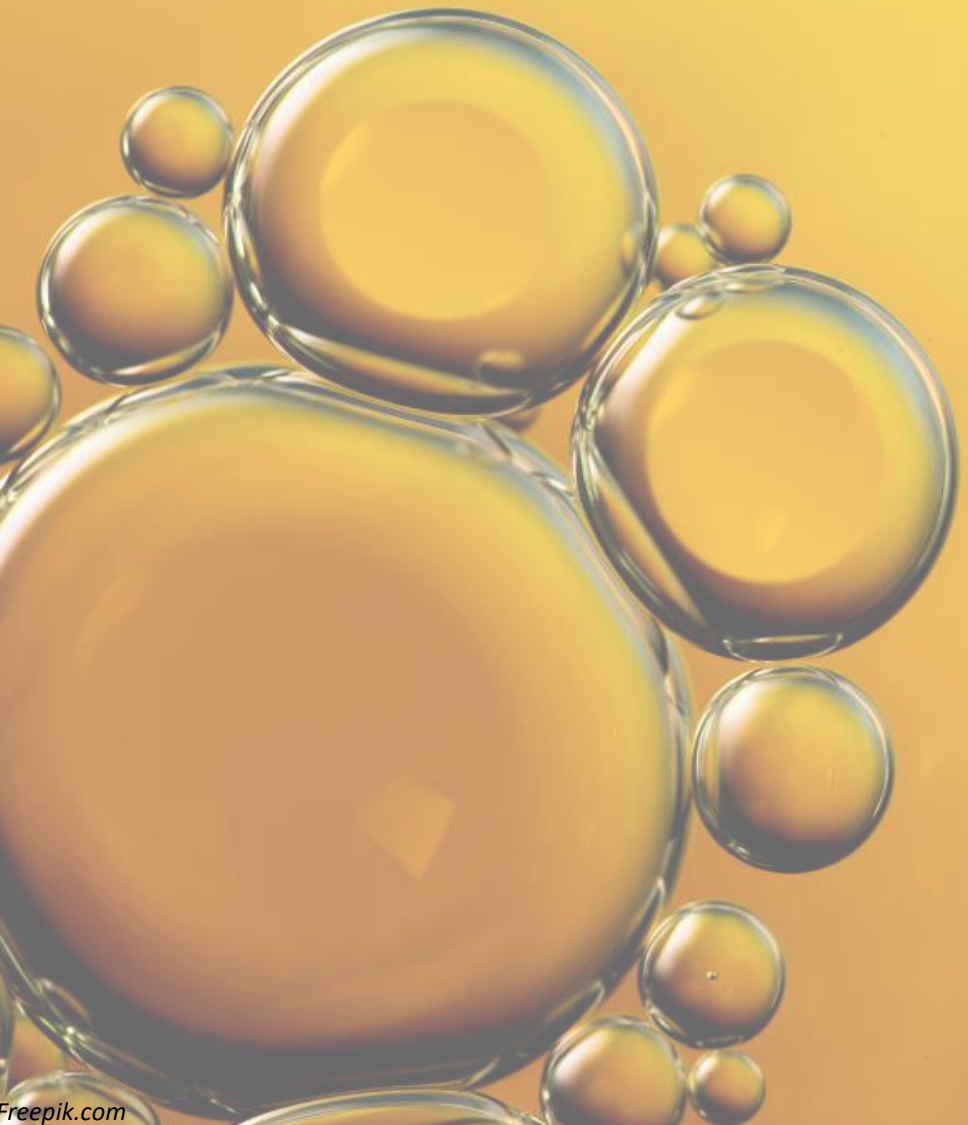
Pulses are of high interest from an agronomy perspective, and their high protein content makes them suitable to produce protein ingredients with interesting emulsifying properties. However, knowledge regarding the full composition and functionalities of these ingredients is still limited. This is partly due to the fact that these parameters are greatly affected by the involved preparation processes.

Hence, lab-made ingredients do not display equal properties as industrially produced ones. Therefore, commercial protein isolates from pea and lupin, along with protein concentrates, were selected for this study. Our objective was to understand and master the ingredients' properties all the way from their composition, behavior upon model food formulation and to their digestive fate.

MATERIALS & METHODS

PULSES › PROTEIN FRACTIONATION › FUNCTIONAL INGREDIENTS › FOOD STRUCTURING › DIGESTIVE FATE

Sustainable protein forum poster session,
Tuesday, April 30, 5 pm



2024 AOCS Annual Meeting & Expo

April 29th, 2024

Thank you for your attention !

Eléna Keuleyan, elena.keuleyan@inrae.fr

Supervision:
Anne Meynier, Alain Riaublanc, Claire Berton-Carabin

Freepik.com