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## 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

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**2024 AOCS Annual Meeting & Expo**

April 29<sup>th</sup>, 2024

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

**Eléna Keuleyan<sup>a</sup>, Jeanne Kergomard<sup>a</sup>, Adeline Boire<sup>a</sup>, Elisabeth David-Briand<sup>a</sup>, Véronique Vié<sup>b,c</sup>, Anne Meynier<sup>a</sup>, Alain Riaublanc<sup>a</sup>, Claire Berton-Carabin<sup>a,d</sup>**

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<sup>d</sup> *Wageningen University & Research, Laboratory of Food Process Engineering, The Netherlands*

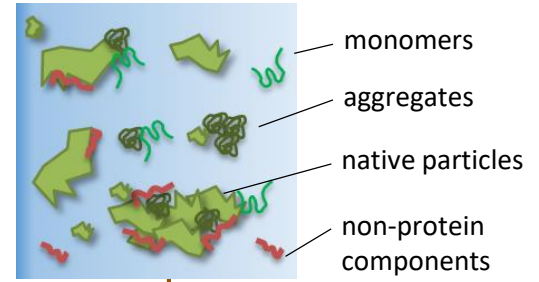
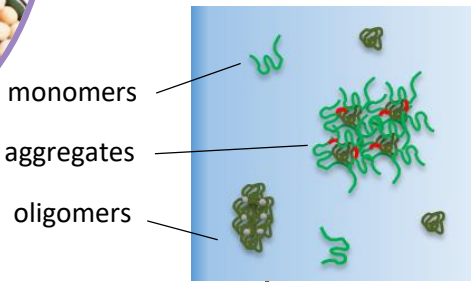


Protein transition



Protein isolates (> 80 wt.% proteins)

Protein concentrates (> 50 wt.% proteins)



Emulsion

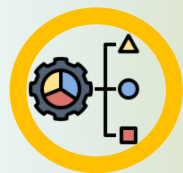


Pea  
Seed composition  
14 - 40% (d.m.) proteins  
1 - 4% (d.m.) lipids

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)



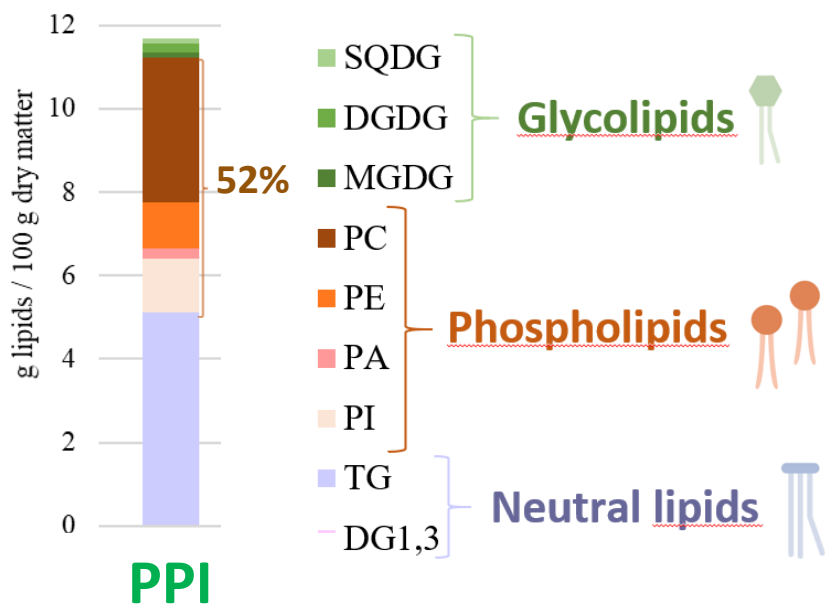
To characterize the fine chemical composition of commercial ingredients



Proteins  
In their colloidal state: native, aggregates...  
Non-proteinaceous compounds  
Focus on endogenous lipids



Chloroform (CHCl<sub>3</sub>) / methanol (MeOH) extraction adapted for protein powders



Surface-active properties



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 <sup>a</sup>, Perrine Gélébart <sup>a</sup>, Valérie Beaumal <sup>a</sup>, Alice Kermarrec <sup>a</sup>, Lucie Ribourg-Birault <sup>a</sup>, Sophie Le Gall <sup>a, b</sup>, Anne Meynier <sup>a</sup>, Alain Riaublanc <sup>a</sup>, Claire Berton-Carabin <sup>a, c</sup>







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



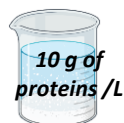
High-pressure homogenization →



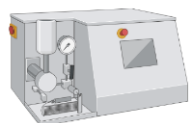
Powders



pH 7 ; 90 mM NaCl ;  
10 mM phosphate



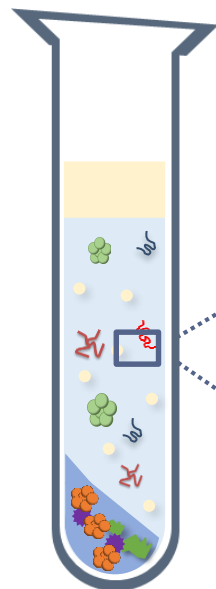
10 g of proteins /L  
Non-treated protein suspension



High-pressure homogenization  
300 bars  
3 min



HPH-treated protein suspension

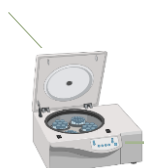


Protein solubility

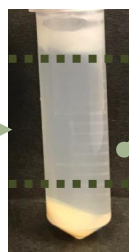
Lipid content



HPH-treated soluble fraction



20 000g  
30 min

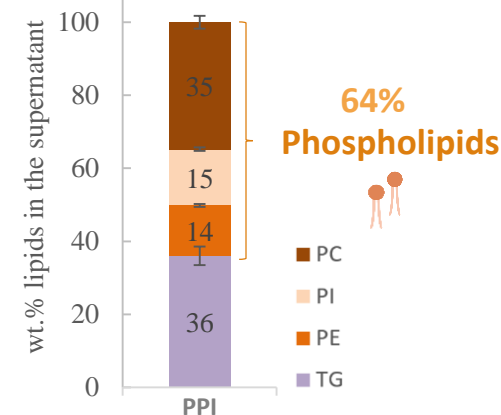


PPI - HPH

Lipid extraction

0.86 ± 0.02 mg lipids /  
g supernatant

Protein-to-lipid ratio  
6 : 1

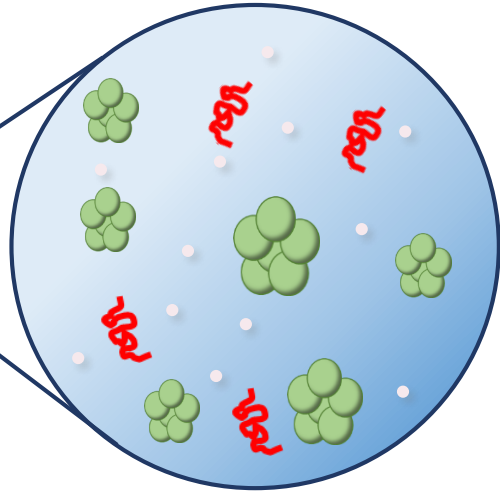





HPH helps to **release endogenous lipids** from the grain powders to the “soluble” phase.

The lipid fraction is **representative** of the total lipid content



PPI - HPH

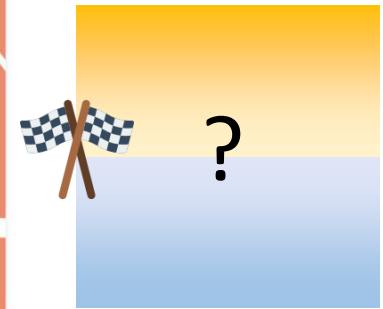


-  Protein aggregates
-  Native proteins
-  Lipid assemblies

+



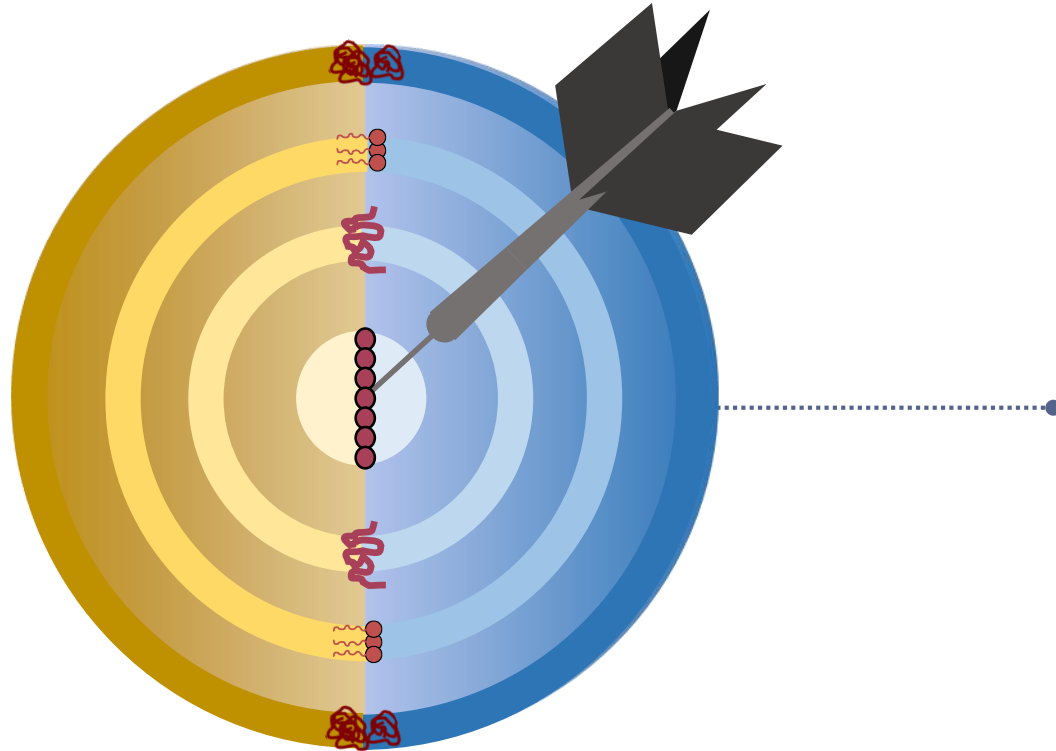
Freepik.com



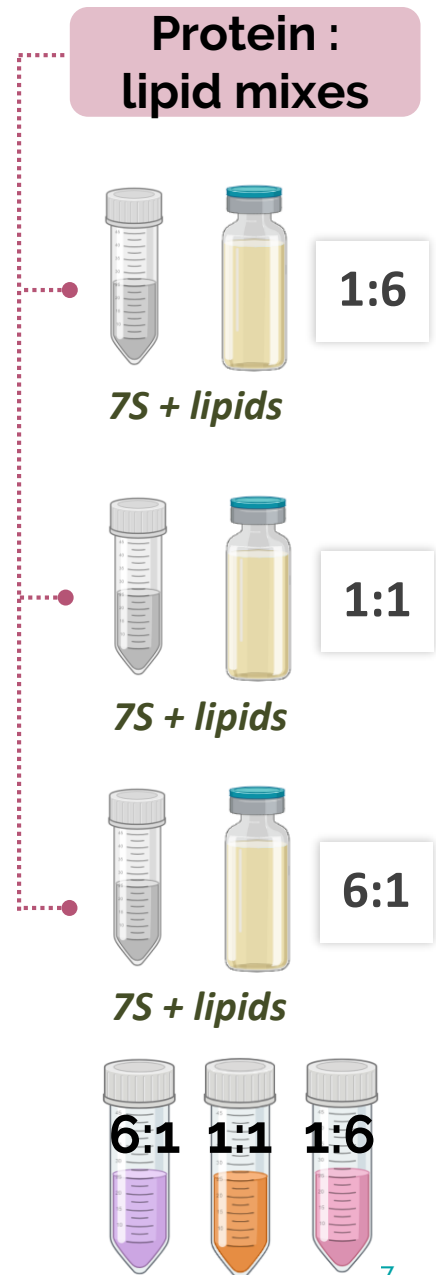
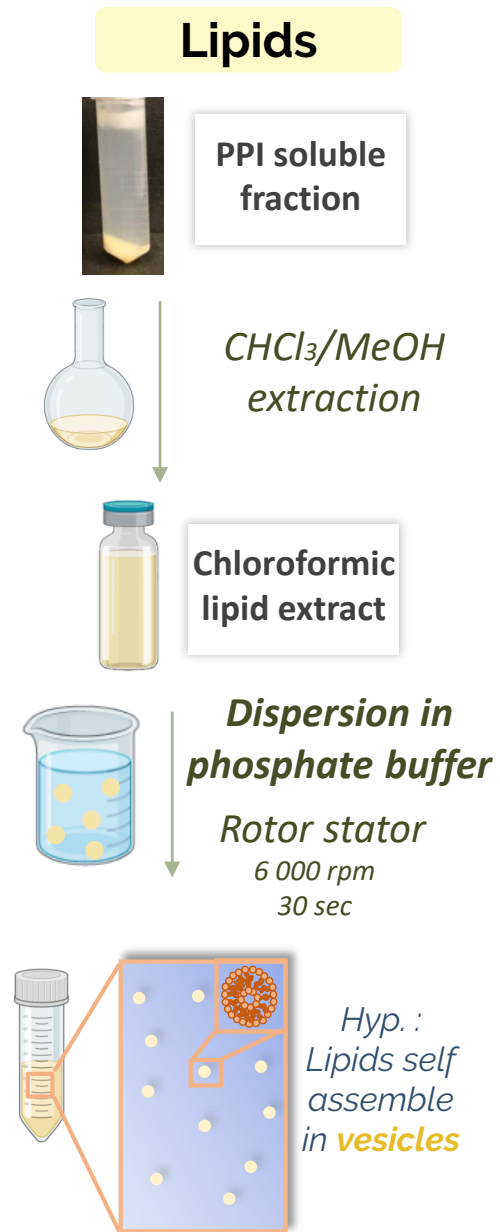
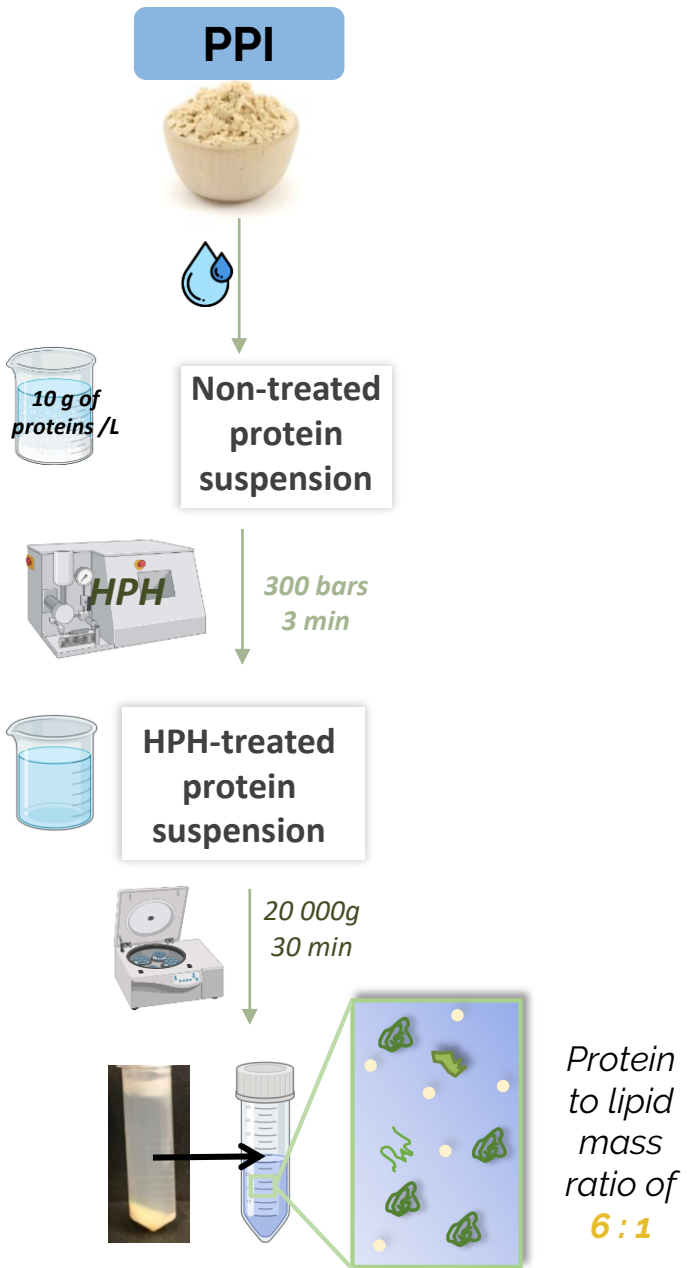
Mackie, A. R. *et al.* (2000), *Langmuir*, 16

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**

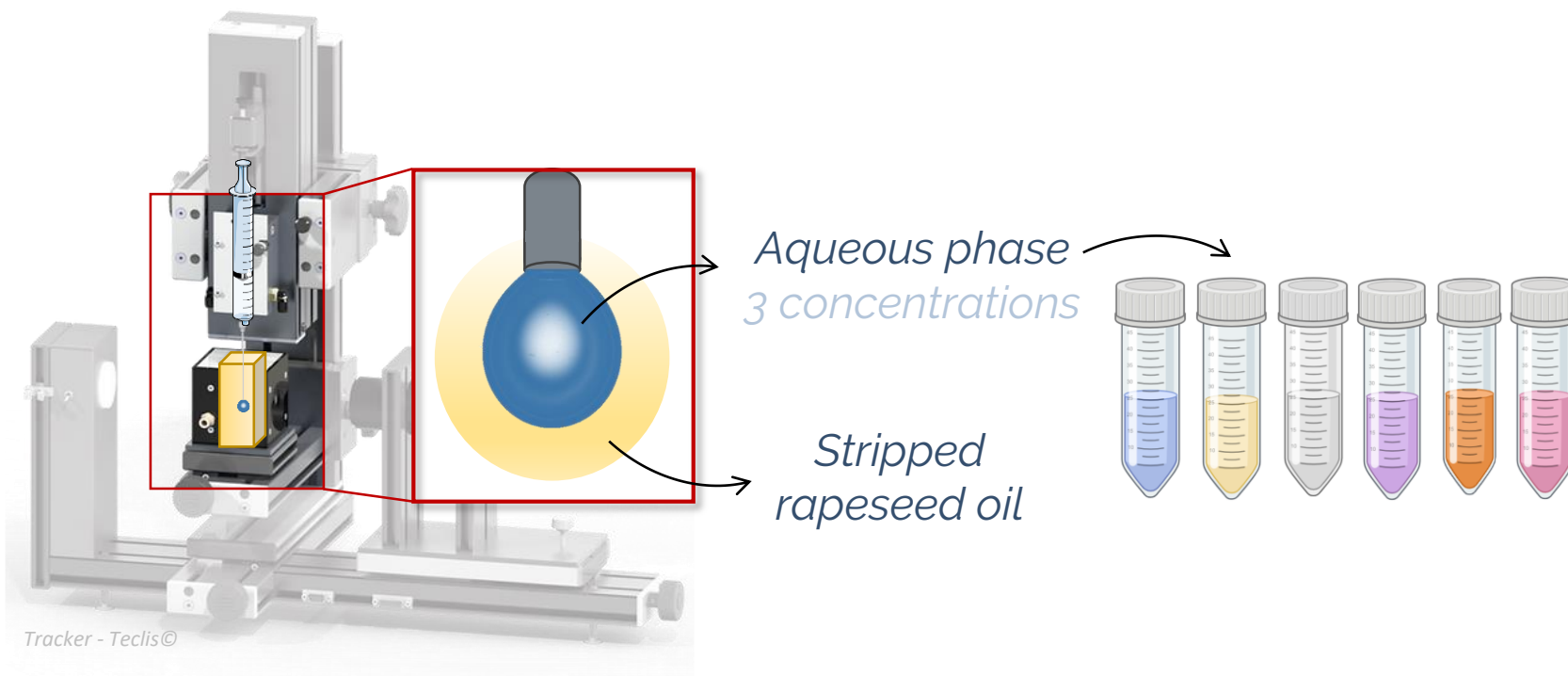






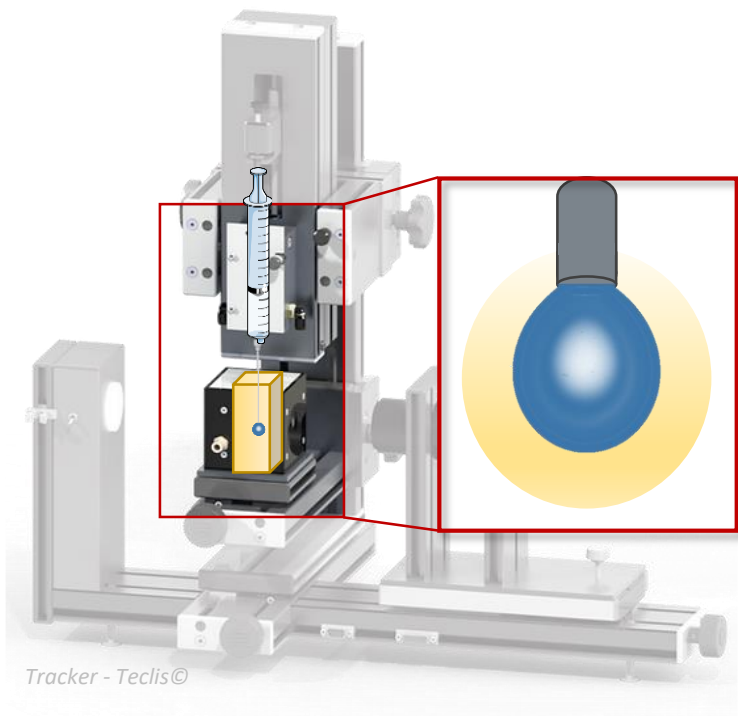
## Drop tensiometry

## Automated drop tensiometer



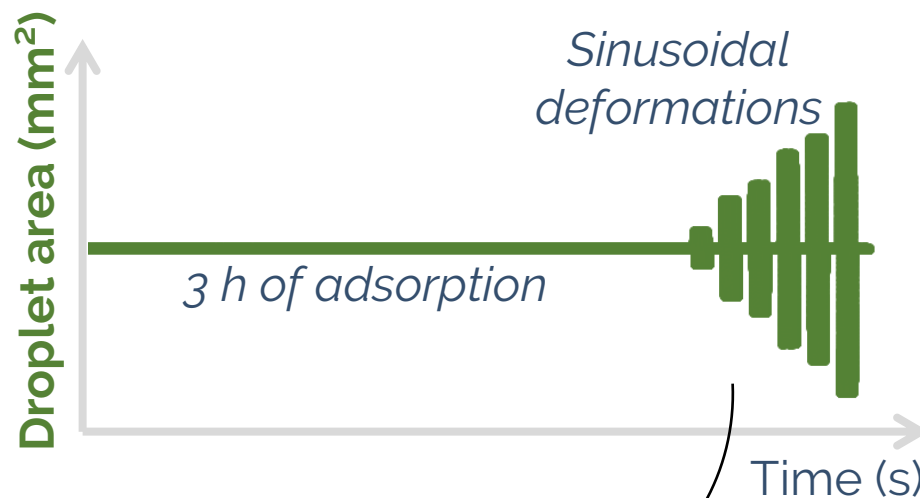


### Automated drop tensiometer



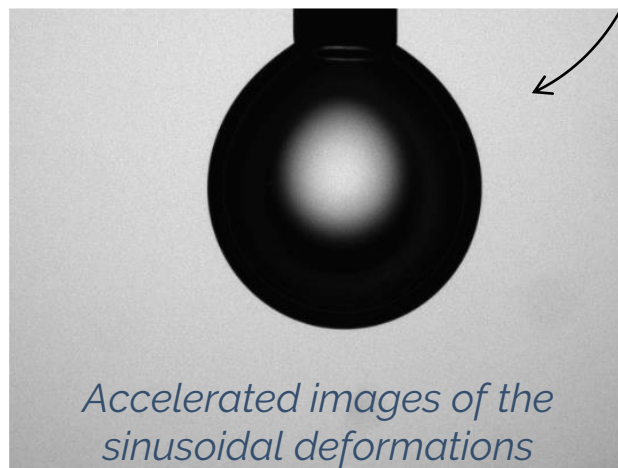
### Drop tensiometry

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



Monitoring of droplet area ( $\text{mm}^2$ )

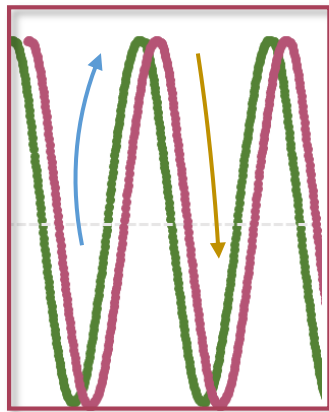
Recording of interfacial tension ( $\text{mN/m}$ )



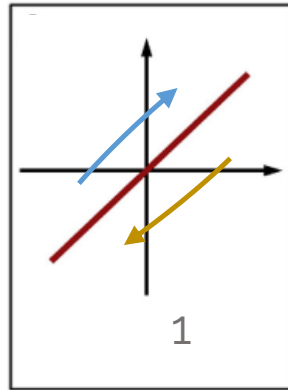
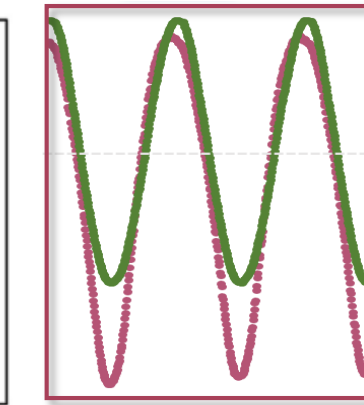


## Drop tensiometry

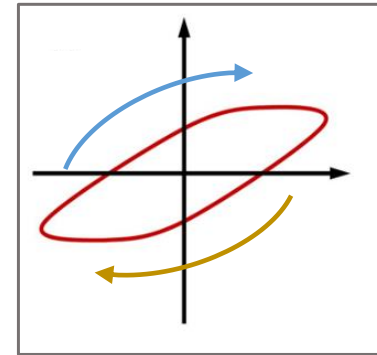
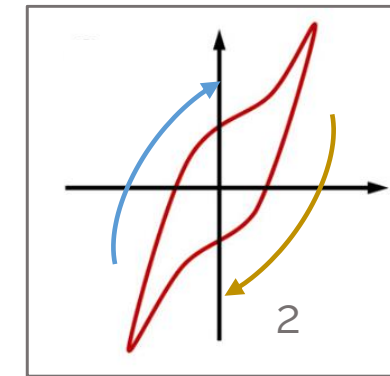
Lissajous plots represent **the amplitude of the interfacial response** ( $\Delta\pi = \gamma - \gamma_0$ ) as a function of **the deformation amplitude**  $\Delta A = \frac{A - A_0}{A_0}$



Linear regime

**Elastic**

Non linear regime

**Strain softening****Strain hardening**

Extension  
Compression

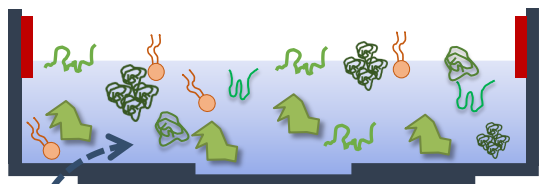
1: Sagis *et al.*, 2014, *COCIS*, 19(6)2: Sagis *et al.*, 2014, *TFST*, 37(1)



Atomic force microscopy (AFM)

1

The aqueous solutions are poured into the Wilhelmy balance

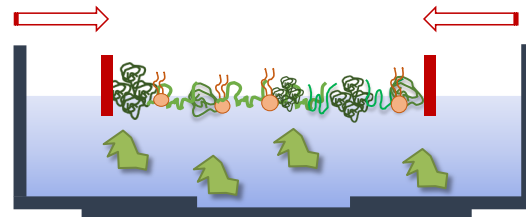


Aqueous phase



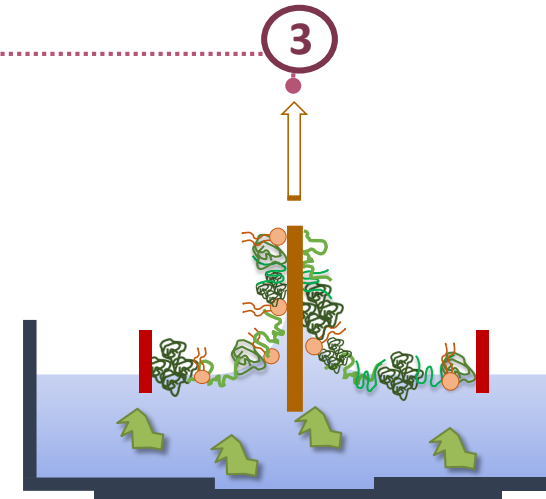
2

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

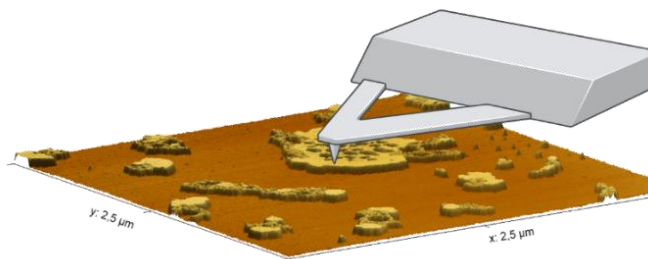


3

The air-water interfacial film is transferred 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

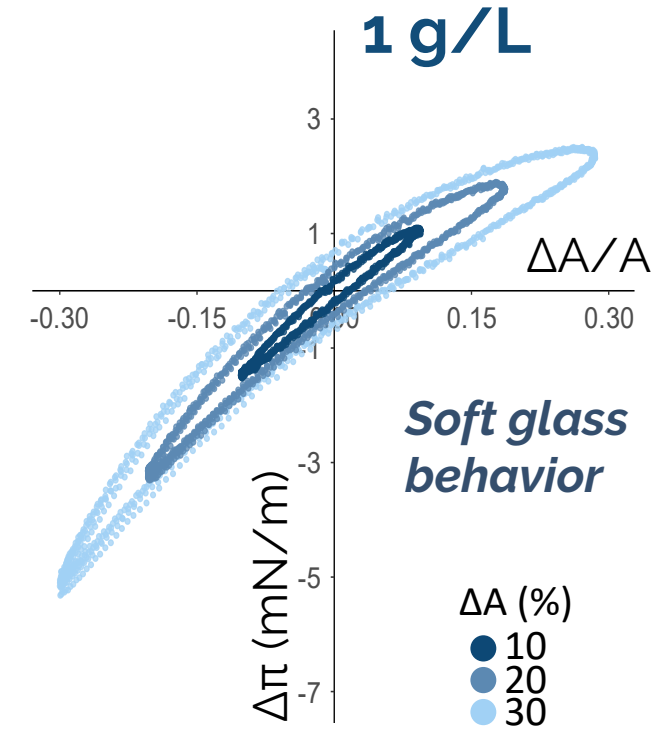
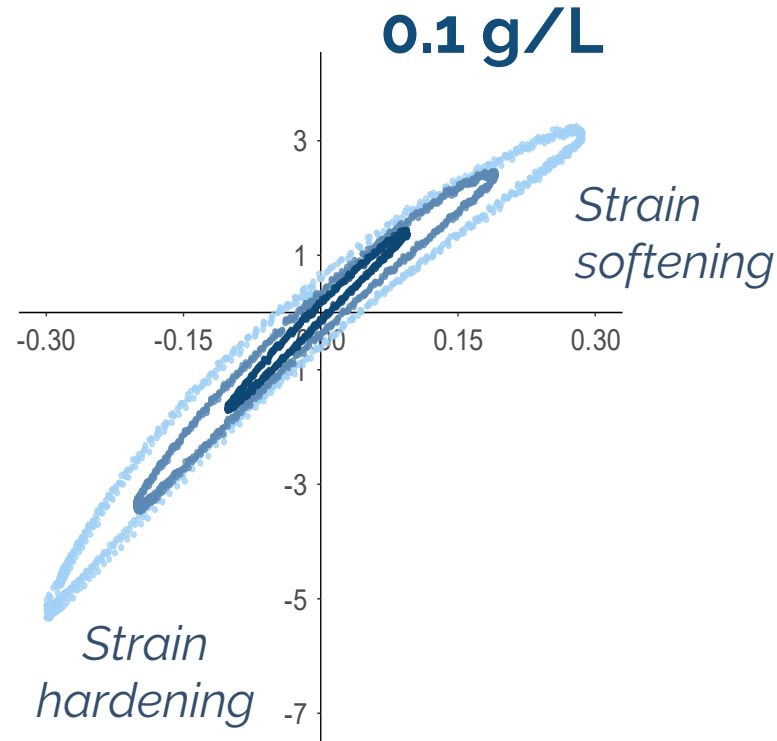
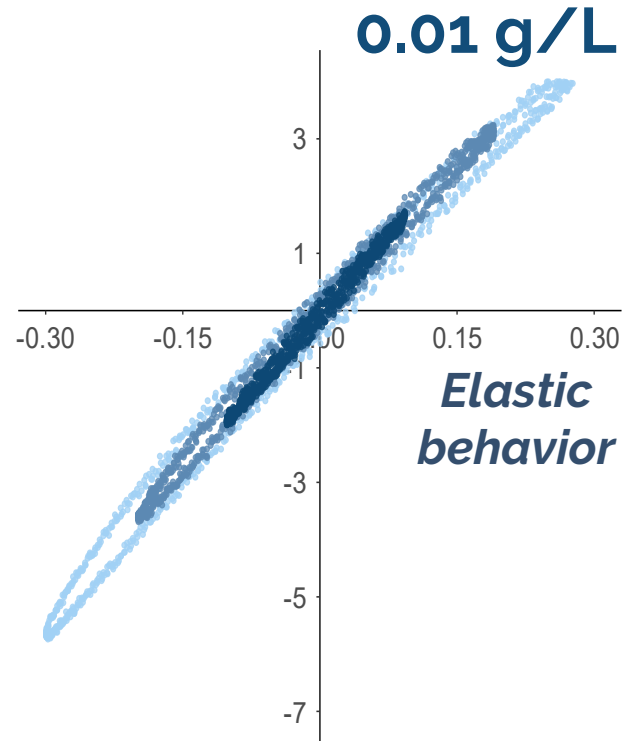
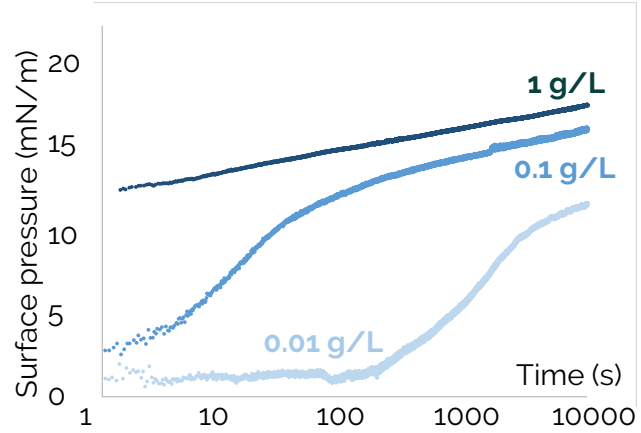
Purified components

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**



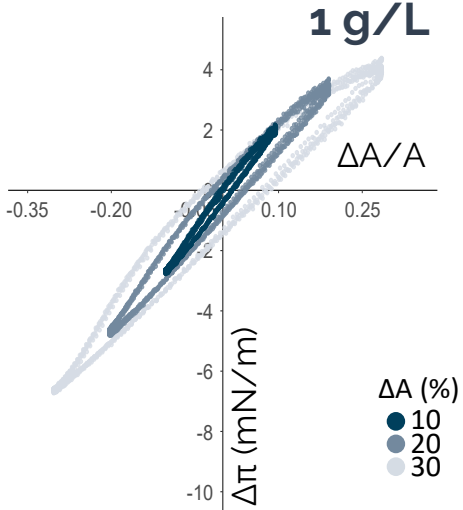
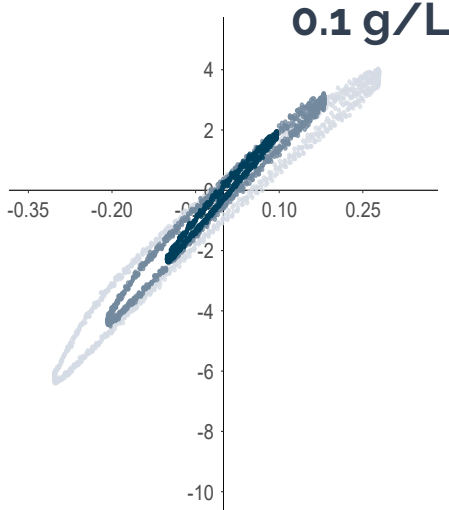
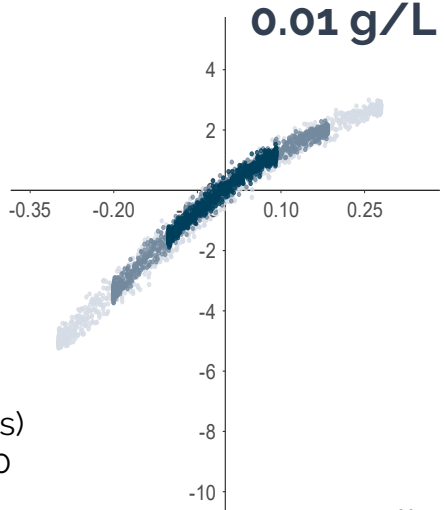
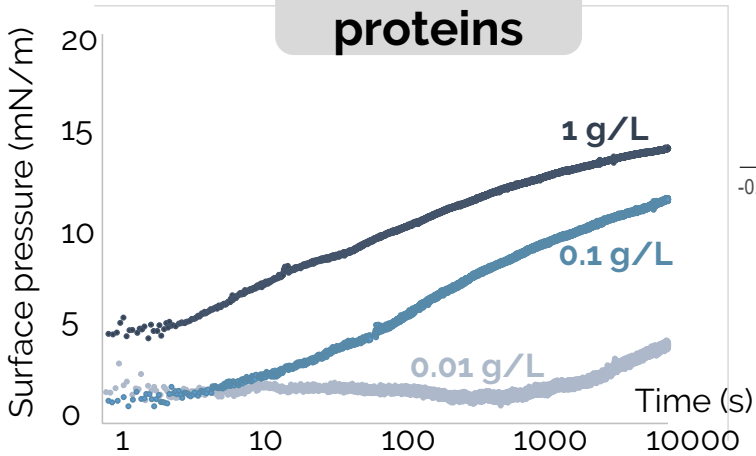
PPI

Purified components

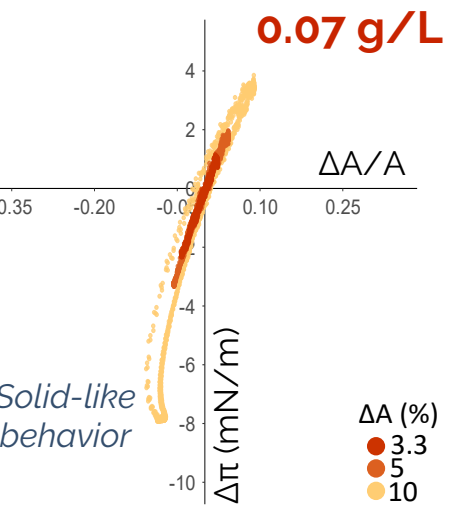
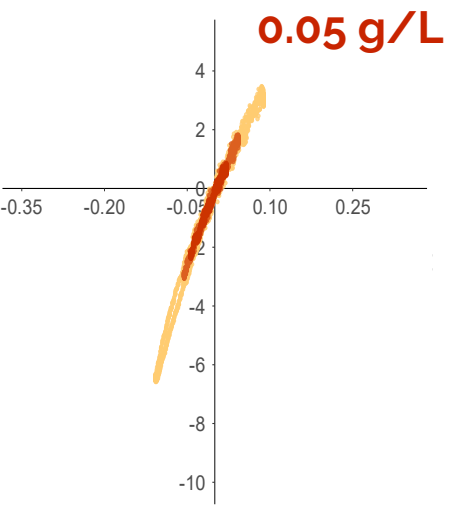
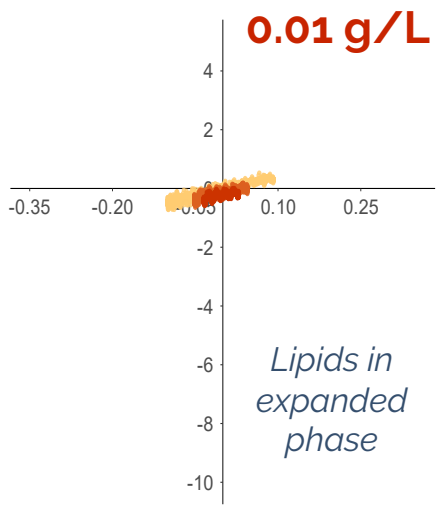
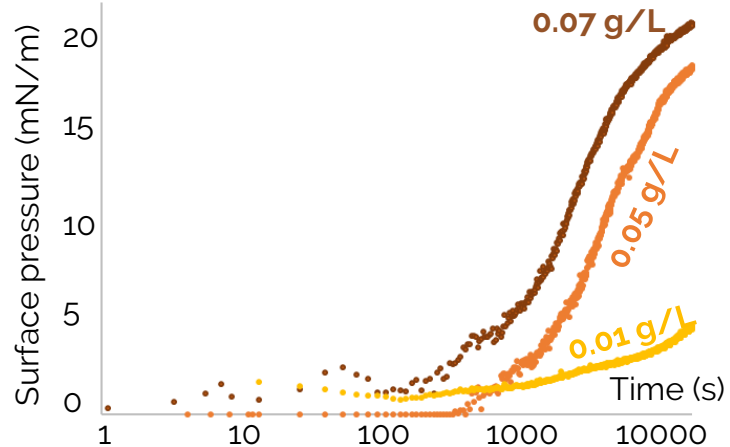
Protein to lipid mixes

AFM

7S pea proteins



Lipids



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

Interface extremely stiff, for very low concentrations.

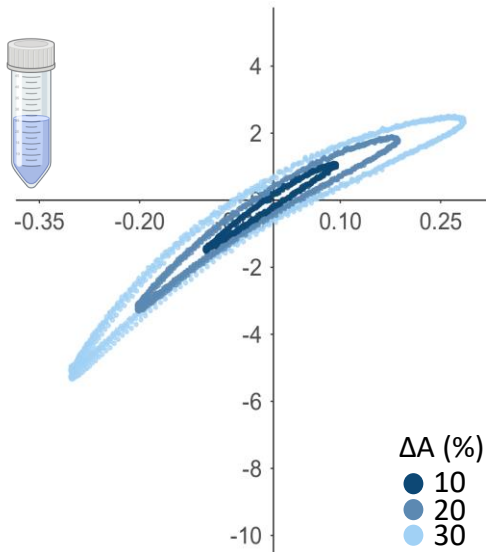
Very strong resistance against the deformation.

PPI

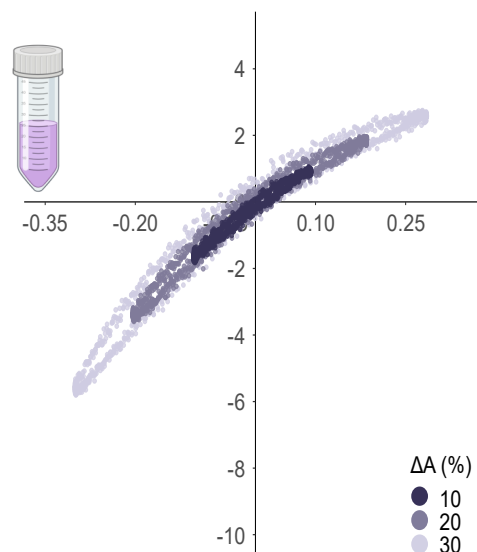
Purified  
componentsProtein to  
lipid mixes

AFM

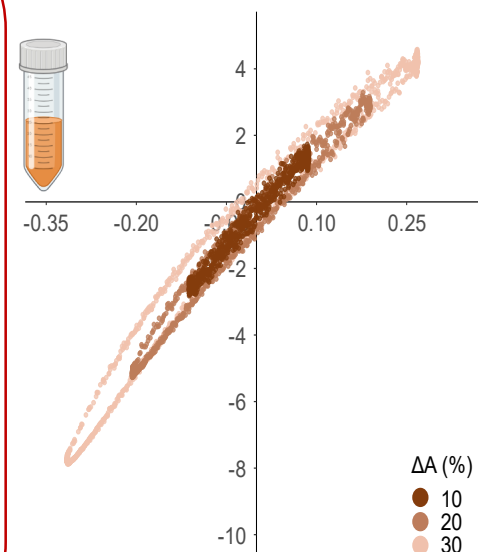
PPI 1 g/L



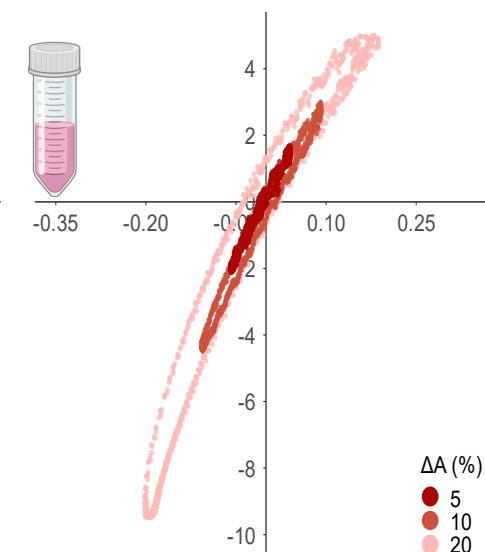
Mix 6:1



Mix 1:1



Mix 1:6



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

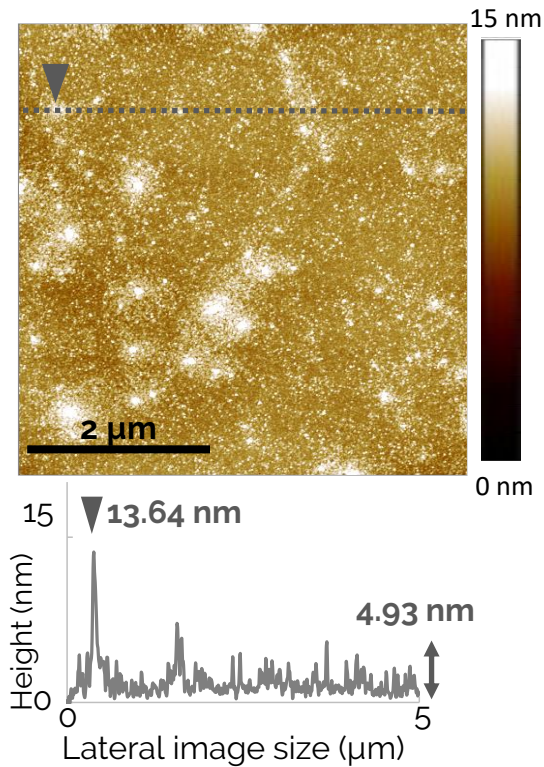
Close signatures between PPI (1 g/L) and the 6:1 system: the interfacial film of PPI **comprises both proteins and phospholipids, competing** for interfacial adsorption.

PPI

Purified  
componentsProtein to  
lipid mixes

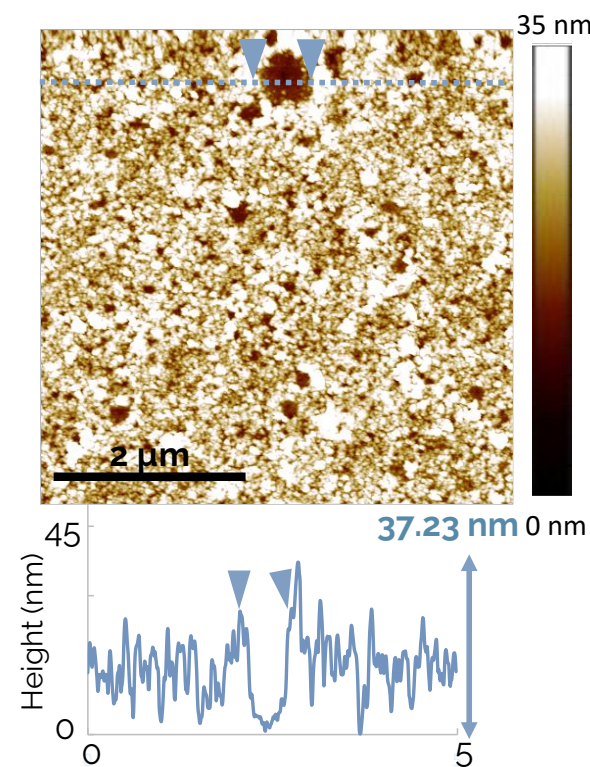
AFM

7S – 0.1 g/L



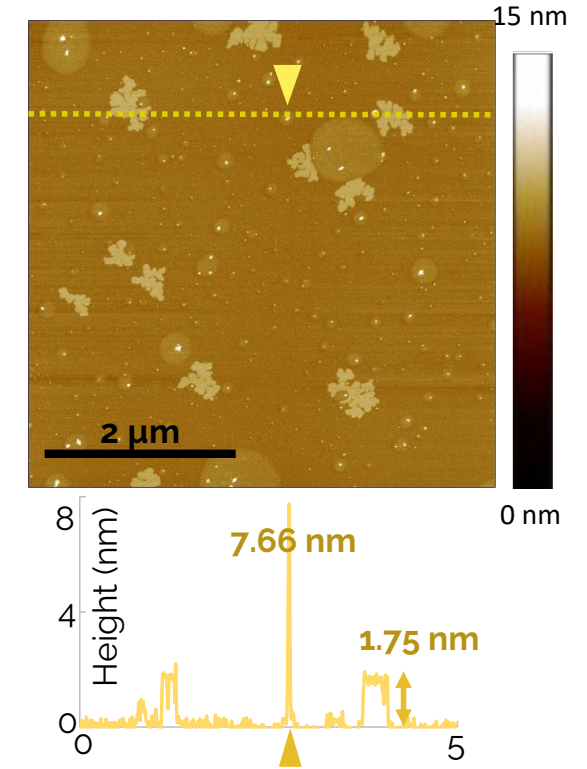
Homogeneous film, with  
**protein aggregates** all  
over the sample.

PPI – 1 g/L



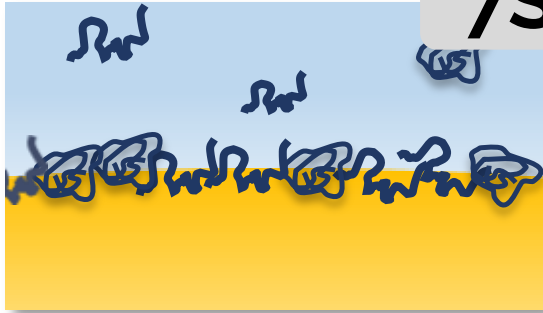
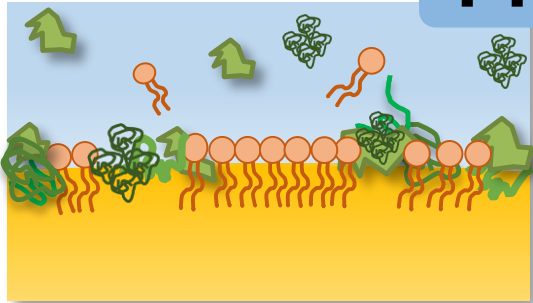
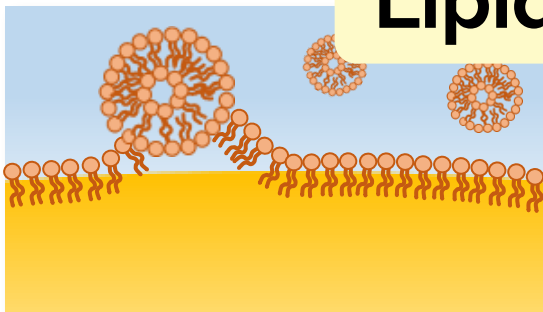
Very **high protein clusters**  
**(aggregates)** amongst which  
circular and low domains are  
forming « islands », very likely to be  
**lipid domains.**

Lipids – 0.07 g/L



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



**7S****PPI****Lipids**

**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.

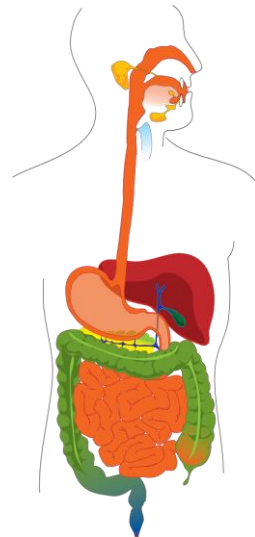
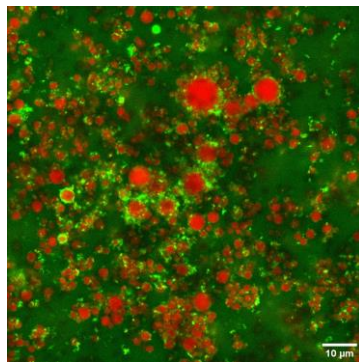
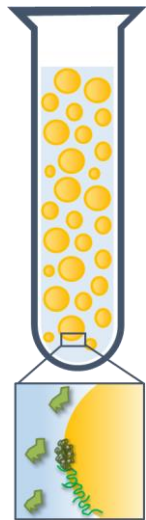
# Top-down approach from the formulation to the digestion of food emulsions




Pea




Lupin





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



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**Eléna Keuleyan<sup>1</sup>, Anne Meynier<sup>1</sup>, Alain Riaublanc<sup>1</sup>, Claire Berton-Carabin<sup>1,2</sup>**

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

**Pea**

**Lupin**

**Wet fractionation**

- Good protein purity
- Poor sustainability
- Poor functionality

**Dry fractionation**

- Complex composition
- Good sustainability
- Functionality maintained

**Commercial ingredients sourcing**

Pea protein isolate (PPI)

Lupin protein isolate (LPI)

Pea protein concentrate (PPC)

Lupin protein concentrate (LPC)

**Protein aggregates** 50-200 nm

**Native proteins** ± 50 nm

**Protein monomers** ± 10 nm

**Non-proteinaceous compounds**

- Lipids
- Polysaccharides
- Minor compounds (polyphenols, antinutritional factors)

**Non-treated protein suspension**

Phosphate buffer 10 mM, 90 mM NaCl, pH 7.0

**HPH-treated protein suspension**

High pressure homogenization (HPH)

**Emulsion**

HPH-treated suspension, Rapeseed oil, Acator-stator, HPH

**In vitro static digestions**

Oral phase

Gastric phase only

Gastric + Intestinal phases

Lipid digestibility & bioaccessibility

Infogest 2.0 protocol (Brodhorst et al., 2019)

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



**2024 AOCS Annual Meeting & Expo**

April 29<sup>th</sup>, 2024

**Thank you for your  
attention !**

**Linked in**



**Eléna Keuleyan**, [elena.keuleyan@inrae.fr](mailto:elena.keuleyan@inrae.fr)

**Supervision:**

Anne Meynier, Alain Riaublanc, Claire Berton-Carabin