

➤ Hemp seed oil bodies for food applications

**Thierry Chardot¹, Marc Anton², Bruno Novales^{2,3}, Hanitra Rabesona²,
Magalie Weber² & Christelle Lopez²**

¹ INRAE, AgroParisTech, Université Paris-Saclay, Institut Jean-Pierre Bourgin, Versailles, France

² INRAE, UR BIA, Nantes, France

³ INRAE, BIBS facility, Nantes, France

➤ Outline

1. Context

2. From hemp seeds to hemp seed aqueous extract and OBs

3. Hemp seed OBs: chemical composition/ nutritional interest

4. Hemp seed OBs in stress environment

- pH
- Minerals: NaCl, CaCl₂

5. Conclusions



> Context

- Consumers do expect more natural, **minimally processed**, of high sensorial quality, nutritionally interesting, and healthy food products (Battacchi et al., 2020; Roman et al., 2017).
- **The ever-increasing demand of consumers for plant-based diets**, including foods containing plant-based proteins of nutritional interest and lipids rich in poly-unsaturated fatty acids (PUFA; mainly n-3 FA) and other bioactive compounds, **is in the spotlight**.



Oilseeds are among the most promising sustainable plant-based food sources:
contain proteins and **oil bodies (oleosomes, lipid droplets)**

Oil bodies (OBs) are **lipid-based nature-assembled colloidal structures**, such as LDL in egg yolk and milk fat globules.

OBs synthesised by plants could therefore be further valorized in human diet, for example in dressings, sauces, dips, beverages, desserts, in plant-based emulsion products such as mayonnaise (Nikiforidis et al., 2014; Romero-Guzman et al., 2020) and in complex food systems such as fermented products.

1. Essential to find and characterize **new sources** of plant nutrients
2. The current lack of understanding of the microstructure and surface properties of OBs in response to changes in pH and mineral environment, associated to the physical stability of the emulsion poses a significant barrier to the development of OBs-based new food products.

> Context

Growing interest in the last decade for **hemp (Cannabis Sativa L.)**

*Hemp cultivation has a low environmental impact and is adapted to mild climates

*Whole hemp can be valorized and provides **hemp seeds of nutritional interest and health benefits** (Farinon et al., 2020; Rupasinghe et al., 2020; Sorrentino, 2021)



The **objectives of this work** were :

- to use **minimal processing** including aqueous extraction and grinding to recover OBs from hemp seeds
- to **characterize the lipid composition** of natural hemp seed OBs
- to **examine their microstructure**,
- to **determine the impact of pH and minerals** (various NaCl and CaCl₂ concentrations) on their surface properties and the physical stability of the emulsions.
- to determine the impact of homogenization on the physical stability of hemp seed extracts for long-time storage (not presented here)

Lopez, C., Novales, B., Rabesona, H., Weber, M., Chardot, T., Anton, M., 2021. Deciphering the properties of hemp seed oil bodies for food applications: Lipid composition, microstructure, surface properties and physical stability. *Food Research International* 150, 110759. <https://doi.org/10.1016/j.foodres.2021.110759>

➤ Outline

1. Context

2. From hemp seeds to hemp seed aqueous extract and OBs

3. Hemp seed OBs: chemical composition/ nutritional interest

4. Hemp seed OBs in stress environment

- pH
- Minerals: NaCl, CaCl₂

5. Conclusions

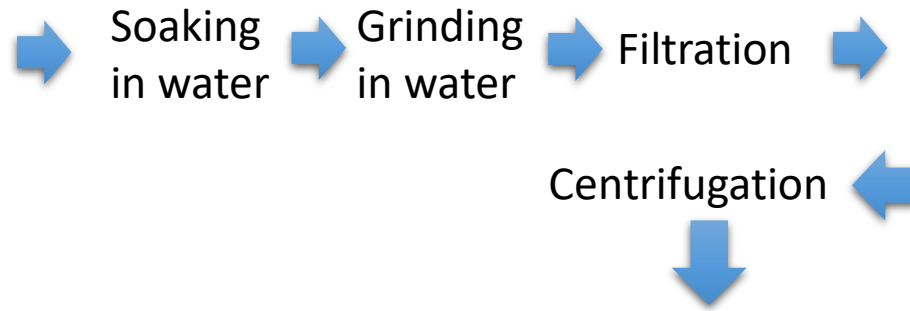


➤ From hemp seeds to hemp seed aqueous extract and OBs

Hemp seeds



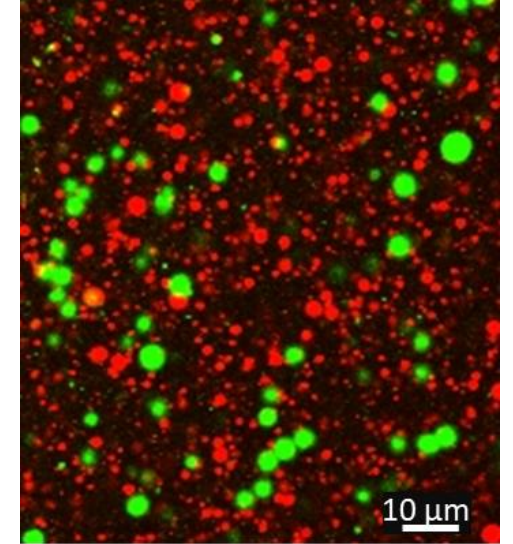
Minimal processing



Hemp seed aqueous extract



Confocal microscopy

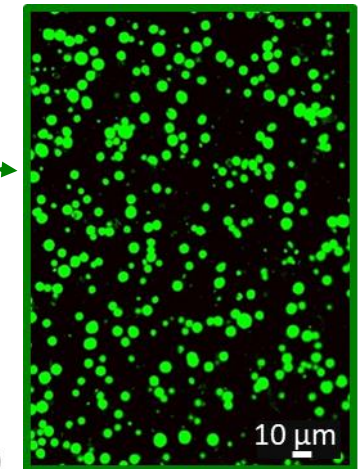
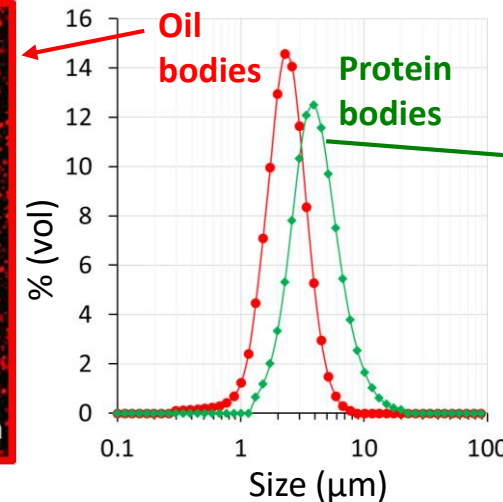
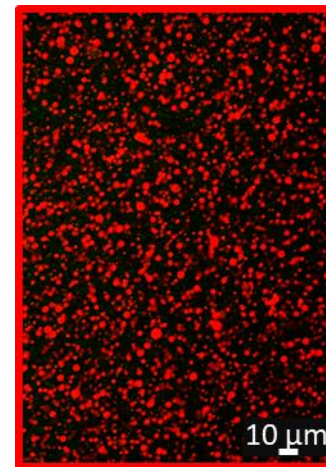


■ Hemp seed OBs
■ Hemp seed protein bodies

Confocal microscopy

Hemp seed OBs

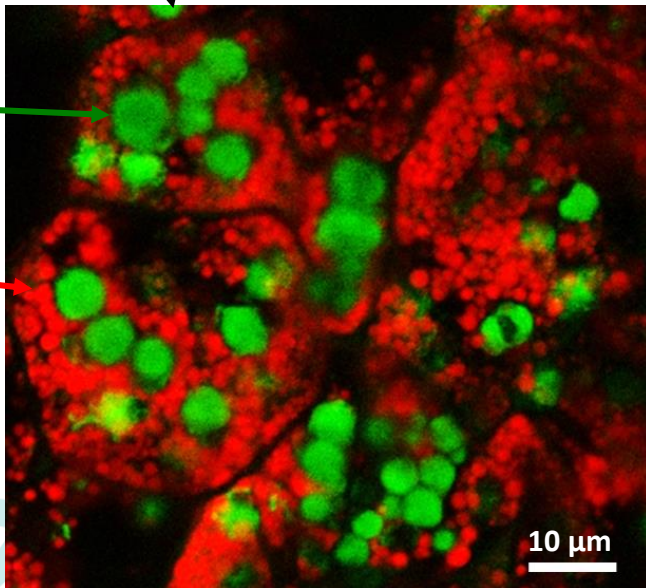
Size distribution: 0.3 to 8 μm
Mean diameter: 2.3 ± 0.1 μm



PBs:
4.1 ±
0.3 μm

Protein bodies (PBs)

Oil bodies (OBs)



➤ Outline

1. Context

2. From hemp seeds to hemp seed aqueous extract and OBs

3. Hemp seed OBs: chemical composition/ nutritional interest

4. Hemp seed OBs in stress environment

- pH
- Minerals: NaCl, CaCl₂

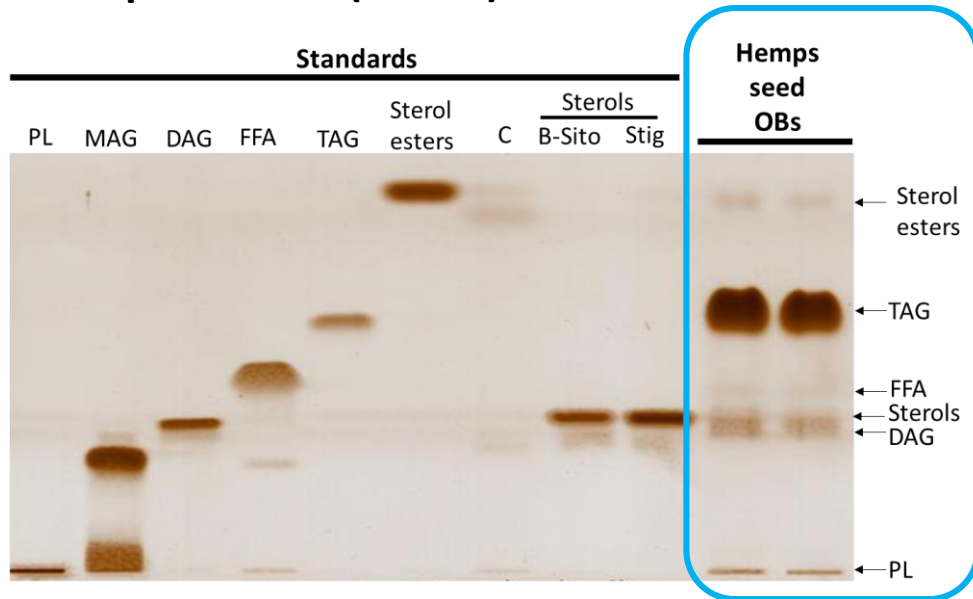
5. Conclusions



➤ Hemp seed OBs: chemical composition / nutritional interest

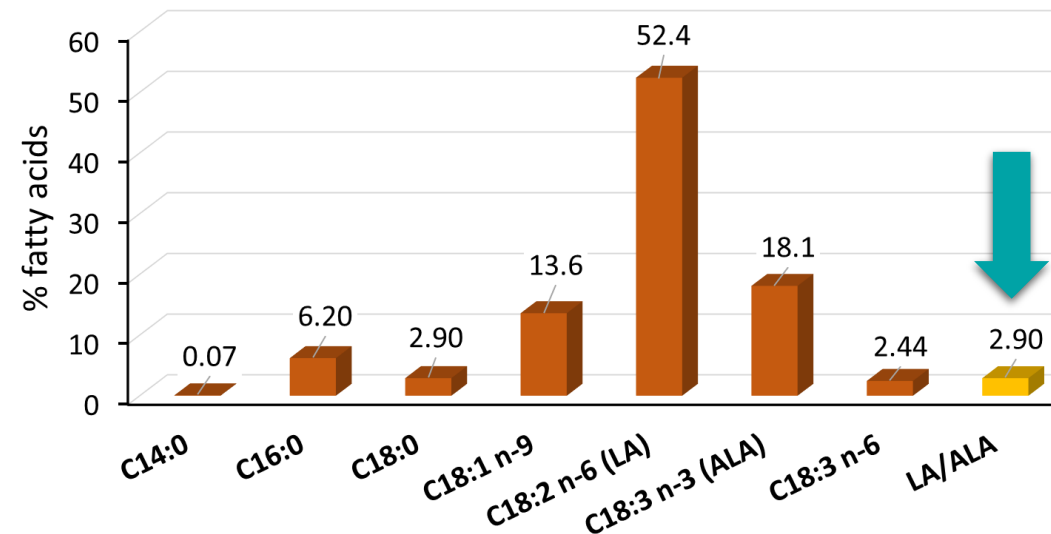
Lipid content: 33.6 ± 0.5 g of total lipids/100 g of seeds

Main lipid classes (HPTLC)



- Mainly TAG (~ 95% of lipids)

- Fatty acid composition (GC)



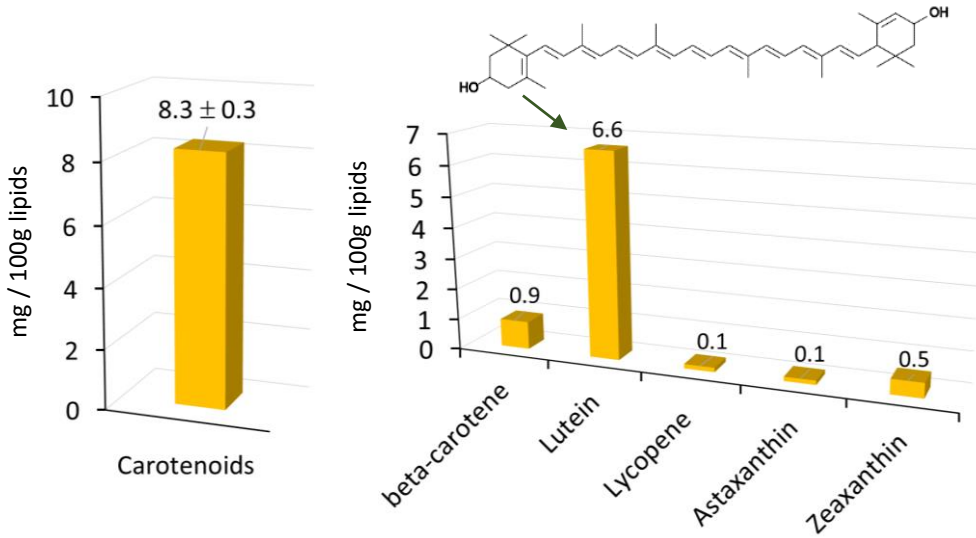
PUFAs of the oil amounted to **72.9%** of the total of FAs
linoleic acid (C18:2n-6; LA), α-linolenic acid (C18:3n-3; ALA)

The hemp seed OBs deliver the essential **PUFAs n-6 and n-3 in the proportion 3:1** that is the best ratio recommended by medical research and the most advanced theories in the field of nutrition.

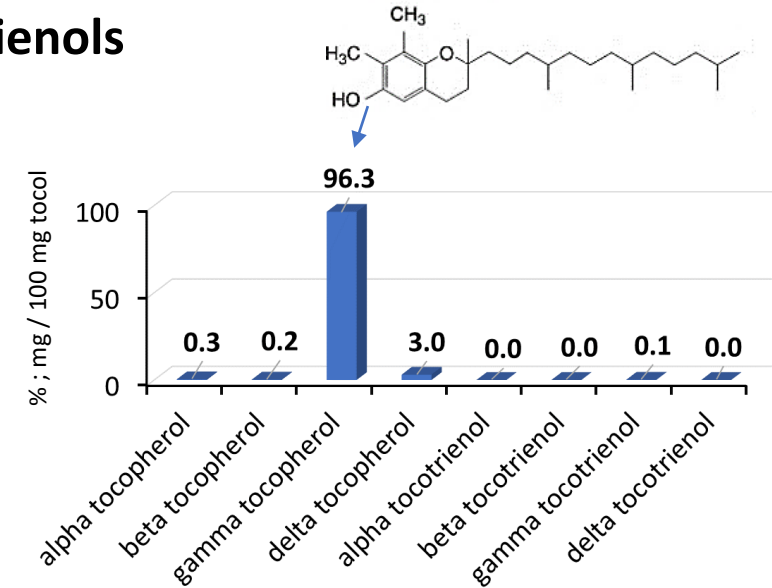
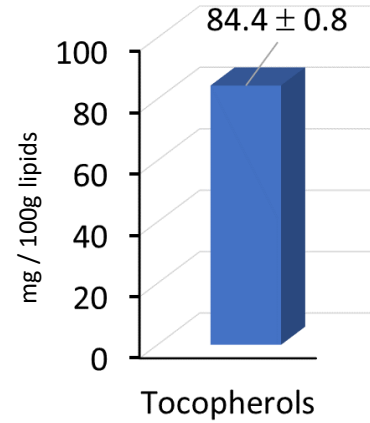
➤ Hemp seed OBs: chemical composition / nutritional interest

Carotenoids: 8.3 ± 0.3 mg/100g lipids

mainly lutein



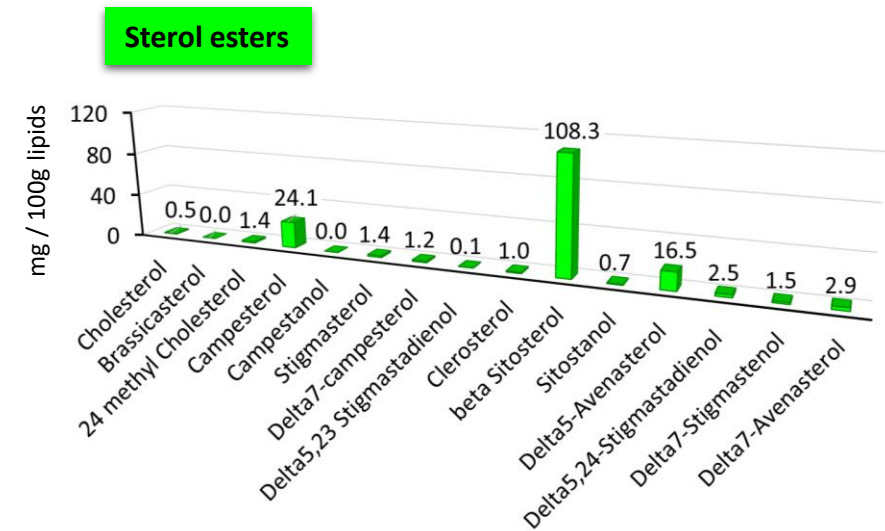
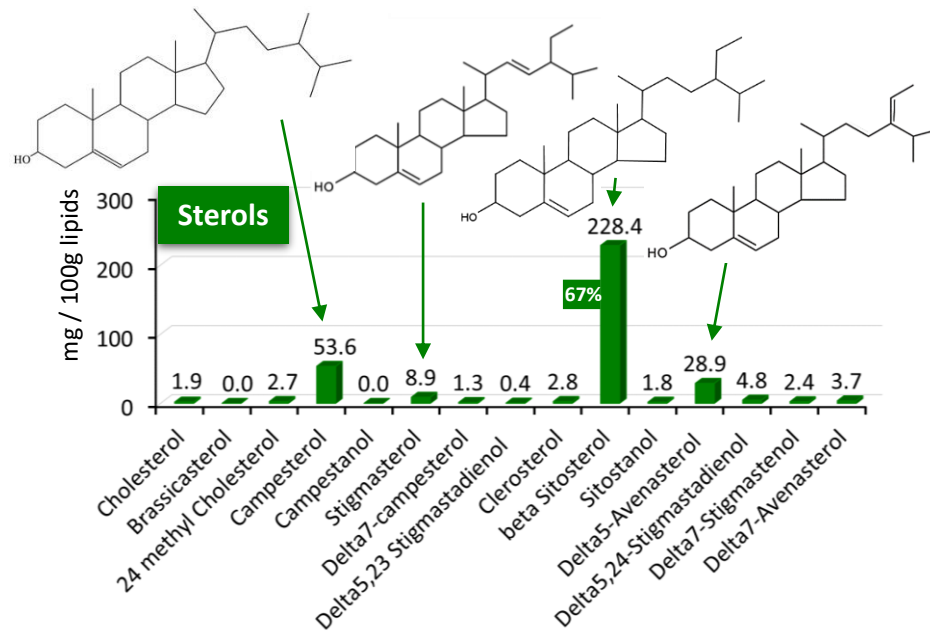
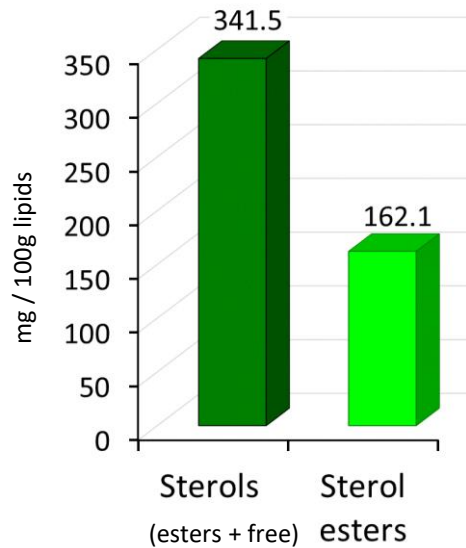
Tocopherols and tocotrienols



➔ **Natural protection against oxidation**

➤ Hemp seed OBs: chemical composition / nutritional interest

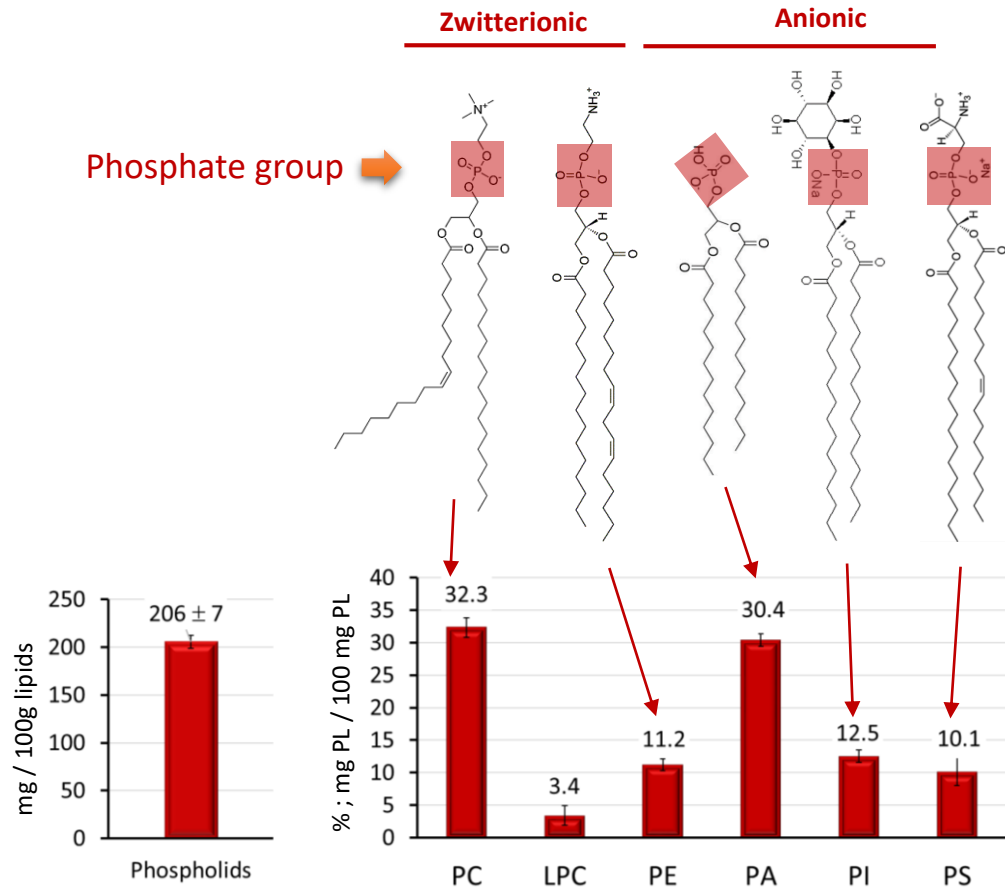
Sterols: 341.5 mg/100g lipids; mainly β -sitosterol; about 50% sterol esters



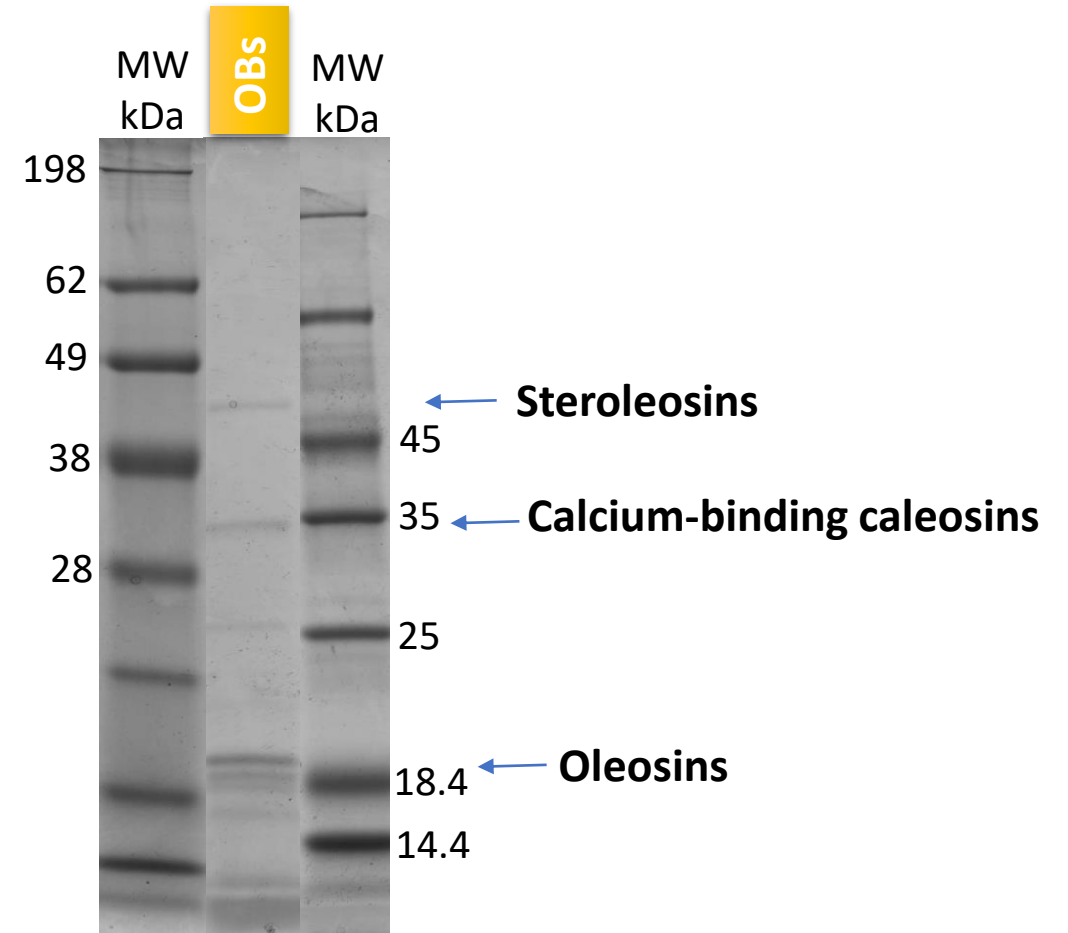
- ➔ Diversity of sterols
- ➔ High amount of sterols

➤ Hemp seed OBs: chemical composition / nutritional interest

Phospholipids: zwitterionic and anionic



Surface proteins

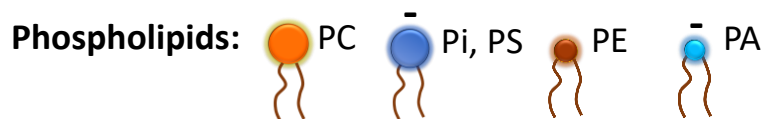
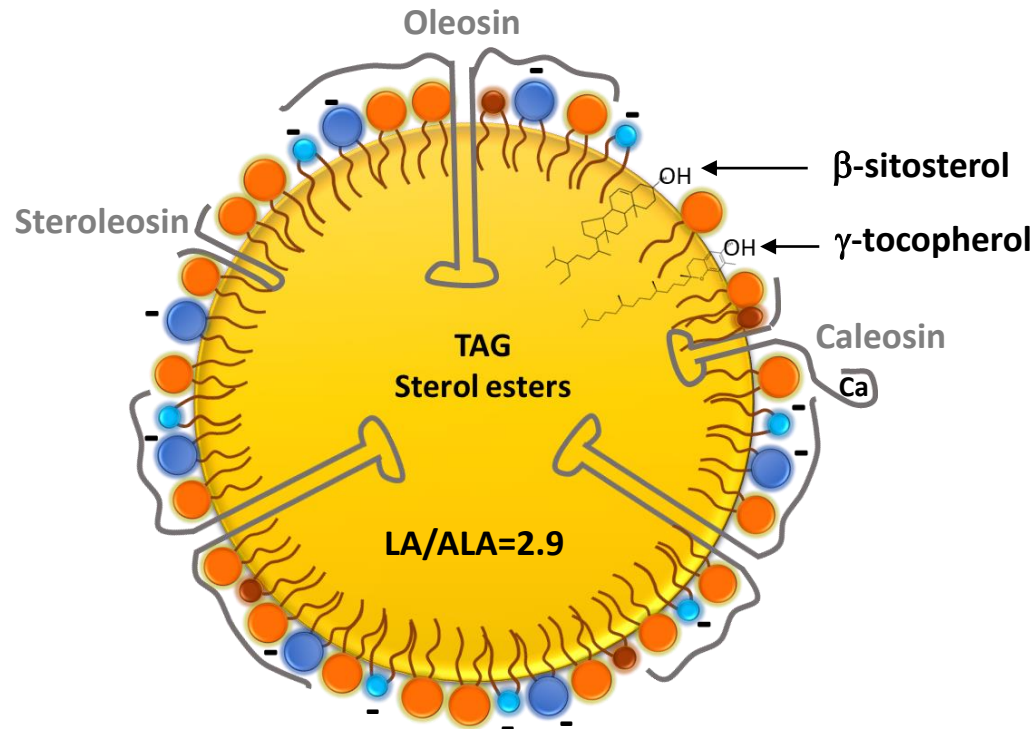


➤ Hemp seed oil bodies

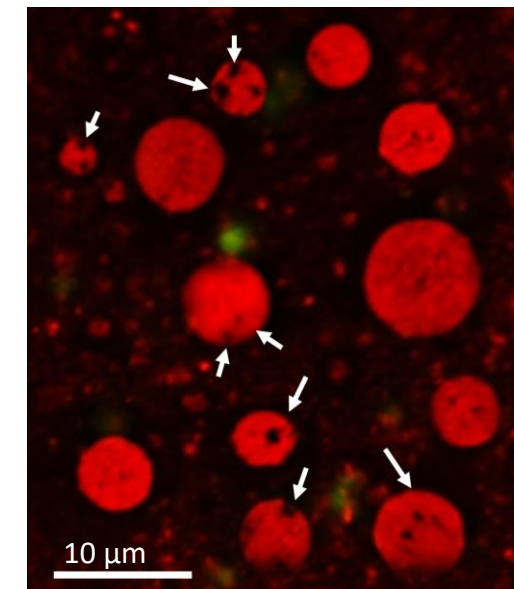
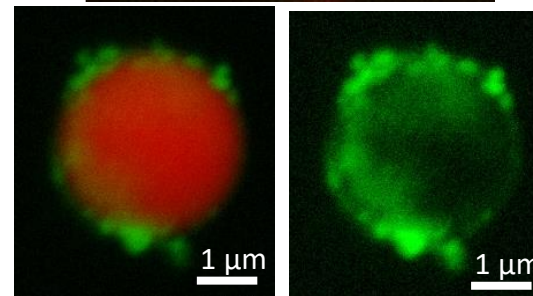
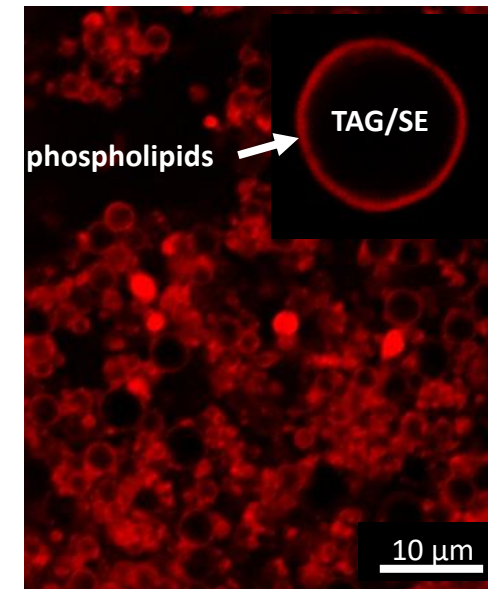
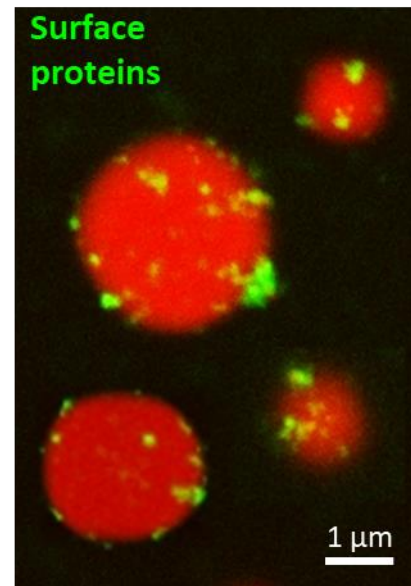
Confocal microscopy images

Schematic representation

Lopez et al. 2021 Food Res Int



Proteins: Oleosins, calcium-binding caleosins, steroleosins



Surface heterogeneities: sterols ?

➤ Outline

1. Context

2. From hemp seeds to hemp seed aqueous extract and OBs

3. Hemp seed OBs: chemical composition/ nutritional interest

4. Hemp seed OBs in stress environment

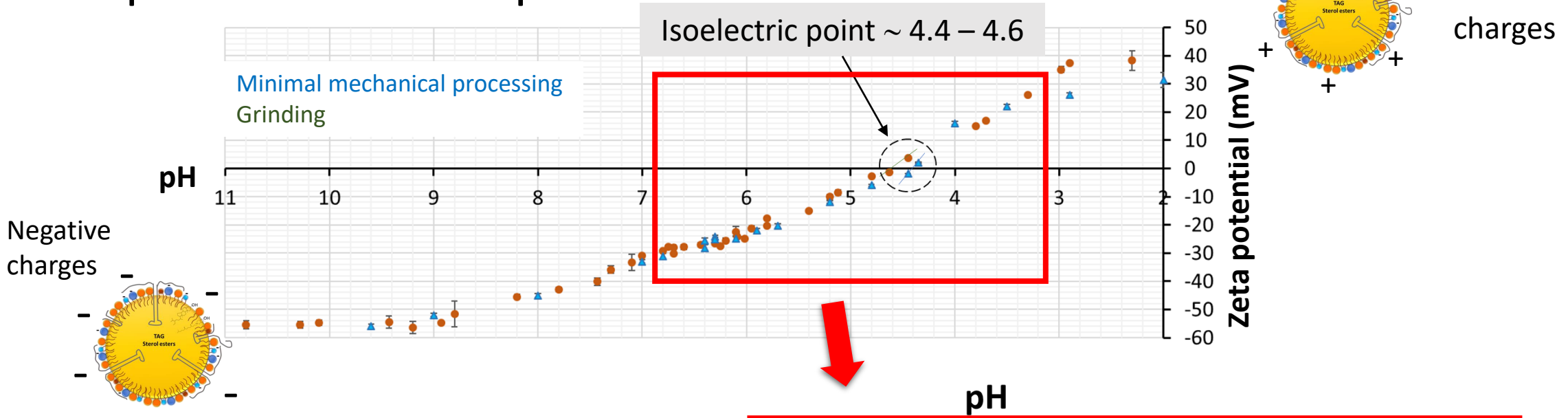
- pH
- Minerals: NaCl, CaCl₂

5. Conclusions

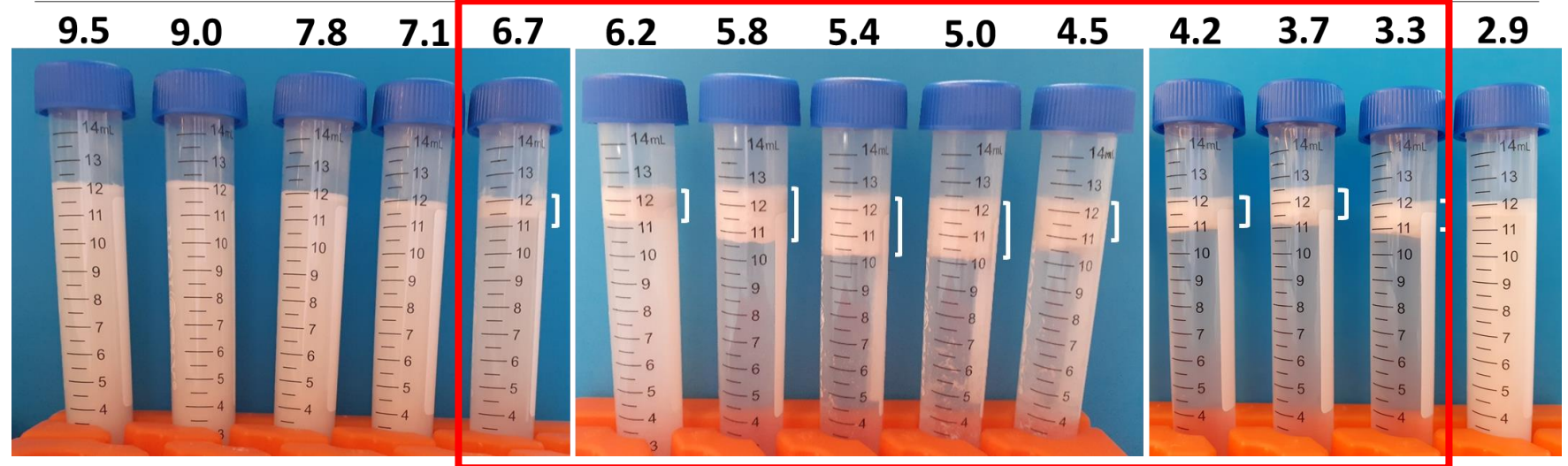


➤ Hemp seed OBs in stress environment: impact of pH

■ Zeta potential as a function of pH



■ Physical (un)stability of hemp seed oil bodies suspensions as a function of pH



➤ Hemp seed OBs in stress environment: impact of pH

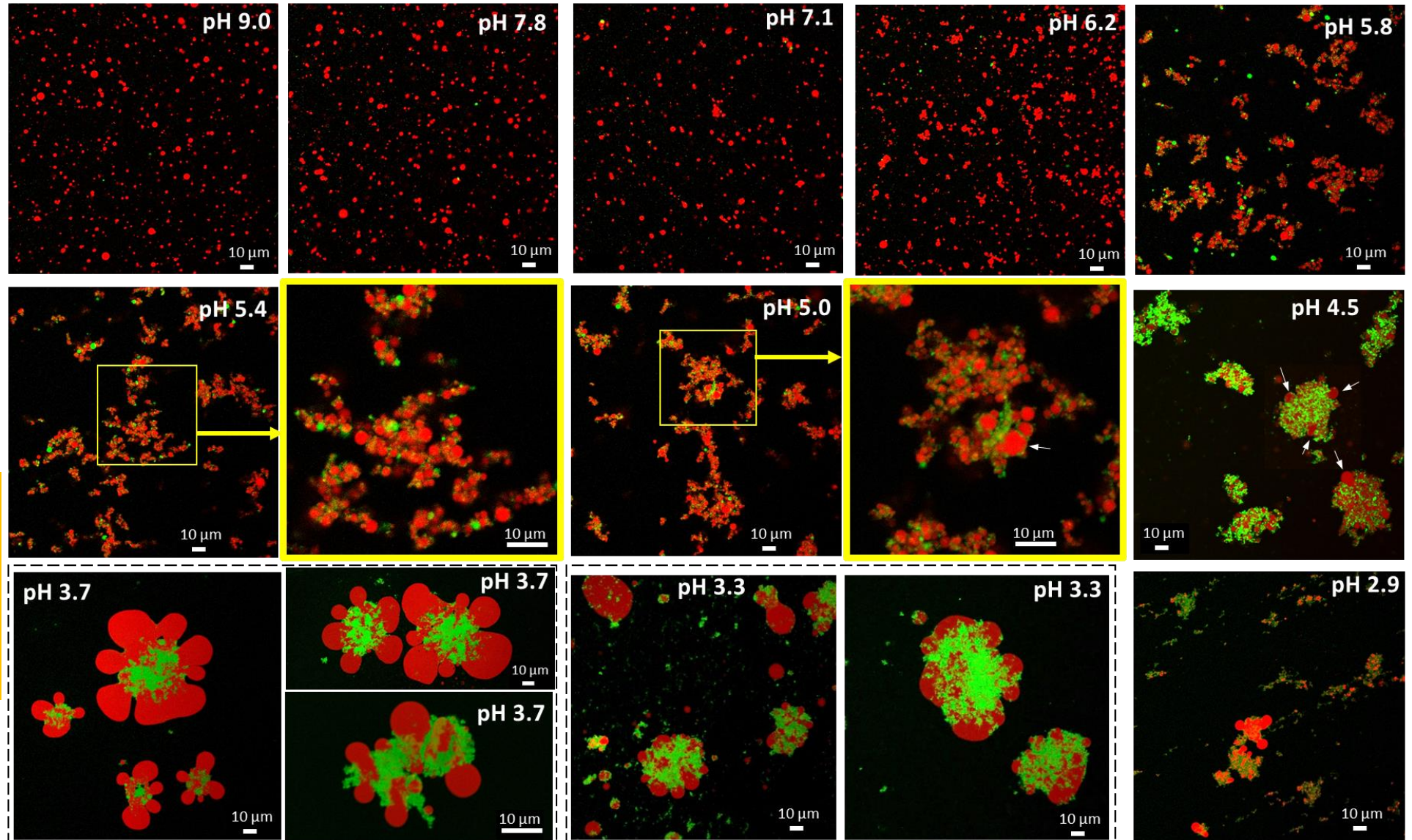
▪ Microstructure as a function of pH

Aggregation
for pH < 7

Coalescence
for pH < 4

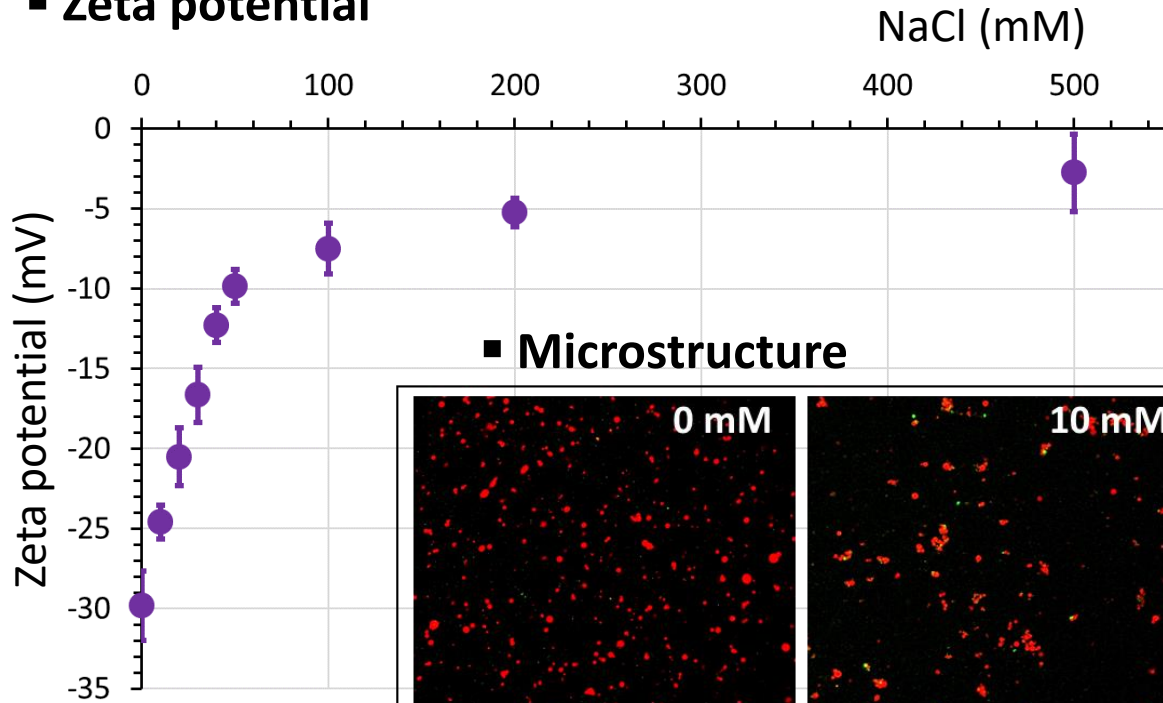
Creaming

Structural changes of
hemp seed OBs in the
gastro-intestinal tract
upon digestion:
to be evaluated

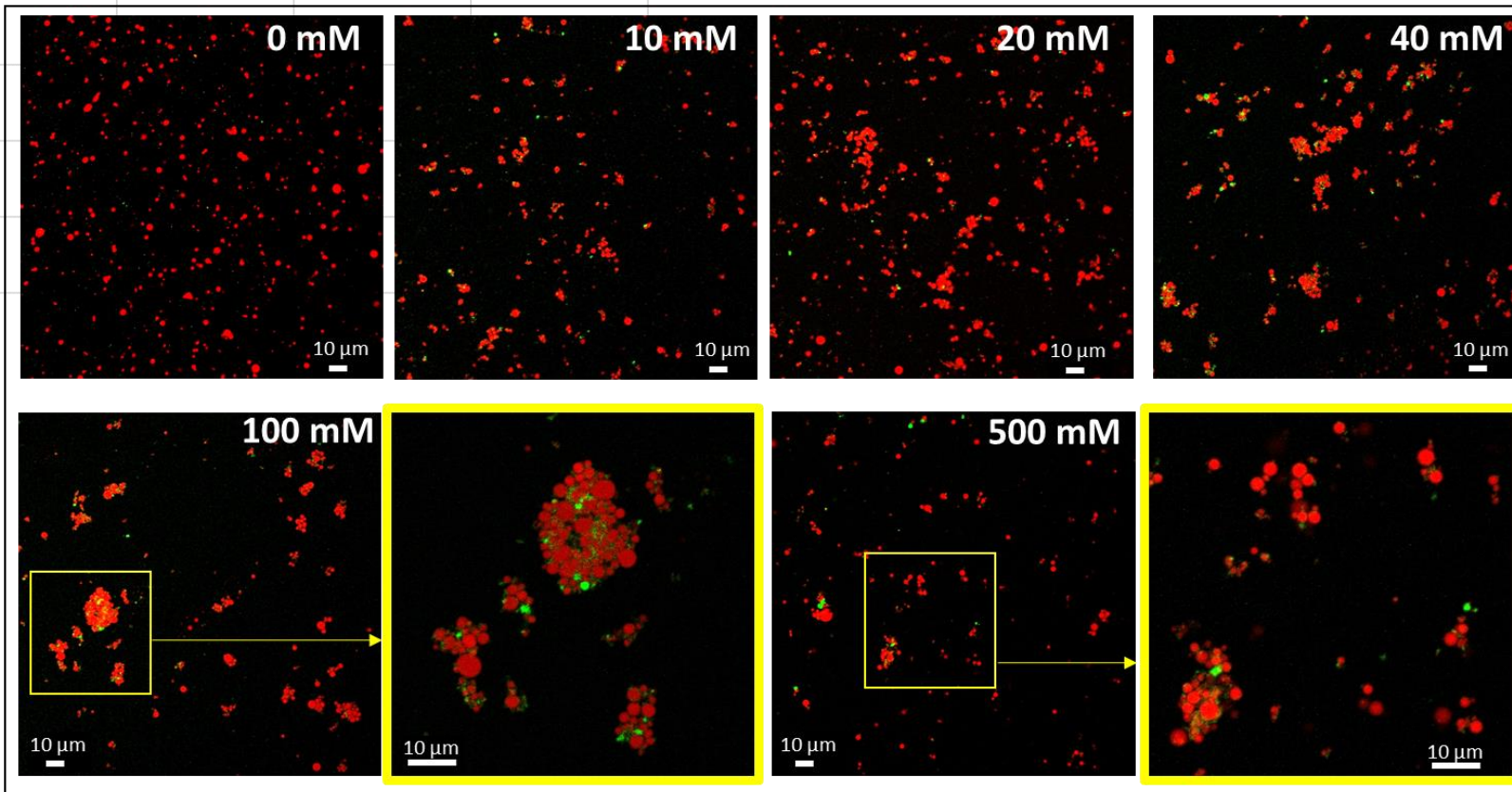


➤ Hemp seed OBs in stress environment: impact of NaCl

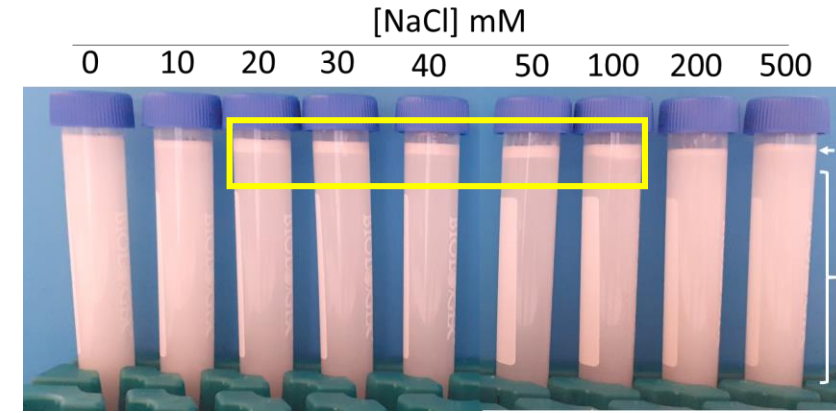
■ Zeta potential



■ Microstructure



■ Physical (un)stability



NaCl:

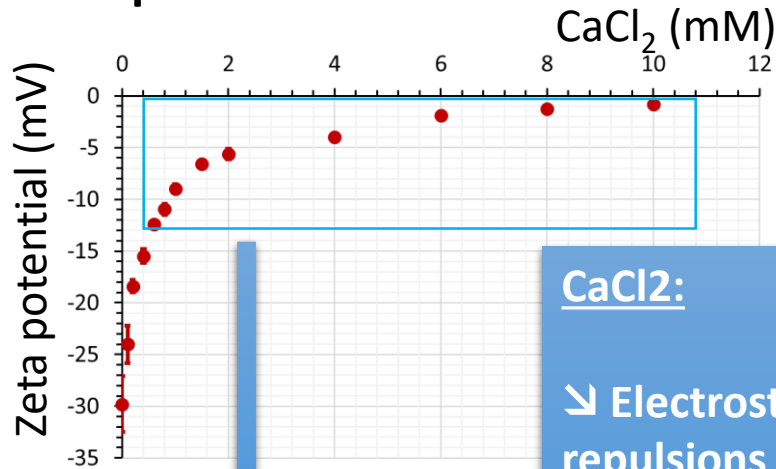
↘ Electrostatic repulsions

Aggregation of OBs for $[\text{NaCl}] \geq 20 \text{ mM}$

Creaming of OBs aggregates

➤ Hemp seed OBs in stress environment: impact of CaCl₂

■ Zeta potential



CaCl₂:

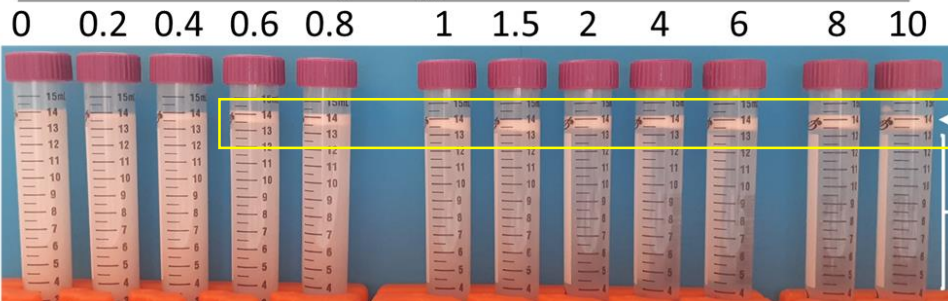
↘ Electrostatic repulsions

Aggregation and coalescence

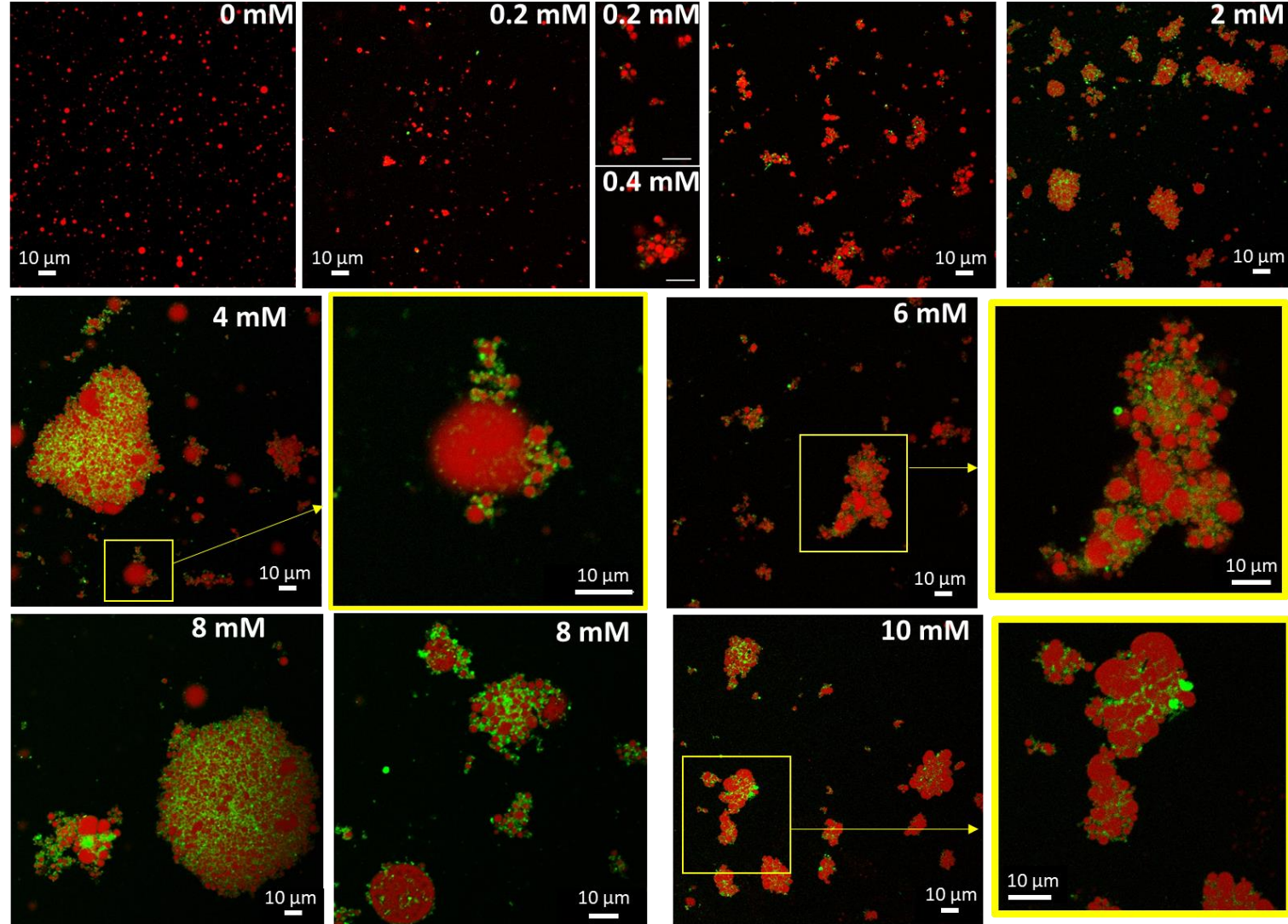
Creaming

■ Physical (un)stability

[CaCl₂] mM

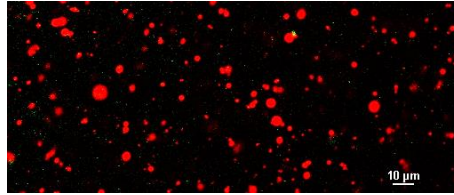


■ Microstructure

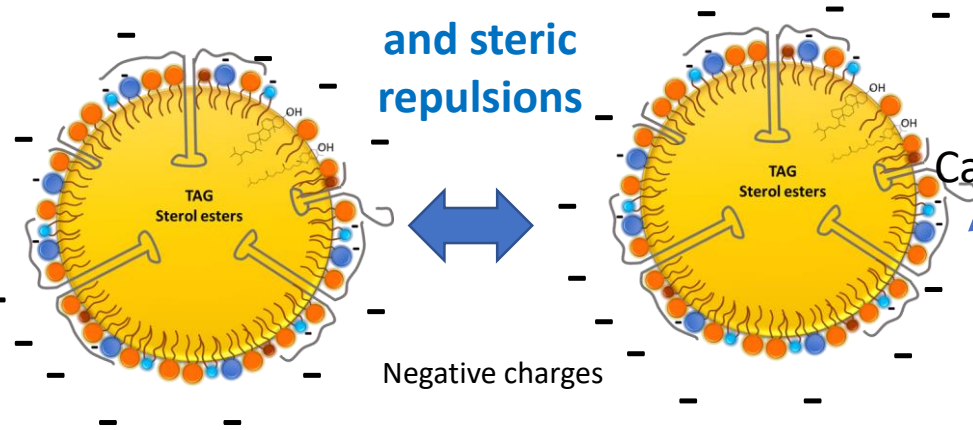


➤ Hemp seed OBs in stress environment: interpretations

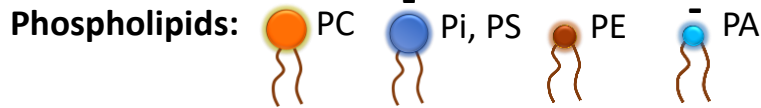
pH above 7
Absence of salts



Electrostatic
and steric
repulsions



Negative charges



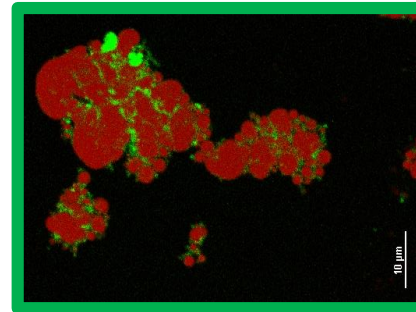
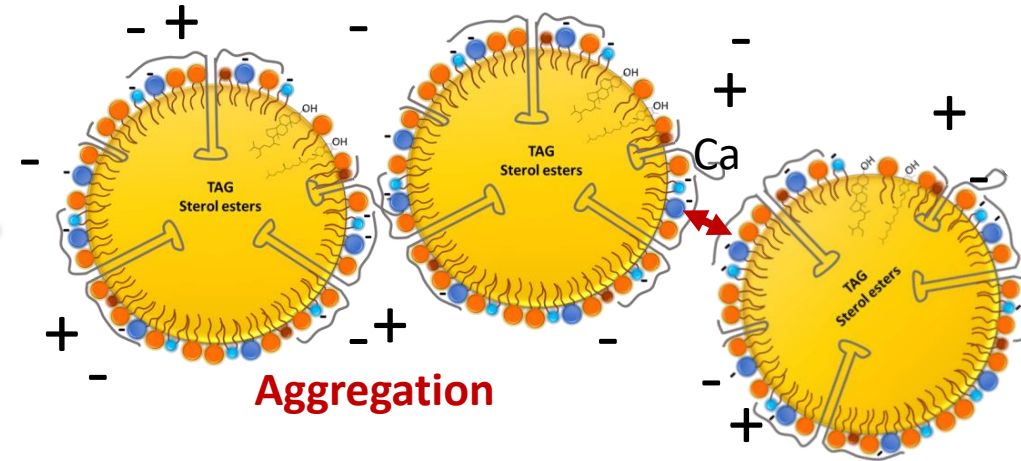
Calcium-binding
caleosin

pH: H⁺ ions
NaCl: Na⁺ ions



CaCl₂: Ca²⁺ ions

Changes in surface properties:
proteins and phospholipids



Calcium favours **membrane-fusion mediated coalescence** of OBs through

- the formation of PS – PS complexes: 10% PS/PL in hemp seed OBs
- calcium-binding caleosins

The physical instability of OBs induced by cations (pH, Na⁺, Ca²⁺) should be considered for
-extraction from the seeds and purification
-food applications

➤ Outline

1. Context

2. From hemp seeds to hemp seed aqueous extract and OBs

3. Hemp seed OBs: chemical composition/ nutritional interest

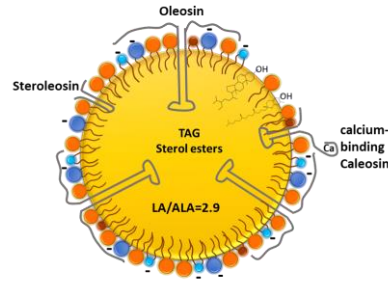
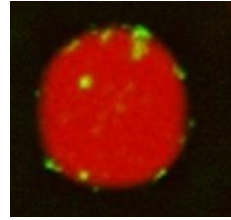
4. Hemp seed OBs in stress environment

- pH
- Minerals: NaCl, CaCl₂

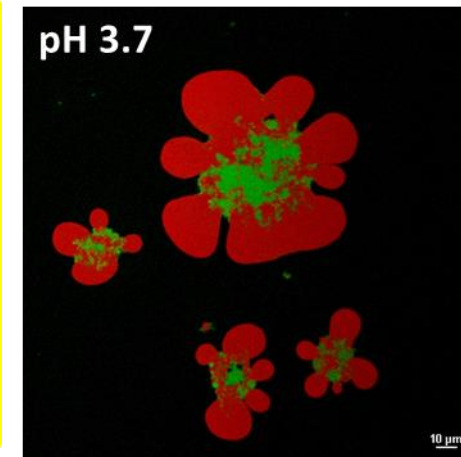
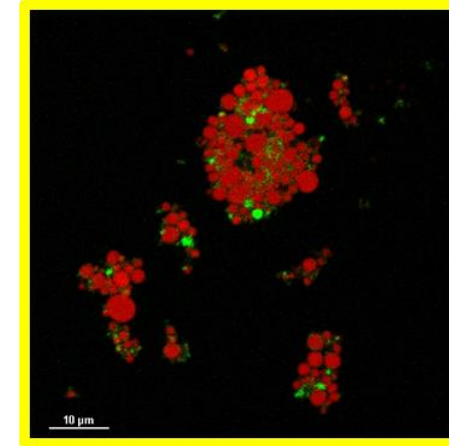
5. Conclusions



➤ Conclusions : take home message



pH
minerals



1./ The **extraction of hemp seed OBs** can be performed by **minimal processing**, including soaking of the seeds, grinding, filtration and centrifugation

The valorization of full hemp seed aqueous extracts, containing both the OBs and the protein bodies, should be performed.

2./ **Hemp seed OBs are interesting nutritional sources** of **fatty acids** with a well-balanced LA/ALA ratio (LA/ALA = 2.9) but also of other bioactive compounds: **carotenoids (mainly lutein), phytosterols, and antioxidant molecules (γ-tocopherol)**

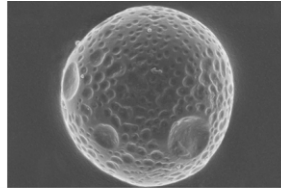
3./ The mean diameter of HSOBs was $2.3 \pm 0.1 \mu\text{m}$ with an isoelectric point in the range of pH 4.4 to 4.6.

4./ **Hemp seed OBs are highly sensitive to ions !**

The composition of their surface (mainly the proteins and phospholipids) governs their physical stability/unstability.

Decreasing the pH and the addition of salts induce aggregation of OBs.

Calcium induce both aggregation and membrane-fusion mediated coalescence of hemp seed OBs by involving probably the anionic phospholipids together with membrane proteins.



Hemp seed oil bodies for food applications

Thierry Chardot¹, Marc Anton², Bruno Novalés^{2,3}, Hanitra Rabesona²,
Magalie Weber² & Christelle Lopez²

¹ INRAE, AgroParisTech, Université Paris-Saclay, Institut Jean-Pierre Bourgin, Versailles, France

² INRAE, UR BIA, Nantes, France

³ INRAE, BIBS facility, Nantes, France

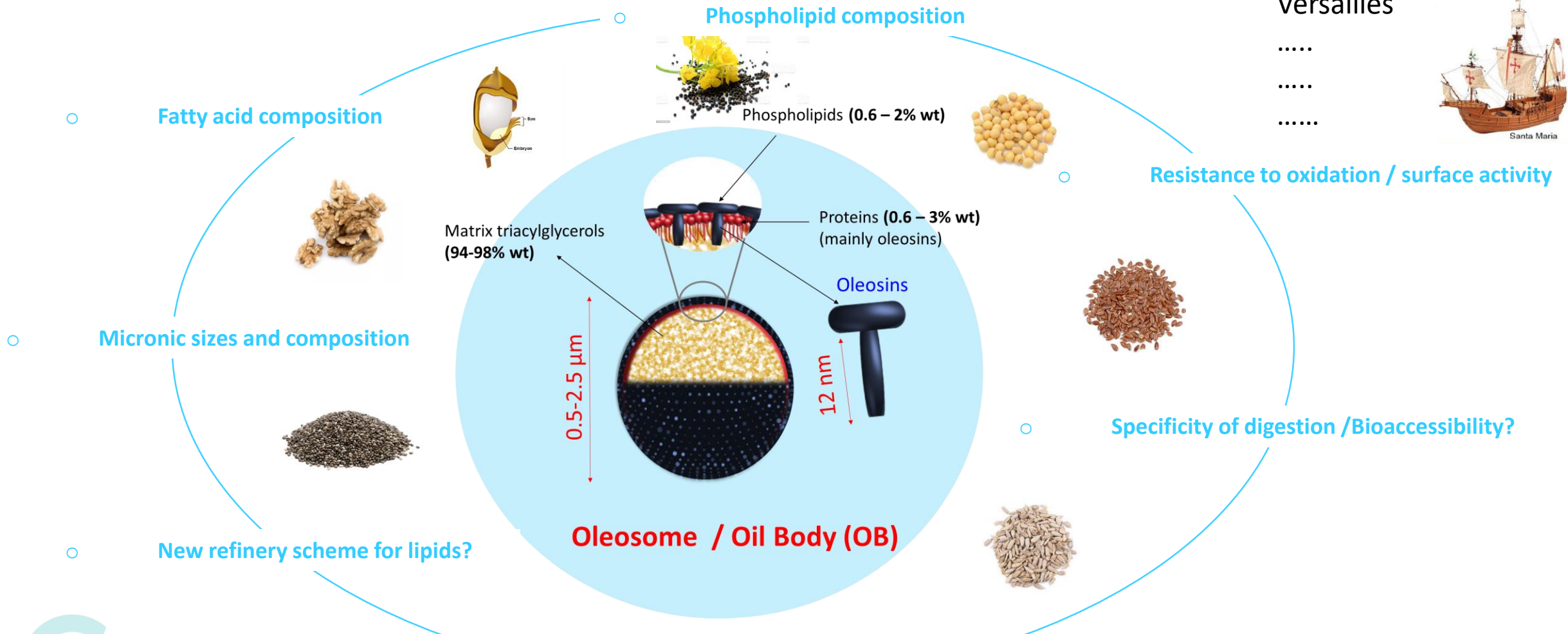


Thank you for your attention



➤ Storming through plant biodiversity to extract oleosomes for sustainable food applications

INRAE
Montpellier
Nantes
Toulouse
Versailles



- ➔ To highlight the physicochemical properties of various OB in the spotlight of the OBTAIN** consortium
- ➔ To present the green refinery scheme proposed for their extraction



About *OCL Journal*



- Peer-reviewed scientific journal (>50% of submitted articles are rejected)
- Devoted to oil and protein crops, fats and lipids (Agronomy, chemistry, nutrition)
- Edited by EDP Sciences, the publishing house of learned societies with a century of publishing experience.
- About **60 articles published each year** :
 - entirely free of charge for authors,
 - and the journal is open source for readers.

NO FEES



Gold open source

Costs are borne by a number of French institutions (Terres Univia, Terres Inovia, ITERG, FNCG, SFEL).

Citation



Indexed in:

- CABI Abstracts),
- DOAJ (Directory of Open Access Journals)
- "Emerging Citation Index" Web of Sciences (Clarivate Analytics, formerly Thomson Reuter),
- FSTA (Food Science and Technology
- Ebsco Discovery,
- Proquest,
- My Science work,
- CNKI, CNPIEC,
- ISTIC,
- Wanfang Data,
- Google scholar
- and Scopus.

Indicators (2020)

- **Scopus**
 - CiteScore = 2.9
 - #104/347 (Q2) in Agronomy and Crop Science
- **Web of Science**
 - Journal Citation Report = 0.54
 - Facteur d'impact en attente
 - #53/122 (Q2) in Agronomy (Emerging Sources Citation Index)

High consultation statistics (2020)

- **599 153 articles downloaded**
- **i.e. ≈ 50 000/month**

Topical issues



- Operates by focussing in turn on specific themes
- One of them = “**Lipid droplet**”.
- Opportunity to valorize the congress
- The basic administrative details are as follows:
 - Submission deadline: Mid-2022.
 - Length: approximately 35 000 characters but each author should feel free to adapt as necessary
 - Language: English.
 - Instructions to authors: <https://www.ocl-journal.org/author-information/instructions-for-authors>

**OCL IS PRESENT
AT MAJOR WORLD EVENTS**
In order to maximize the impact of the author’s work, printed copies are provided to attendees during targeted conferences, seminars or workshops, when the event’s scope matches the specific subject area in *OCL*.