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Interfacial adsorption and lipolysis behavior of gPLRP2 onto natural lipid membrane extracts from microalgae *Chlorella vulgaris*, walnuts, and almonds

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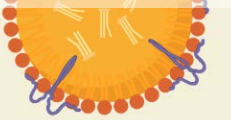
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Context

Oilseed OB membranes vs Photosynthetic plant membranes

Rich in phospholipids
Rich in $\omega 6$ and $\omega 3$ PUFA
(C18:2 and C18:3)

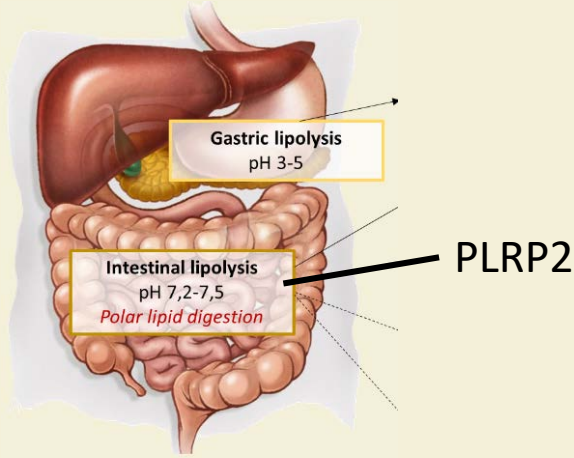


Oil bodies (OB)

Rich in galactolipids
Rich in $\omega 3$ PUFA
(C16:3 and C18:3)



Chloroplast



Objectives

This study is focused on the adsorption and lipolytic activity of the pancreatic lipase related type 2 protein (PLRP2) on natural lipid extracts. Two sources, OB (walnuts and almonds) or chloroplasts (microalgae), were selected. Complementary biophysical tools (tensiometry, ellipsometry, Atomic Force Microscopy-AFM) were used to characterize firstly the membrane model organisation and secondly, to quantify the adsorption and activity of PLRP2.

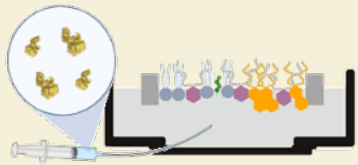
Material and methods

Systems studied

- Walnut OB
- Chlorella vulgaris*

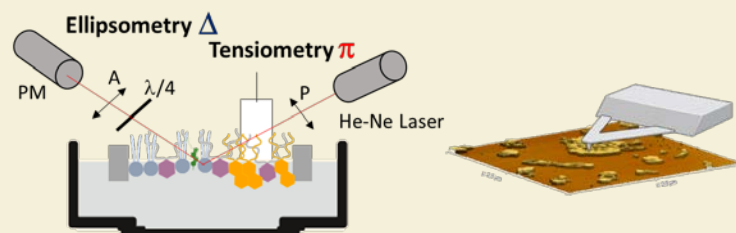
Polar lipid extraction

Membrane model



Polar lipid monolayer

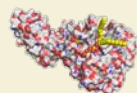
Characterization



π (surface pressure, mN/m)
→ Lateral molecular interactions at the air/water interface

Δ (ellipsometric angle, °)
→ Molecular film thickness (measured on 1 μ m below the film)

Enzymes



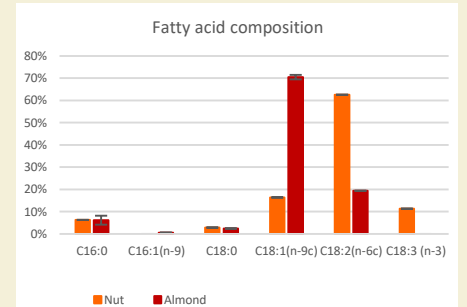
Guinea pig PLRP2 (gPLRP2)

Results

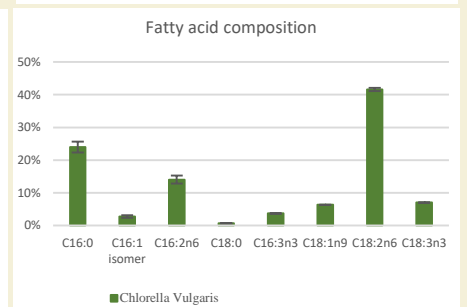
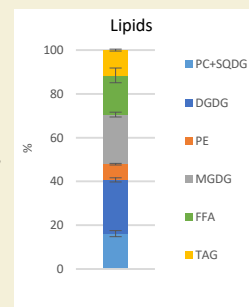
Chemical lipid characterization



Phospholipids only
Saturated FA ~10%
Walnut : $\omega 6 \gg \omega 3$
Almond : $\omega 9 \gg \omega 6$



Chlorella vulgaris



Galactolipids > Phospholipids

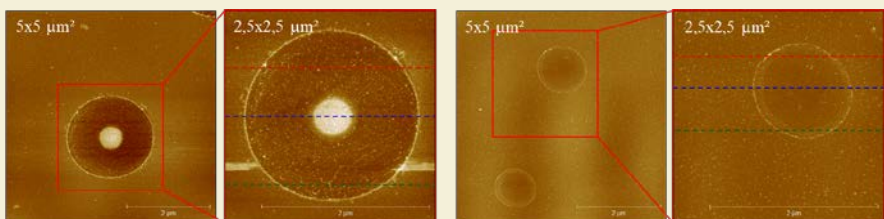
Saturated FA ~22%
 $\omega 6 \gg \omega 3$ (~walnut)

Results

Characterization of membrane model

Walnut OB

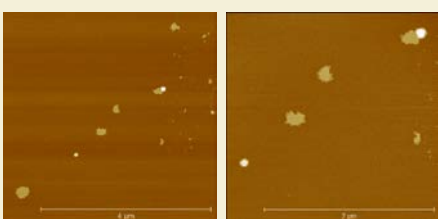
Almond OB



$\pi=20.1 \pm 0.5$ mN/m, $\Delta=8.1 \pm 1^\circ$ $\pi=20.1 \pm 0.5$ mN/m, $\Delta=9.1 \pm 0.5^\circ$

Phase separation on fluid phase due to the complexity of AG mixtures

C. vulgaris



$\pi=19.7 \pm 0.5$ mN/m, $\Delta=8.1 \pm 0.5^\circ$

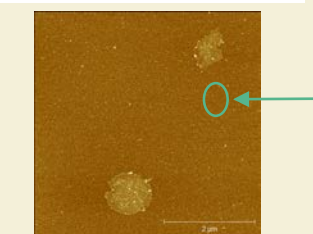
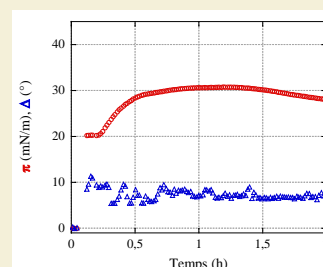
h=2,2 nm
h=1,5 nm
200 nm

Small liquid condensed domains with 2 different heights

Results

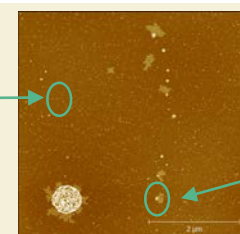
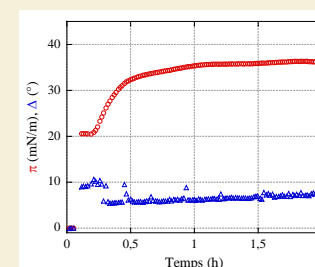
Kinetics adsorption and lipolytic activity of gPLRP2 (0.128 mg/L)

Walnut OB + gPLRP2



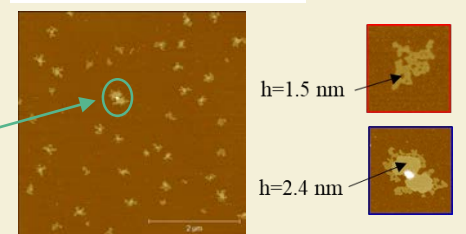
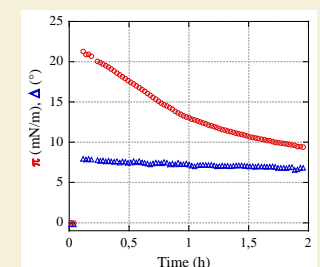
$\pi=28.0$ mN/m, $\Delta=8.1^\circ$

Almond OB + gPLRP2



$\pi=36.2$ mN/m, $\Delta=8.0^\circ$

C. vulgaris + gPLRP2



$\pi=9.4$ mN/m, $\Delta=6.9^\circ$

Adsorption of gPLRP2
Insertion in fluid phase and condensed phase
Film reorganization
Digestion products on almond extract film?

Adsorption of gPLRP2
Insertion in condensed phase
Domains of digestion products

Conclusion

The organisation of lipid extracts was observed by AFM and showed phase separation related to the FA composition. The interaction with the gPLRP2 was followed by ellipsometry and tensiometry, both signals allow to discriminate between adsorption, adsorption and insertion, adsorption and digestion. The lipolytic activity on galactolipids is clearly observed for the *chlorella vulgaris* extract.

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