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Galactolipids from microalgae as a new source of omega 3 and naturally structured surfactants for human nutrition



CNIS







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CONTEXT

Fatty acids

Essential

Non essential

✓ Global spread of obesity, overweight and diet-related chronic diseases with its burden of comortalities. How can we turn the tide off?

https://www.fda.gov/news-events/fda-voices/improving-nutrition-turn-tide-diet-related-chronic-disease

RDA* (% TEI)

cardiovascular diseases (17.9 million deaths globally/y), cancers (9 million deaths/y), and diabetes (1.6 million deaths/y) (WHO, 2019)

✓ Global picture but lipid nutrition matters (35-40 % TEI)

Lauric + myristic + palmitic FA

LA (C18:2, ω6)

Total saturated FA

Oleic FA (C18:1, ω9)

$LA(C18.2, \omega 0)$	4 %	10-12 %	
ALA (C18:3, ω3)	1 %	2.5-3 %	*RDA = Recommended Dietary
Docosahexaenoic acid, DHA (C22:6 ω3)	250 mg		allowances, TEI=Total energy intake, TFA=total fatty acids of lipid source
Eicosapentaenoic acid, EPA (C20:5 ω3)	250 mg		···· j··· j··· · · · · j , · · · · ·
Lauric + myristic + palmitic FA	≤ 8 %	20-23 %	(ANSES 2010 recommandations)

30-34 %

38-50 %

% TFA*

10 10 0/

1 0/

≤12 %

15-20 %







EPA (C20:5), DHA (C22:6) (250 mg/d)

(ANSES 2010 recommandations)

<mark>@6/</mark>@3=4





 \checkmark

 \checkmark

- Balancing our lipid intake by regulating our intake of omega-6 fatty acids and increasing our intake of omega-3 fatty acids = strong nutritional recommendation from the WHO
- Unbalanced diets (excess omega-6) promote the onset of chronic inflammatory diseases
- High imbalance in $\omega 6/\omega 3$ ratio, still very strong in infants and adolescents (INCA3; Chuy et al., Chevreul Congress 2023; Simopoulos, Biomed. Pharmacol., 2002)



To rebalance intakes what are the sources that can be used in nutritional strategies ?



Seeds and nuts rich ω3 precursors – main molecular form TAG >> PL



Fish and marine resources rich ω**3 VLCPUFA – main molecular form TAG >>PL**



Phospholipids (PL)



Photosynthetic vegetals from marine or terrestrial sources



(Cohn & al., Nutrients, 2010; Sahaka et al., Food & Function, 2020)

Triacylglycerols (TAG) 80 g/d



In some microalgae, fatty acids present under specific molecular forms: glycolipids





Digested by specific enzymes (pancreatic lipase related type 2 (PLRP2) or cholesterol ester hydrolase (CEH) and not by human pancreatic lipase (HPL)

 When structured under form of thylakoid membrane
 => inactivation of pancreatic lipase and weight loss (Köhnke et al., Scand. J. Gastrol., 2009; Köhnke et al., Phytotherapy Res., 2009; Tabrizi & Farhangi, FRI, 2021)

(Kergomard et al. CRFSN 2021; Sahaka et al. Food Funct. 2020; Vors et al., Cur Op Clin Nutr Metab Čare 2020)





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Antioxidant – DGDG > MGDG > TAG (Yamaguchi et al., J. Oleo Sci., 2012; Hazahari, et al., Food Nutr. Sci., 2018)

Natural surfactants

- content in omega 3 and its plasticity
- content in galactolipids
- potential presence of oxylipins
- 2) Interfacial behaviour of microalgae lipids
- 3) Digestion of microalgae galactolipids







е	
Omega-6-pro	duction
linoleic acid C18:2, LA	$ \begin{array}{c} \omega 6 \\ \downarrow \\ 6 \\ 4 \\ 2 \end{array} $
L ⁵ C18:3, GLA ↓ el C20:3, di-homo- ↓ Δ ⁵ Arachidonic ac C20:4, AA	5 3 1 desaturase Ongase GLA desaturase id

	PUFA (% wof total FA)			
	AA	ALA	EPA	DHA
Isochrysis galbana	0.7	1.2	22.6	8.4
Phaeodactylum tricornutum	3.4	0.6	29.8	0.8
Porphyridium cruentum	23	1.0	23.9	0.2
Crypthecodinium chnii	-	-	-	51.12
Schizochytrium sp.		-	-	25-50
Aurantiochytrium sp.	-	-	-	23-64
Nitzschia sp.	-	-	16- 23	-
Cod liver oil	2.7	0.8	12.5	9.2
Tuna oil	0.92	-	7.81	24.56
Shark liver oil	0.05	-	0.05	0.28
Soft-shell turtle oil	0.64	-	0.19	0.42
Lemuru oil	2.00	-	14.36	4.60
Flaxseed, whole, (1 tbsp)	2.35 g/serving	-	-	-
English walnuts (1oz)	2.57 g/serving	-	-	

(Katiyar et Arora, Algal Res., 2020)



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2

4

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(Katiyar et Arora, Algal Res., 2020)

2011)







\checkmark Plasticity of FA profiles and molecules on which these FA are esterified



Trophic mode	[Glucose] (g/L added to BBM*)	% lipids in the dry biomass	Abbreviati on	ω6/ω3
Photo- autotrophy	0	22	Lab-PA	2.4
Mixotrophy	2	20	Lab-M2	4.5
Mixotrophy	5	13	Lab-M5	3.8
Mixotrophy	8	13	Lab-M8	4.2
Mixotrophy	10	16	Lab-M10	3.6





Visual aspect of in-lab cultivated Chlorella Sorokiniana



Fatty acid profiles of in-lab cultivated Chlorella

(Wind, Master 2, 2021; Barouh et al., Chevreul Congress 2023, Paris; Couto et al., Algal Res. 2020)

\checkmark Plasticity of FA profiles and molecules on which these FA are esterified

	Origin	Species	Year of production	Specifications	Abbreviation	ω6/ω3
	Mongolia or	C. vulgaris	2021	Broken cell wall	Com-P-21	5.2
	Hainan island	C. vulgaris	2022	Broken cell wall	Com-P-22	8.9
	France	C. vulgaris	2021	Whole cells	Com-T-21	4.3
	France	C. vulgaris	2021	Whole cells	Com-F-21	5.7
•	France	C. pyrenoidosa	2022	Broken cell wall	Com-I-22	1.3
	Netherlands	C. sorokinana	2021	Broken cell wall - "fermented"	Com-E-21	5.1



Mass percent of total fatty acids

Jasmonic acid

www.aquaportail.com

✓ Presence of oxylipins ?



(Galano et al., Prog. Lipid Res., 2017)

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Main classes of NE oxylipins



Arachidonic acid (AA, C20:4 n-6) et eicosapentaenoic acid (EPA, C20:5 n-3) → Isoprostanes (IsoPs)



Alpha-linolenic acid (ALA, C18:3 n-3) → **Phytoprostanes** (PhytoPs)



Docosahexaenoic acid (DHA)→ Neuroprostanes (NeuroPs) 17 (Galano et al., Prog. Lipid Res., 2017)

✓ Presence of oxylipins ?

Oxylipins	Activity
Ent-16B1t-PhytoP	Neuroprotectivity
Ent-9L1t-PhytoP	Inflammatory
8-F3-IsoP	Pro-arythmia
10-F4t-NeuroP	Artherosclerosis prevention Anti VIDD (Ventilator Induce Diaphragm Disfunction)
4+F4t-NeuroP	Anti-inflammatory Artherosclerosis prevention Anti VIDD (Ventilator Induce Diaphragm Disfunction) Anti-arythmia Neuroprotectivity Sperm capacitation
14-A4t-NeuroP	Anti-inflammatory

(Galano et al., Prog. Lipid Res., 2017; Bosviel et al., Free Rad. Biol. Med., 2017; Martinez Sanchez et al., 2020; Signorini et al. Life, 2021; Moretti et₁gl. Molecular sciences, 2023)

1) Nutritional interest of microalgae lipids

✓ Presence of oxylipins ?



Profile in non enzymatic oxylipins detected in lipid extract of *C. sorokiniana* cultivated in photoautotrophy

(Avila-Roman et al., Pharmacol Res., 2018; Los Reyes et al., Phytochem, 2014; Conde, et al. Int. J. Mol. Sci. 2021)

2) Interfacial behaviour of microalgae lipids



2) Interfacial behaviour of microalgae lipids

Folch extraction of total lipids on commercial or in-lab produced C. sorokiniana extracts

Ellipsometry/Tensiometry







Average molecular area (Å²/molecule or weight of extract)

Atomic Force Microscopy (AFM)



 Δ (ellipsometric angle) \rightarrow amount of matter at the a/w interface

Topographic visualisation at the nanometric seale



2) Interfacial behaviour of microalgae lipids

61 % MGDG 23 % DGDG



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Enzymes at work in Humans





No lipolytic enzyme acting on polar lipids is produced in the gastric compartement



But the stability of emulsions resulting from gastric digestion influences the kinetics of intestinal digestion.

(Infantes-Garcia et al., JAFC, 2021)

Gastric lipase (HGL) – 47 kDa *Responsible for the digestion of 10 to 30% of TAG*

Pancreatic phospholipase A2 (sPLA2) - 14 kDa
 Phospholipase activity in sn-2 position

Colipase-dependent pancreatic lipase (HPL) – 48 kDa Responsible for the digestion of 56% of TAG

Pancreatic lipase related protein 2 (PLRP2) – 50 kDa

(N'Goma et al. 2012)

Responsible for the digestion of galactolipids

(Borgström, Lipids, 1993; Carrière, Gastroenterol. 1993; Kergomard et al., CRFSN, 2021; Andersson et al., J. Lipid Res., 1995, Wattanakul et al. Food Function, 2019)



Lipid monolayers

Giant liposomes



 \checkmark gPLRP2 adsorption and lipolytic activity onto model monolayers at the air/water interface

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5x5 μm<sup>2</sup>
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<mark>∆</mark>=7.5°

System 1 – GL



+ gPLRP2 (0.128 mg/L)







• Enzymatic activity of gPLRP2 on GL monolayer

Optimal activity at 15 mN/m, decrease of π , marked reduction in thickness (Δ).

• Modification of the interfacial organization

Appearance of domains related to the generation of lipolysis products, adsorption of PLRP2 at the interface

• No lipolysis activity for the PLRP2 inactive variant

No evolution of π and Δ , preservation of the PLRP2 structure (central domain 50 Å)



Kergomard et al, 2022 submitted Biochimie

Comparison of gPLRP2 adsorption and lipolytic activity onto biomimetic system (2) and Lipid monolayer from *Chlorella vulgaris* (System 3 Commercial, Com-P-21)



System 2 – GL/SQDG/PG

+ gPLRP2

1h, π=8.5 mN/m, Δ =5.2°

System 3 – Commercial, Com-P-21









In the presence of biliary salts: Galactolipase activity Phospholipase activity

\rightarrow Generation of new surfactant molecules

(Kergomard et al, 2022 PHD unsubmitted work)

Ab: Pancreatic lipase related protein 2 (PLRP2), BSSL : bile salt stimulated lipase, DGDG: digalactosyldiacylglycerol, DGMG: digalactosylmonoacylglycerol, FFA: free fatty acids, MGDG: monogalactosyldiacylglycerol, MGMG: monogalactosylmonoacylglycerol, SQDG: sulfoquinovosyldiacylglycerol, SQMG: sulfoquinovosylmonoacylglycerol, PG: Phosphatidylglycerol



Conclusions

Microalgae galactolipids can be an interesting complementary source of omega 3 PUFA possibly esterified under the \checkmark galactolipids form

Microalgae sources	Terrestrial sources (green leafy vegetables)
VLC omega 3 PUFA	Omega 3 PUFA precursors only
Higher PL/GL ratios in photoautrotrophy, adjustable with environmental conditions	Ratio PL/GL =45:55 in leaves and 30:70 in chloroplasts
~10-15 % (DM) of lipids of which 70-80 % can be GL	2-30 g/kg (DM) MGDG-DGDG
No arable land use / Concentration/ Stabilization energy consuming	Arable land use

- ✓ These galactolipids have specific interfacial behaviour compared to glyceroPL
- \checkmark Can be easily digested by close analogue of human PLRP2
- ✓ Lots of work remained to be done to understand assembly behaviour in the digestive tract

Work to stabilize biomass and maintain molecular form by Maeva Subileau



Preclinical trial on GL terrestrial sources by C. Vors

Thanks to all contributors !!



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Thank you for your attention









EXTRA-SLIDES



Formes moléculaires et phases polymorphiques du quartet lipidique des thylakoïdes

