

Lipid content in *Chlorella* biomass: variability related to biomass origin

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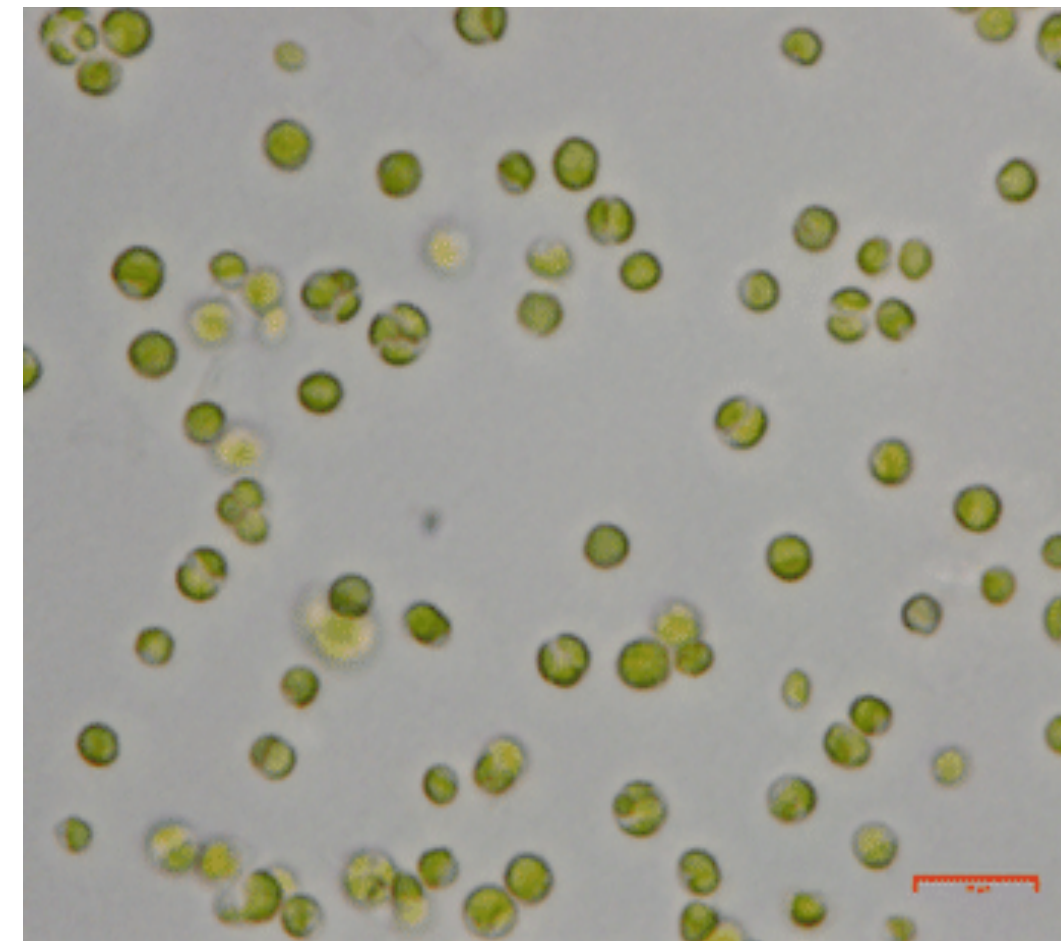
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Microalgae appear as a sustainable source of biomass with valuable nutritional qualities. Under the current regulations, very few biomasses of eukaryotic microalgae species are allowed for human food and most belong to the *Chlorella* genus. *Chlorella* biomass can contain lipids of interest such as polyunsaturated fatty acids (PUFA) ω 3 and ω 6. Yet, the amount of PUFA as well as the ω 6/ ω 3 ratio can vary significantly depending on the species considered as well as the trophic mode used for cultivation (photo-autotrophy, mixotrophy or heterotrophy). While the lipid content of in-lab produced *Chlorella* has been extensively studied, the variability of commercial biomass composition is barely described yet (Canelli *et al.*, 2020). Here we characterized the lipid classes (TAG: triacylglycerols; FFA: free fatty acids; MGDG: monogalactosyldiacylglycerols; DGDG: digalactosyldiacylglycerols; PE: phosphatidylethanolamines; PC: phosphatidylcholines; SQDG: sulfoquinovosyldiacylglycerols) and fatty acid profiles of dried commercial biomasses of different *Chlorella* spp. as well as those of in lab-produced *Chlorella sorokiniana*, grown in photo-autotrophic and mixotrophic conditions (Barouh, et al. 2024). Variations in *Chlorella* lipid content in commercial and in-lab produced biomass. OCL. <https://doi.org/10.1051/ocl/2024005>

Characteristics of commercial and in-lab produced biomasses of *Chlorella* spp.

Freeze dried commercial biomasses

Origin	Species	Year of production	Specifications	Abbreviation
Mongolia or Hainan island	<i>C. vulgaris</i>	2021	Broken cell wall	Cv-A-com-21
	<i>C. vulgaris</i>	2022	Broken cell wall	Cv-A-com-22
France	<i>C. vulgaris</i>	2021	Whole cells	Cv-B-com-21
France	<i>C. vulgaris</i>	2021	Whole cells	Cv-C-com-21
France	<i>C. pyrenoidosa</i>	2022	Broken cell wall	Cp-com-22
Netherlands	<i>C. sorokinana</i>	2021	Broken cell wall - "fermented"	Cs-com-21



Optical microscopic observation of *C. sorokiniana* (x100)

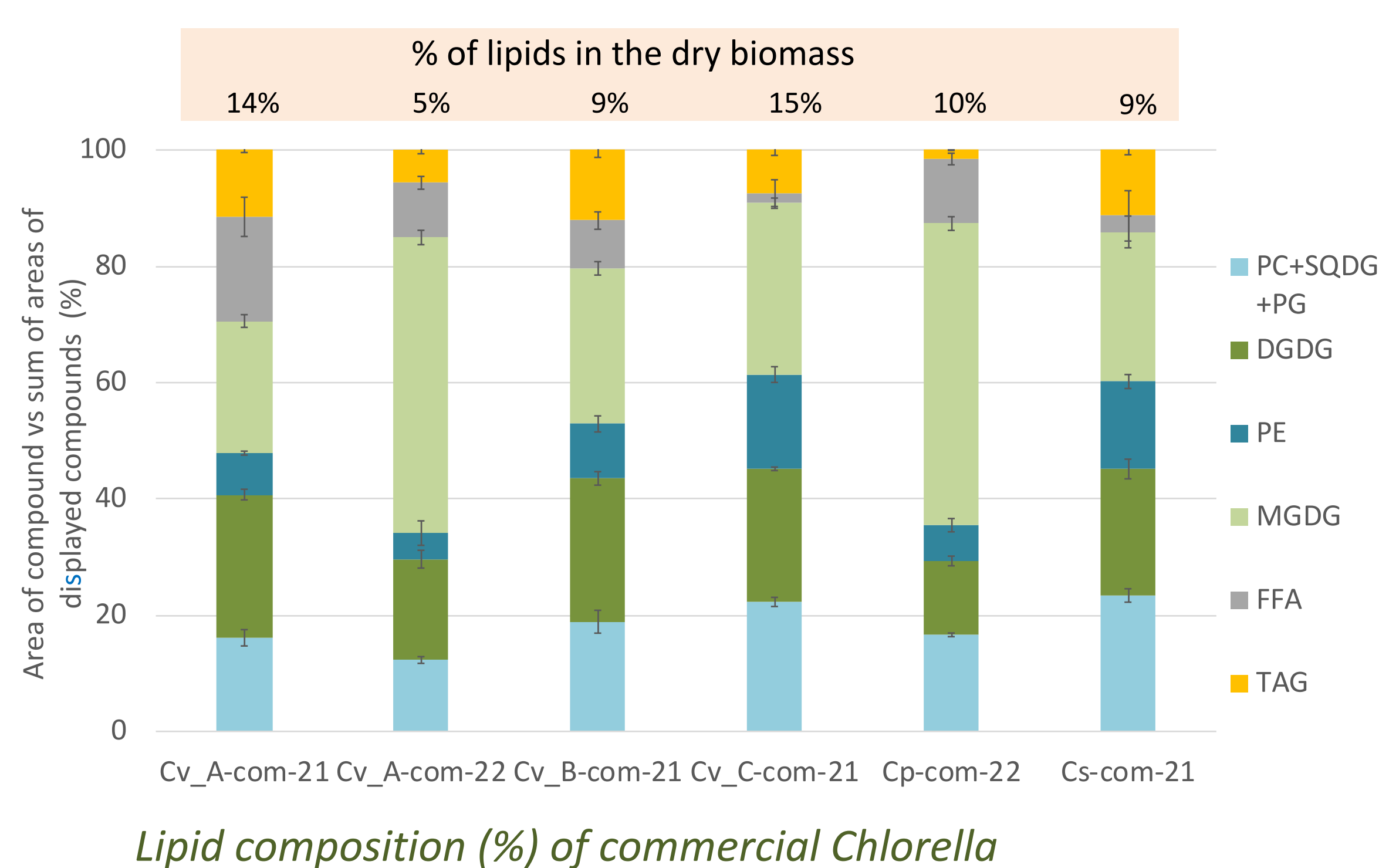
In-lab cultivation conditions of biomasses

Trophic mode	Species	[Glucose] (g/L added to BBM*)	Abbreviation
Photo-autotrophy	<i>C. sorokinana</i>	0	Cs-Lab-PA
Mixotrophy	<i>C. sorokinana</i>	2	Cs-Lab-M2
Mixotrophy	<i>C. sorokinana</i>	5	Cs-Lab-M5
Mixotrophy	<i>C. sorokinana</i>	8	Cs-Lab-M8
Mixotrophy	<i>C. sorokinana</i>	10	Cs-Lab-M10

*Bold's Basal Medium: Bischoff and Bold (1963) derived from Bold (1949)

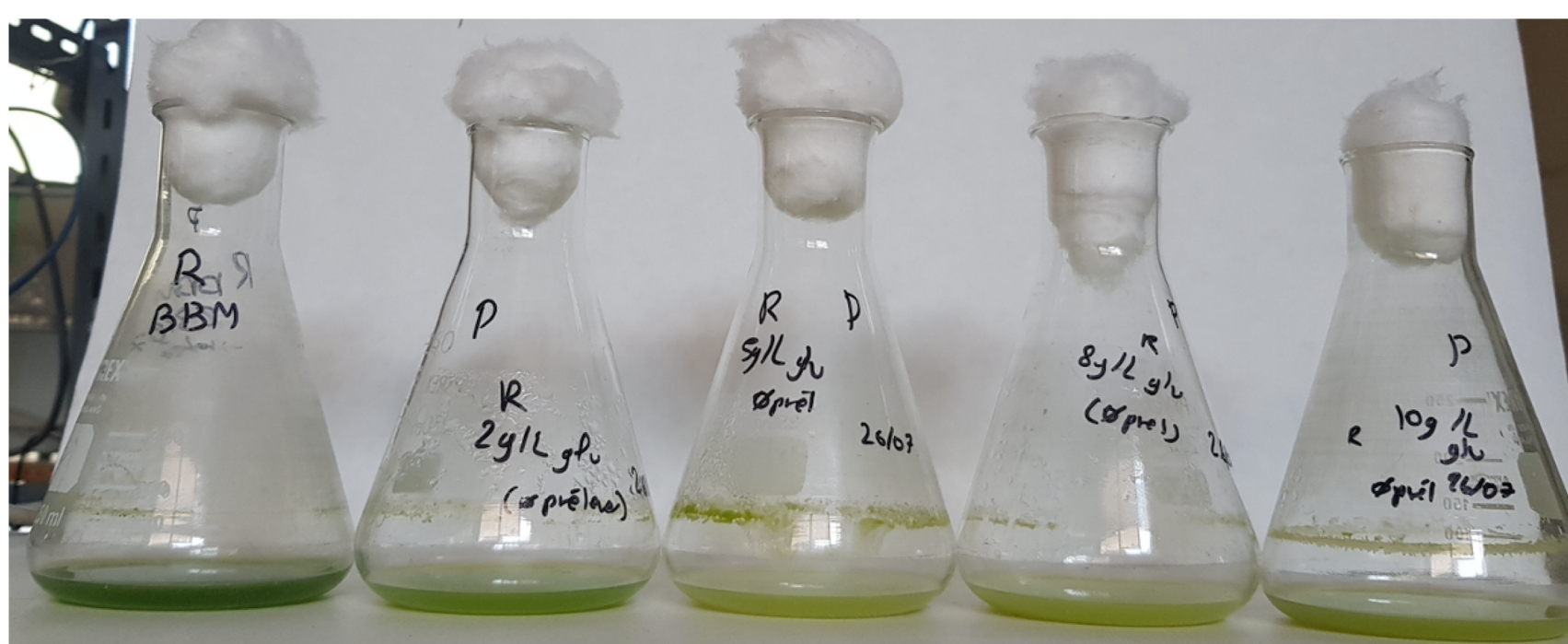
RESULTS

Lipids classes of the biomasses were assessed after extraction of total lipids and TLC analysis (densitometry)



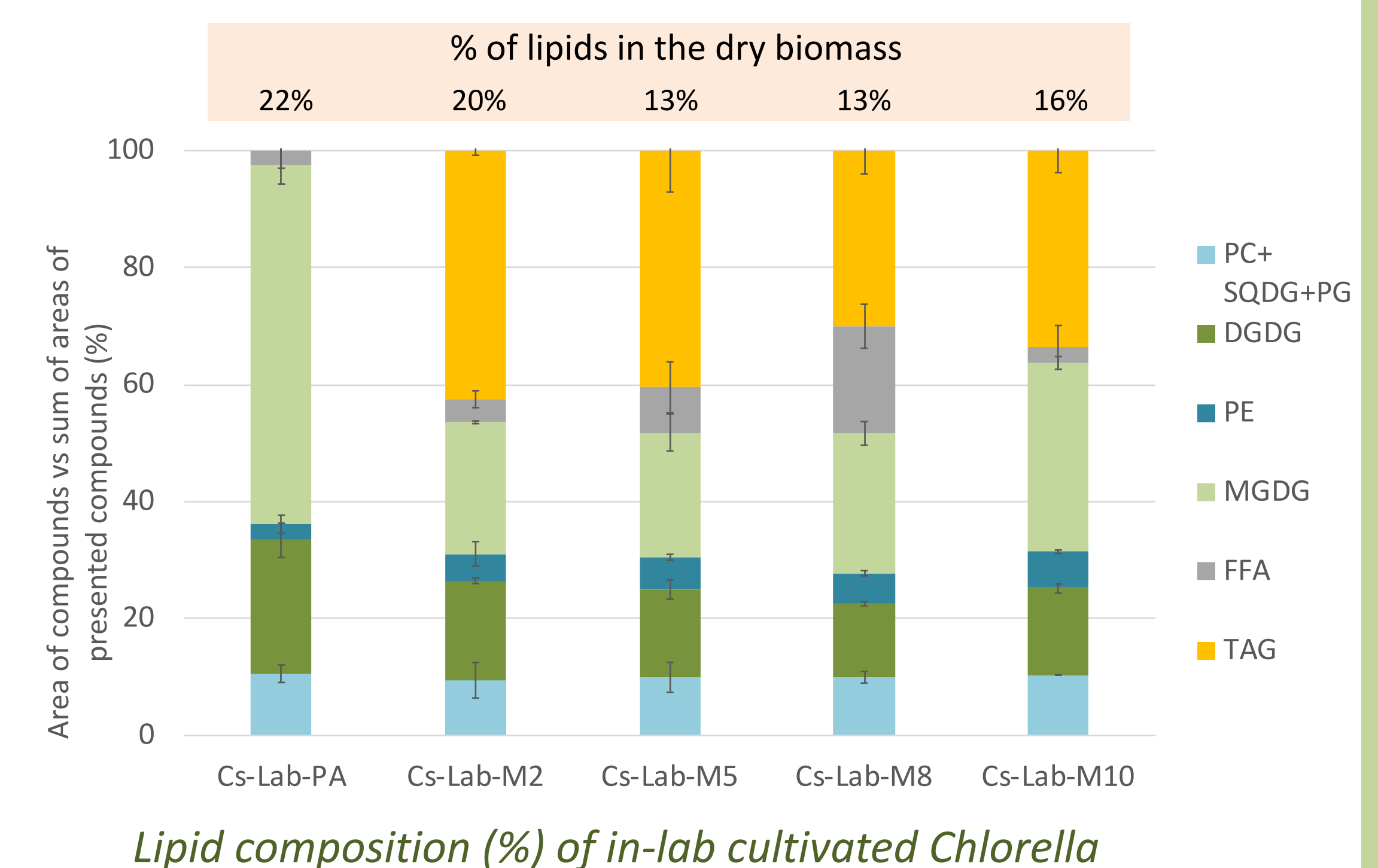
Lipid composition (%) of commercial *Chlorella*

Visual aspect of in-lab cultivated *Chlorella*



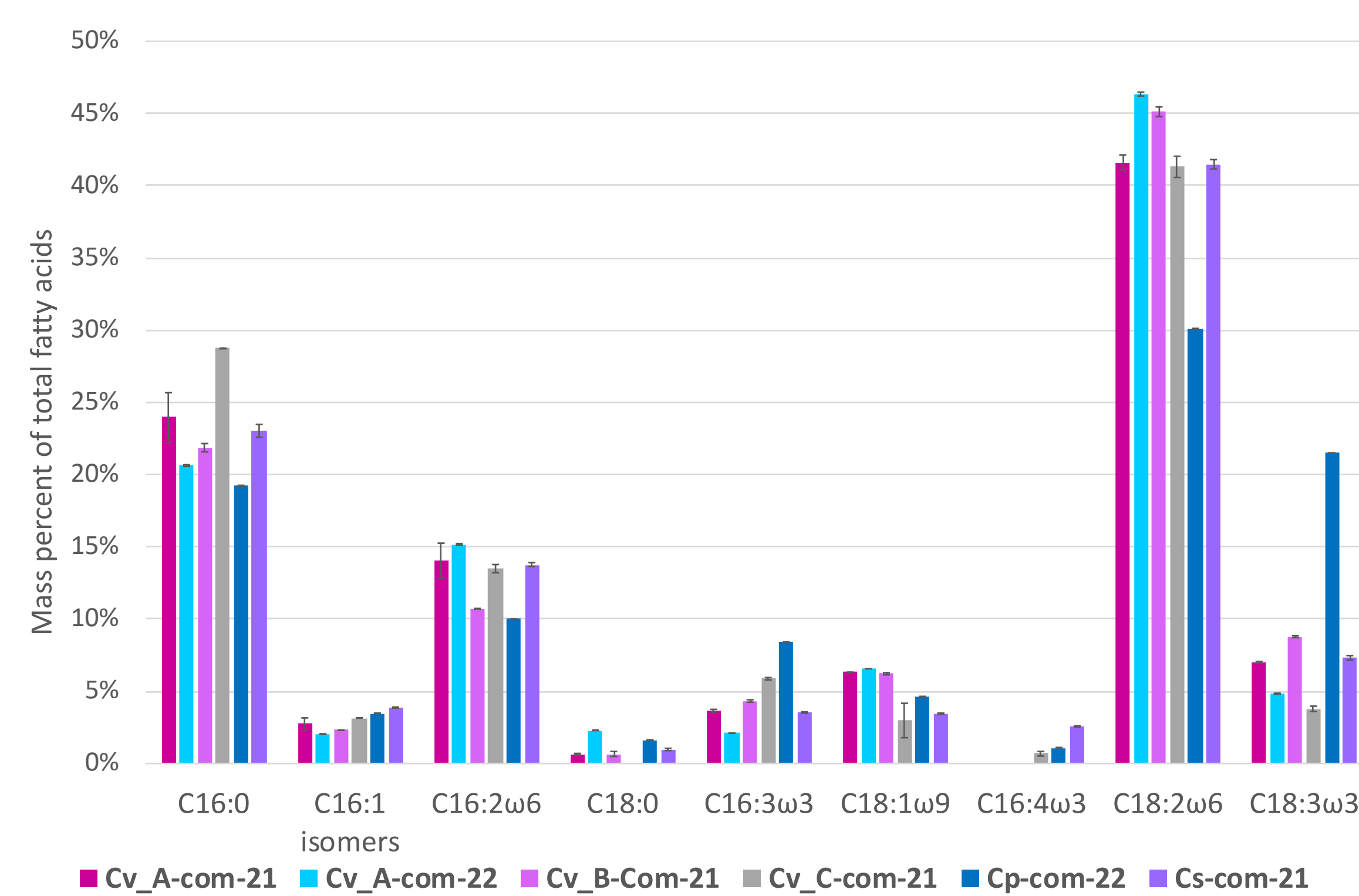
Visual aspect of in-lab cultivated *Chlorella*

*In photo-autotrophic conditions, all lipids were polar membrane lipids, mostly MGDG (61%) and DGDG (21%), while the addition of glucose for mixotrophy led to significant accumulation of TAG (~40%)



Lipid composition (%) of in-lab cultivated *Chlorella*

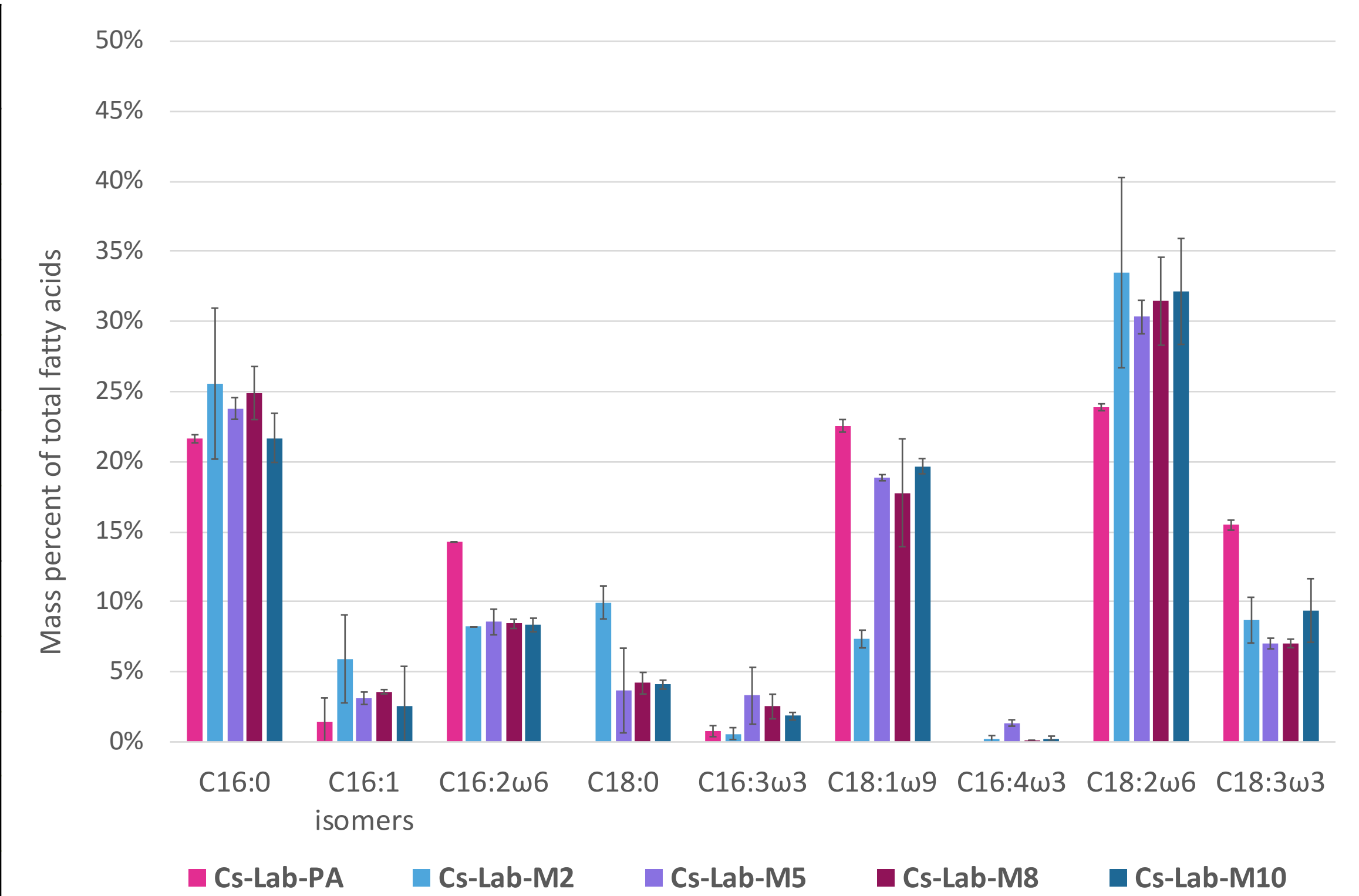
Fatty acid composition of the biomasses were assessed after extraction of total lipids, methylation and GC-FID analysis



Fatty acid profiles of commercial *Chlorella*

Exp.	ω 6/ ω 3**	LA/ALA**
Cv-A-com-21	5.2	5.9
Cv-A-Com-22	8.9	9.6
Cv-B-Com-21	4.3	5.1
Cv-C-com-21	5.3	10.9
Cp-com-22	1.3	1.4
Cs-com-21	5.1	5.7
Cs-Lab-PA	2.4	1.5
Cs-Lab-M2	4.5	3.9
Cs-Lab-M5	3.8	4.3
Cs-Lab-M8	4.2	4.5
Cs-Lab-M10	3.6	3.4

** recommendation: ω 6/ ω 3 and LA/ALA = 5 (AFSSA, 2011)



Fatty acid profiles of in-lab cultivated *Chlorella*

CONCLUSION

The *Chlorella* spp. biomasses displayed significant variations in their lipid composition

Biotic and abiotic parameters significantly modified the lipid composition of *Chlorella* biomass.

- The membrane/neutral lipid ratio ranged from 70/30 to 90/10 in commercial biomasses, and from 50/50 to 98/2 in mixotrophy and photo-autotrophy lab-produced biomasses.
- The ω 6/ ω 3 ratio varied from 1.3 to 8.9 in commercial biomasses, and from 2.4 to 4.2 in photo-autotrophy and mixotrophy lab-produced biomasses. Valuable ratios of ω 6/ ω 3 (<4) and triacylglycerols/glycolipids were achieved in *Chlorella* when produced in photo-autotrophy.
- However, the lipid quality of in-lab cultivated *Chlorella* is not directly scalable to that of commercial biomass. As a result, the lipid composition of commercial *Chlorella* biomasses and their related nutritional benefits can vary significantly, even in biomass of the same brand but of different production batches (Cv-A-com-21 and Cv-A-Com-22).

The next step will be to control the culture conditions to modulate the lipid contents, ω 6/ ω 3 ratio, and bioactive polar galactolipids in a targeted manner. Furthermore, free fatty acid levels can vary substantially if the production or post-processing conditions are not well controlled (see Reynolds et al. poster, GERLI 2024). This data highlights the need for well-managed microalgae biomass production to ensure its final nutritional benefits.