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Variations in *Chlorella* spp. lipid content in commercial and in-lab produced biomasses

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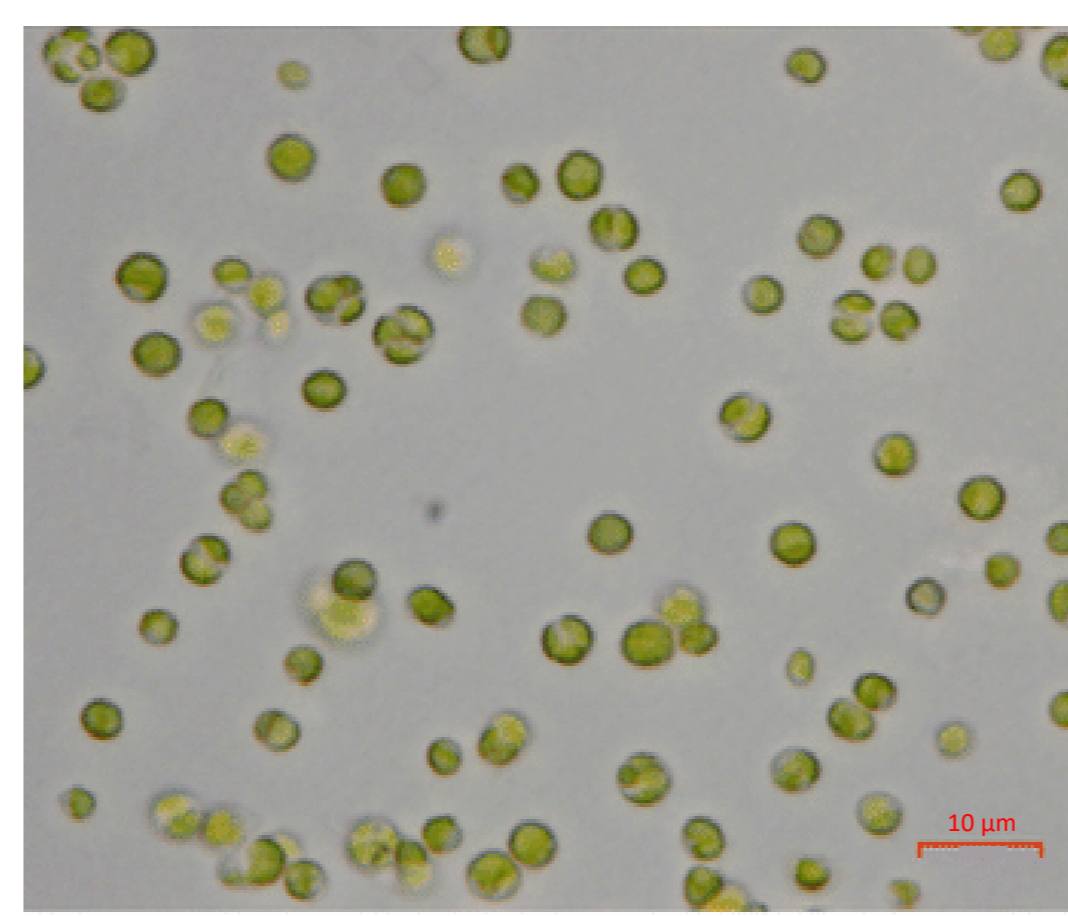
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Microalgae appear as a sustainable source of biomass with valuable nutritional qualities. Under the current regulations, very few biomasses of eukaryotic microalgae species are allowed for human food and mostly belong to the *Chlorella* genus. *Chlorella* 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 (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 commercial biomasses of different *Chlorella* spp. as well as those of in lab-produced *Chlorella sorokiniana*, grown in photo-autotrophy and mixotrophy conditions.

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

Commercial biomasses

Origin	Species	Year of production	Specifications	Abbreviation
Mongolia or Hainan island	<i>C. vulgaris</i>	2021	Broken cell wall	Com-P-21
	<i>C. vulgaris</i>	2022	Broken cell wall	Com-P-22
France	<i>C. vulgaris</i>	2021	Whole cells	Com-T-21
France	<i>C. vulgaris</i>	2021	Whole cells	Com-F-21
France	<i>C. pyrenoidosa</i>	2022	Broken cell wall	Com-I-22
Netherlands	<i>C. sorokinana</i>	2021	Broken cell wall - "fermented"	Com-E-21



Optical microscopic observation of *Chlorella* (x100)

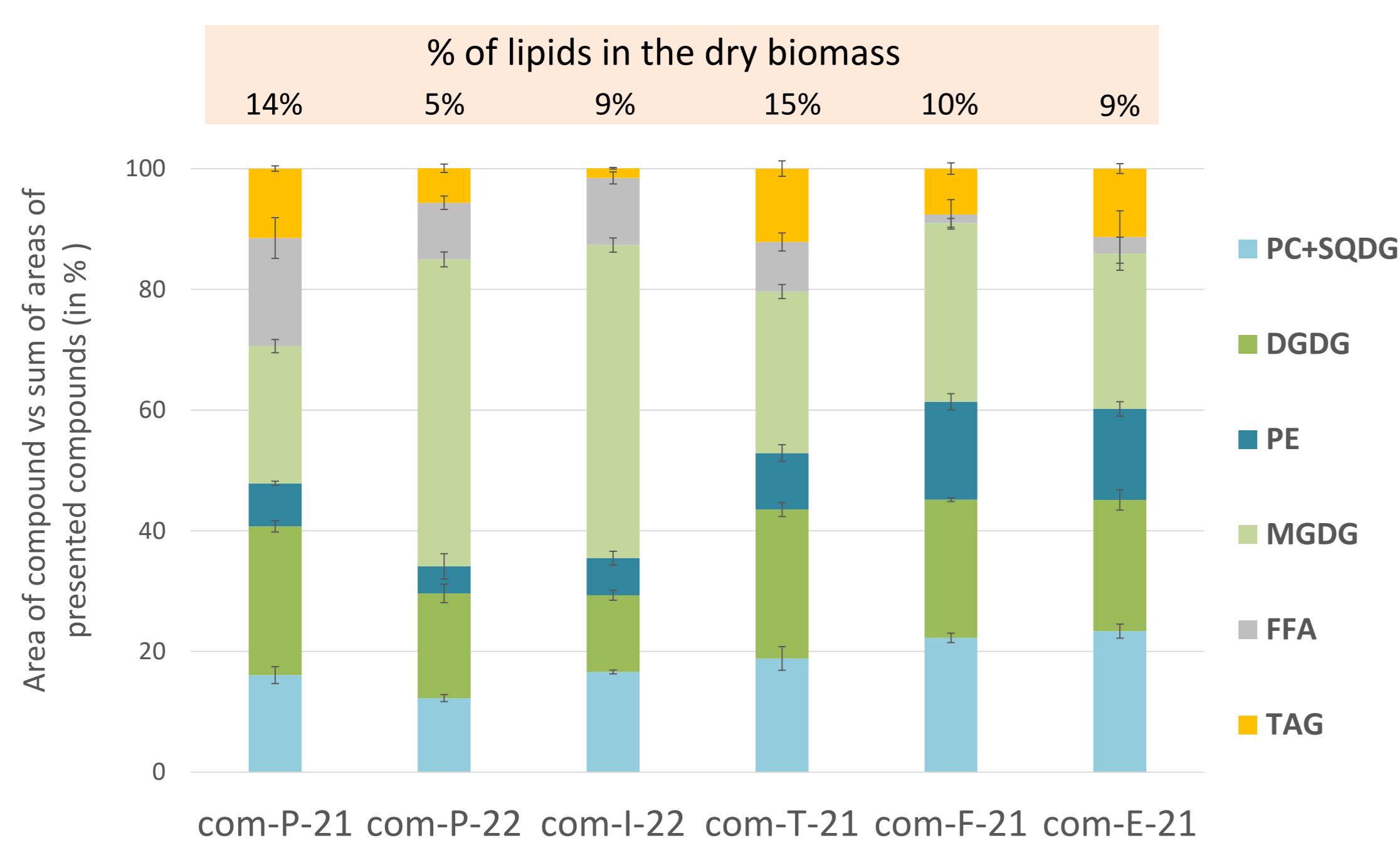
In-lab cultivation conditions of biomasses

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

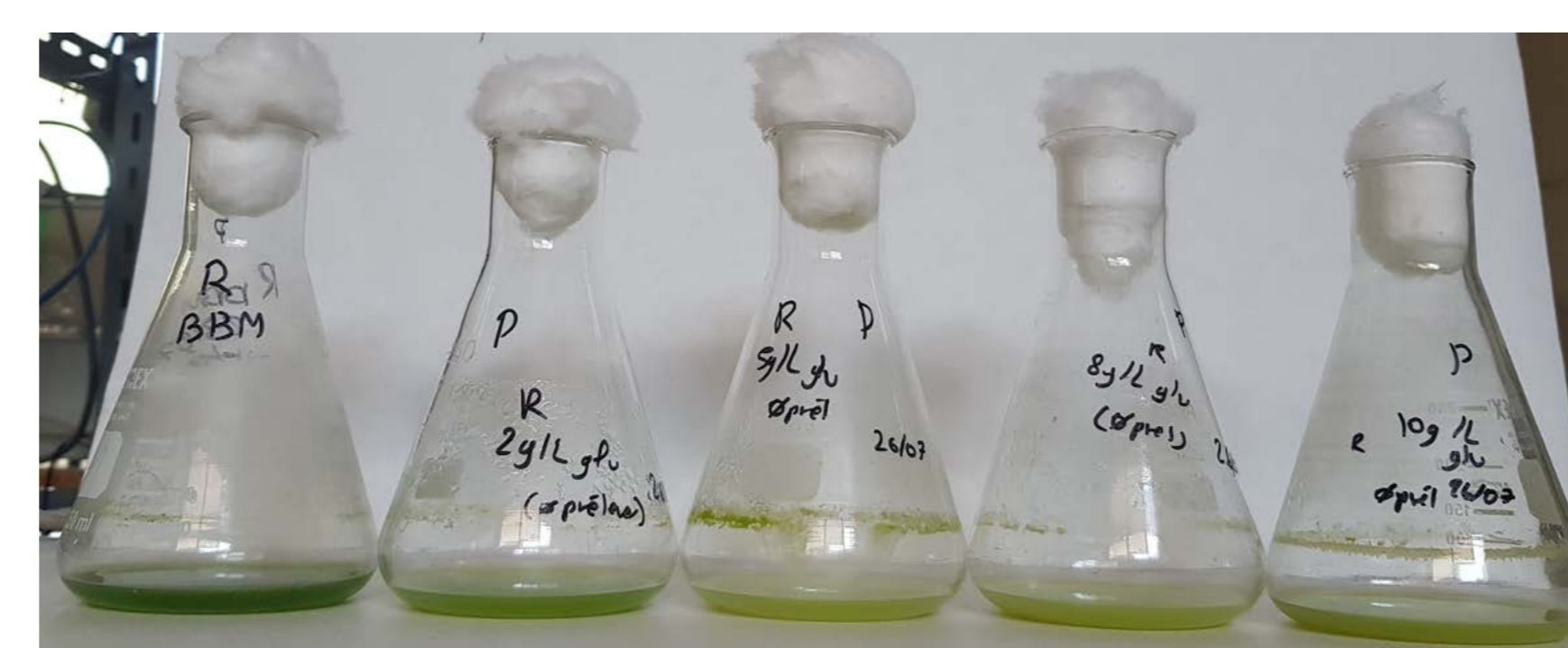
*Bold's Basal Medium: Bischoff and Bold (1963) derived from Bold (1949)

Results

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

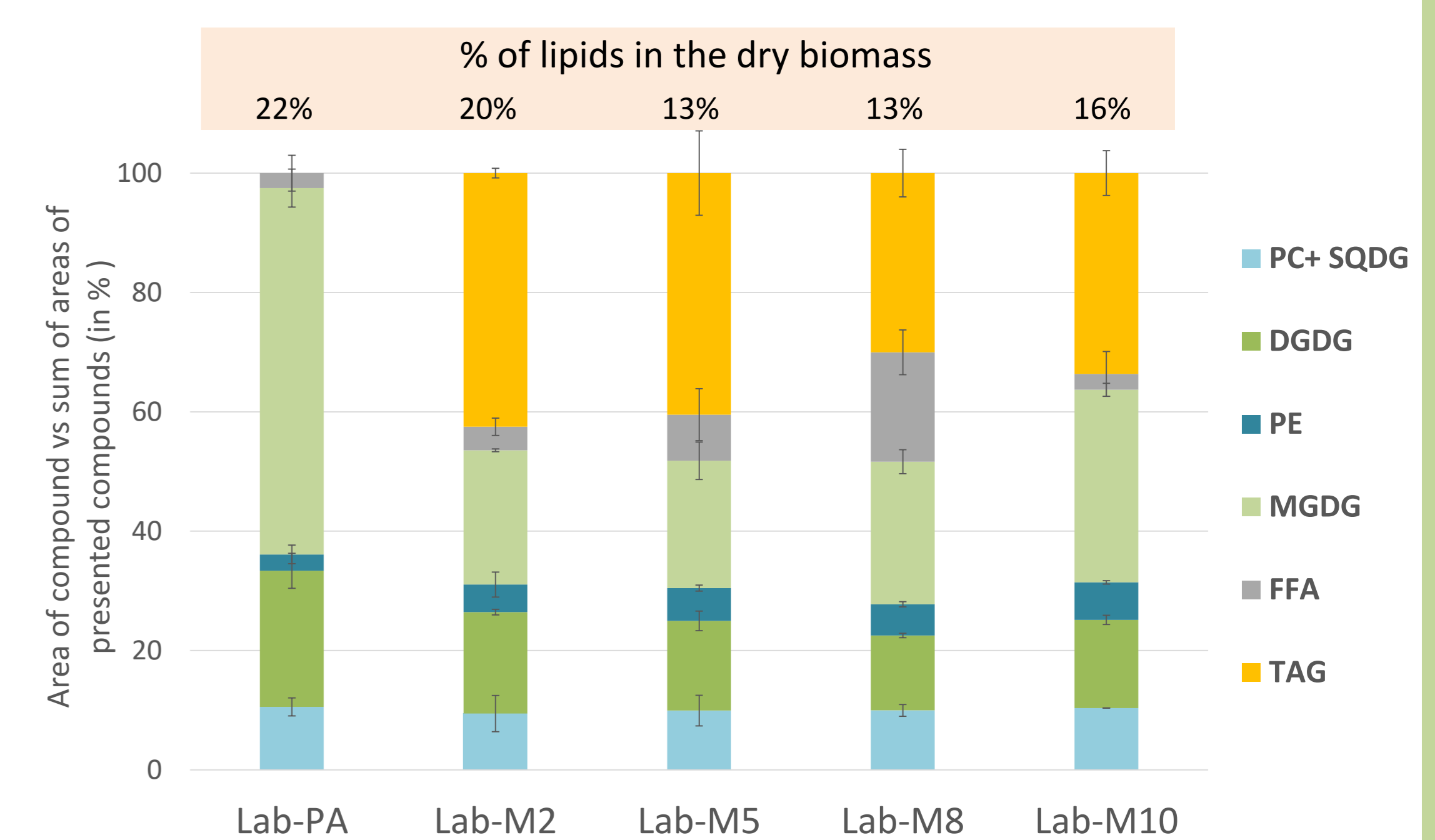


Lipid composition (%) of commercial *Chlorella*



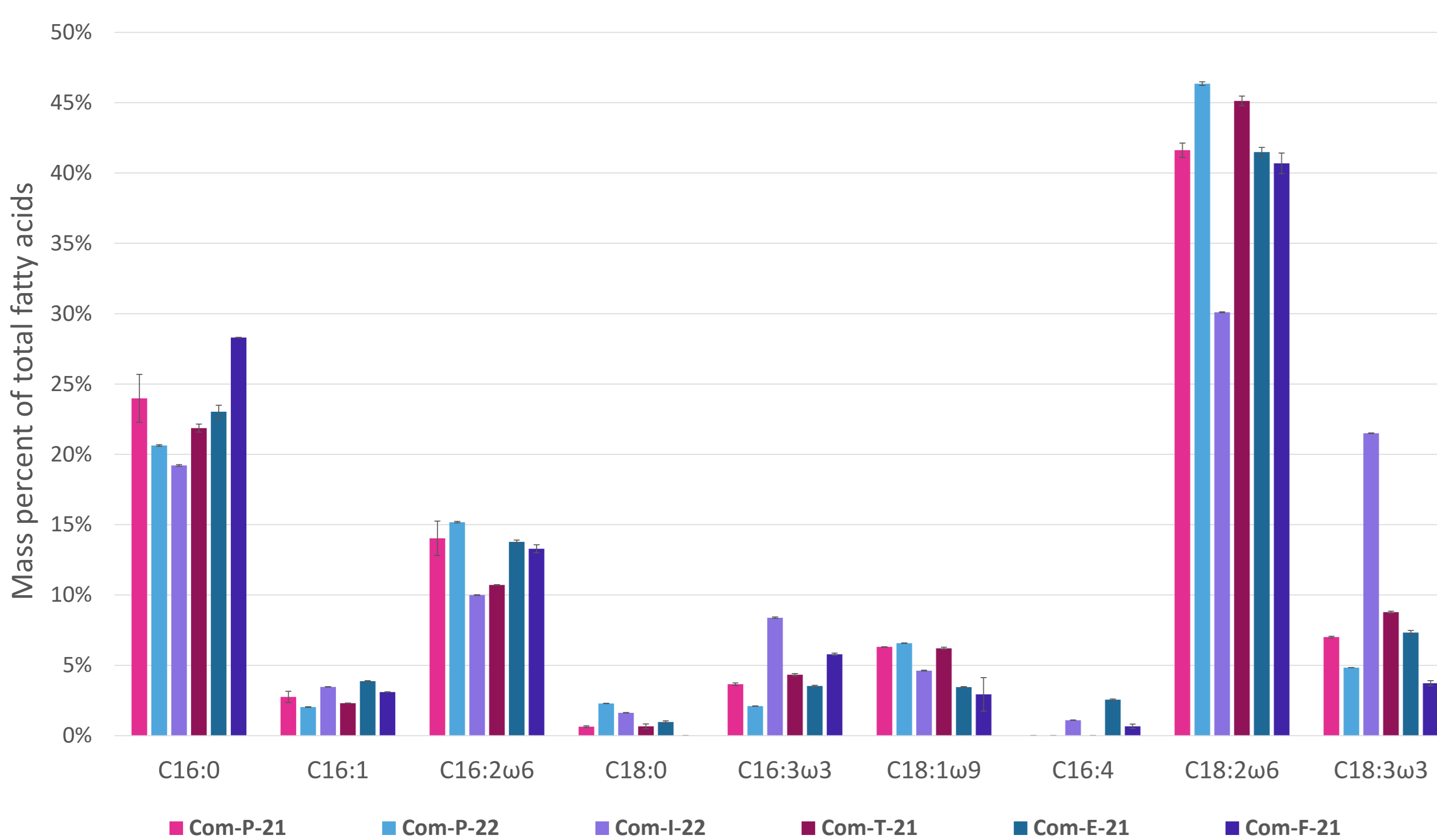
Visual aspect of in-lab cultivated *Chlorella*

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%)



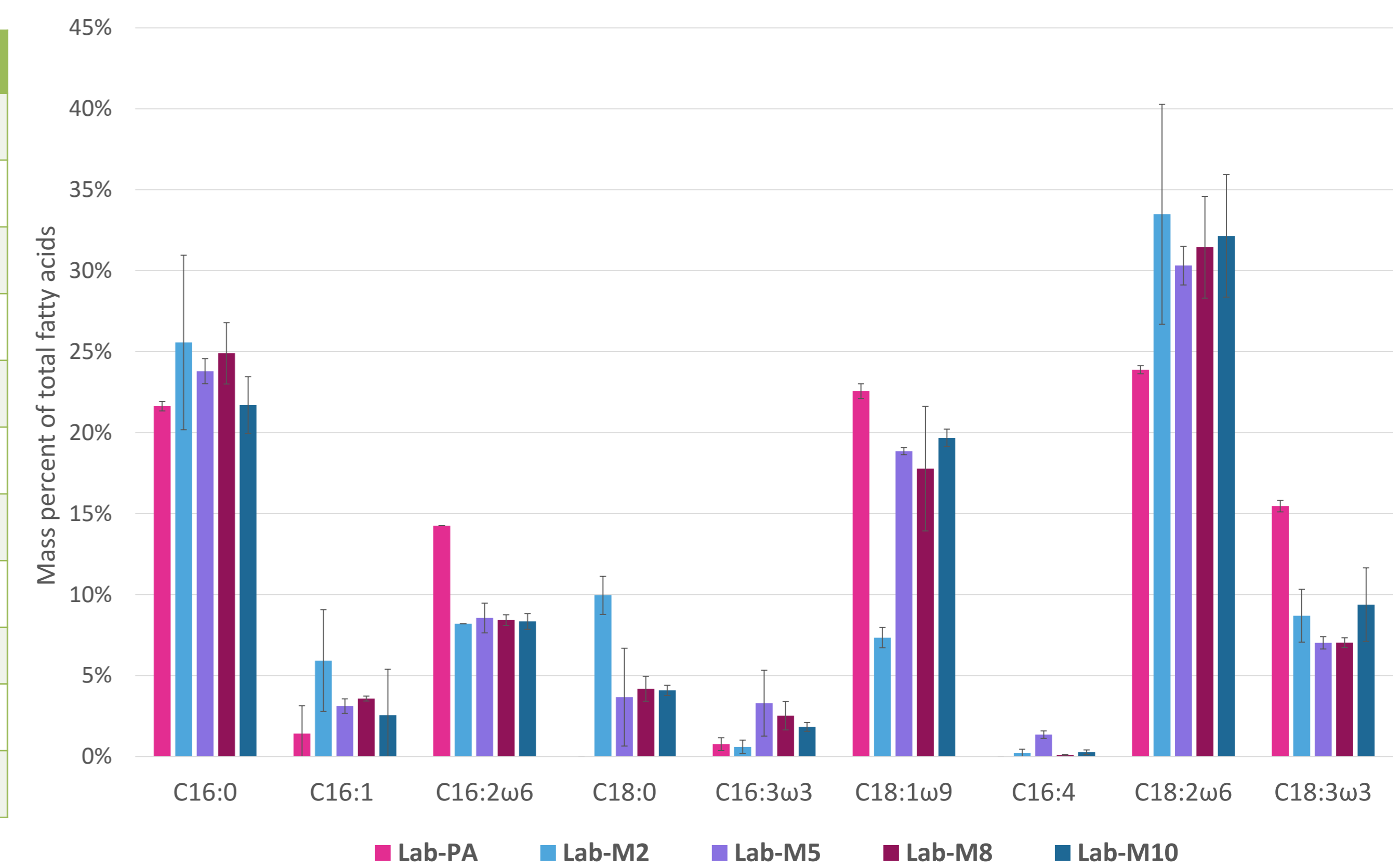
Lipid composition (%) of in-lab cultivated *Chlorella*

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
Com-P-21	5.2
Com-P-22	8.9
Com-T-21	4.3
Com-F-21	5.7
Com-I-22	1.3
Com-E-21	5.1
Lab-PA	2.4
Lab-M2	4.5
Lab-M5	3.8
Lab-M8	4.2
Lab-M10	3.6



Fatty acid profiles of in-lab cultivated *Chlorella*

Conclusion

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

Our results showed significant variations in the lipid composition of the biomasses, *i.e.* the membrane/neutral lipid ratio (70:30 to 90:10 in the commercial biomasses and 50:50 to 98:2 in mixotrophy and photo-autotrophy respectively in-lab produced biomasses) as well as the ω 6/ ω 3 ratio (1.3 to 8.9 in commercial biomasses and 2.4 to 4.2 in photo-autotrophy and mixotrophy lab-produced biomasses). These results thus demonstrate that the biomasses can display important differences in both lipid levels and qualities. Next step will be to control the culture conditions to be able to modulate in a targeted way the lipid contents, the ω 6/ ω 3 ratio and the bioactive polar galactolipids. In addition, free fatty acid levels can be substantially different if the conditions of production or of post-processing are not well controlled. All these data demonstrate the need of well mastered production of microalgae biomass to ensure the nutritional benefits from its consumption.