"Analysis and Evaluation of Nannochloropsis sp. as Renewable Source for Valuable Chemicals"

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"**Third Latin American Conference on Biorefineries**"
November 19-21, 2012
Pucón, Chile

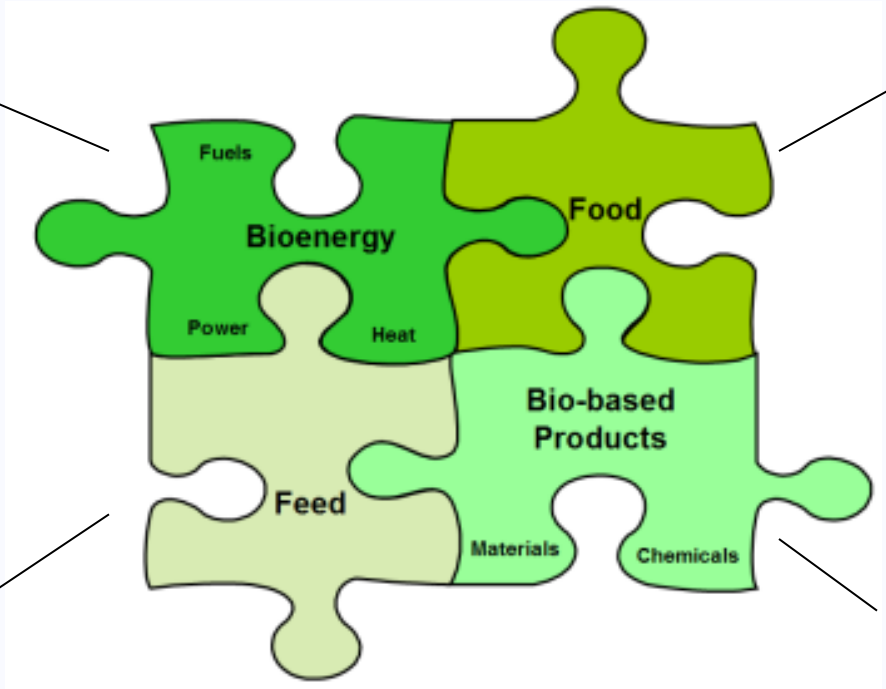
Agenda

- Introduction: Microalgae for biorefineries
- National project „PHOTOCHEM“
- Extraction techniques for *Nannochloropsis occulata*
- Comparison of different extraction solvents
- Qualitative and quantitative characterization of extracts
- Yield of omega-3 and total fatty acids
- Summary and conclusions

Microalgae within a biorefinery concept

research stage

on the horizon



in development

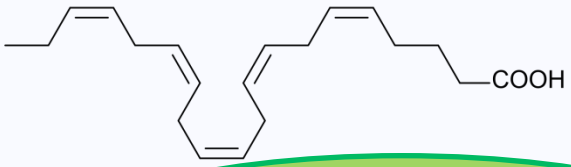
partially developed:
omega-3, vitamins

biorefinery approach is crucial for economic viability!!

Value Added Products

multiple products dependent on strain, growth state and conditions

Eicosapentaenoic acid
(all-(Z)-C20:5 n-3)



„Phyto“sterols

Proteins

Hydrocarbons

Pigments

Lipid (pro-)vitamins

Fatty acids, particularly polyunsaturated fatty acids (PUFAs)

- → omega-3 PUFAs currently produced from fish oil
- → fish are not the primary producers (microalgae are!)
- → drawbacks of production from fish
  - unpleasant odour
  - concerns about contamination with heavy metals, etc.
  - neglects needs of vegetarian and vegan diets

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M.Mittelbach, Biorefineries, Pucon, 2012

UNI GRAZ

National Project Photochem 2010-2012

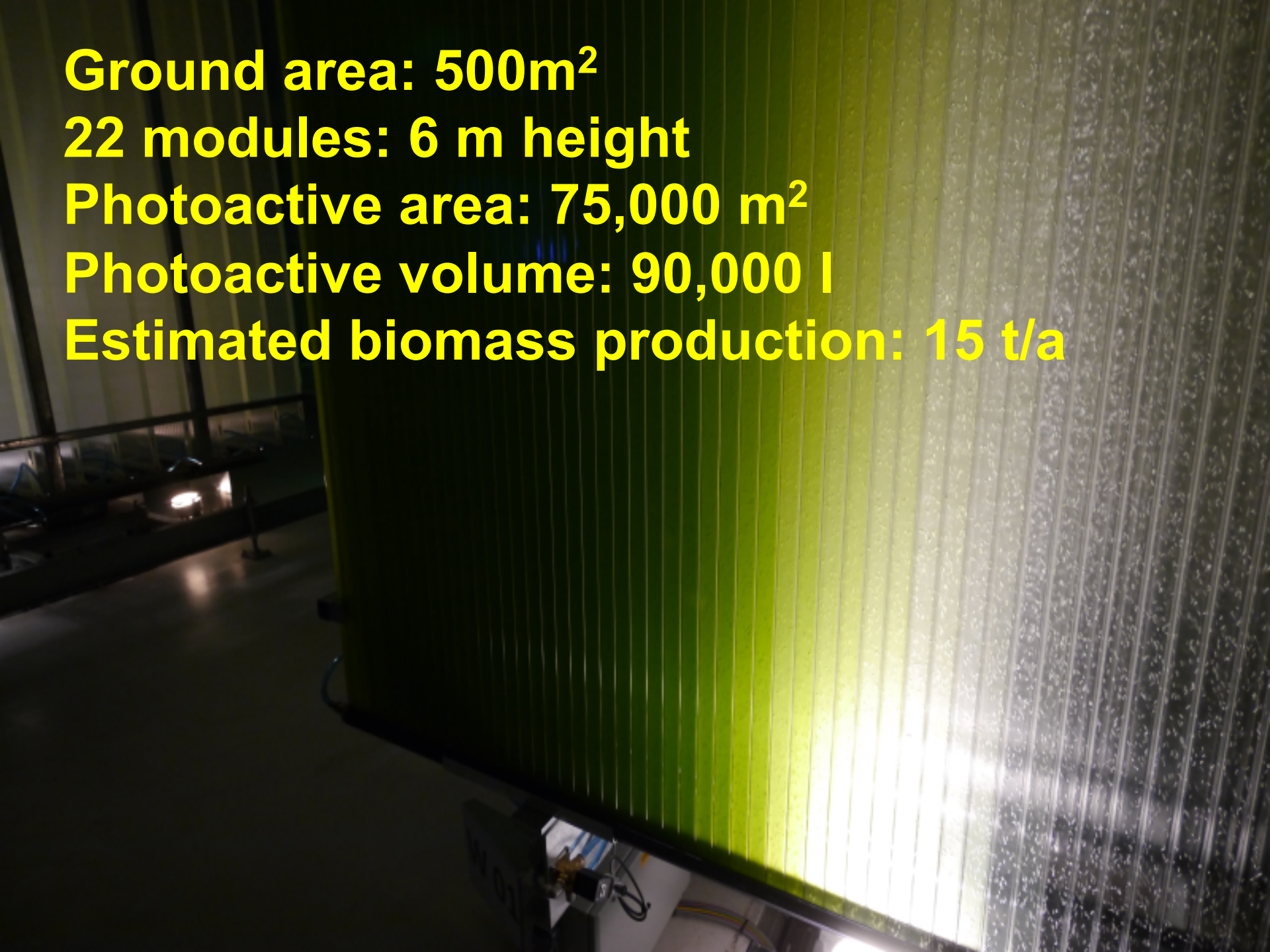
Partners:

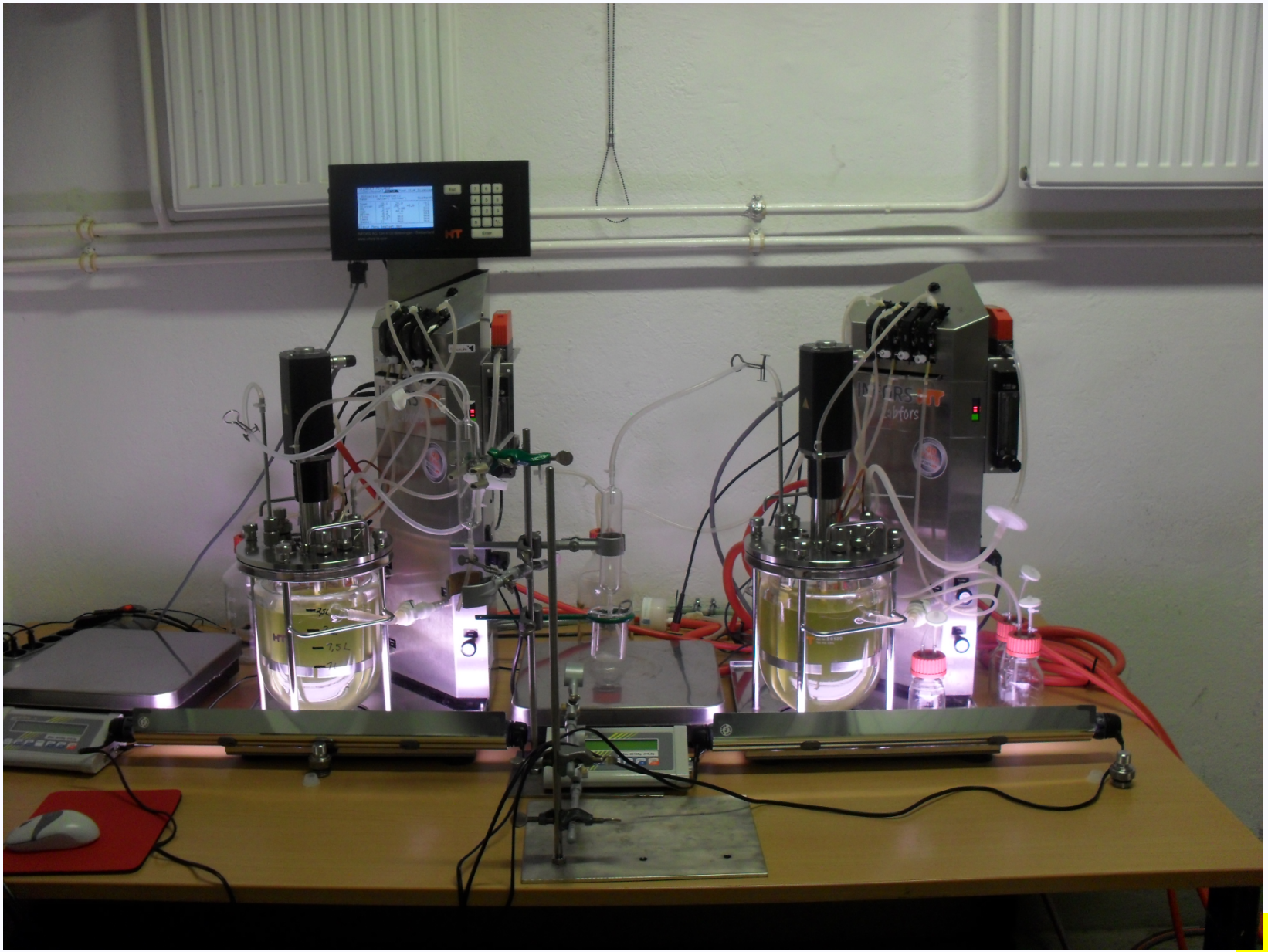
University Graz
University of Technology Graz
Joanneum Research, Graz

Objectives:

- 2-step cultivation of Nannochloropsis occulata:
  mixotrophic 2-step continuous cultivation
- comparison of different extraction techniques
- qualitative and quantitative characterization of extracts

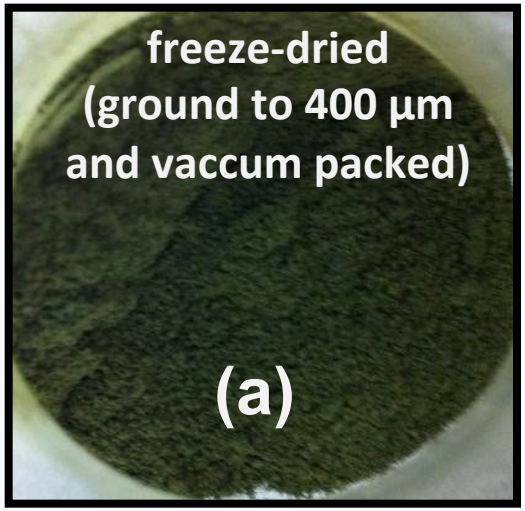
Largest Indoor- Production Unit of Microalgae
Ecoduna, Bruck/L., Austria
Opening: September 2012

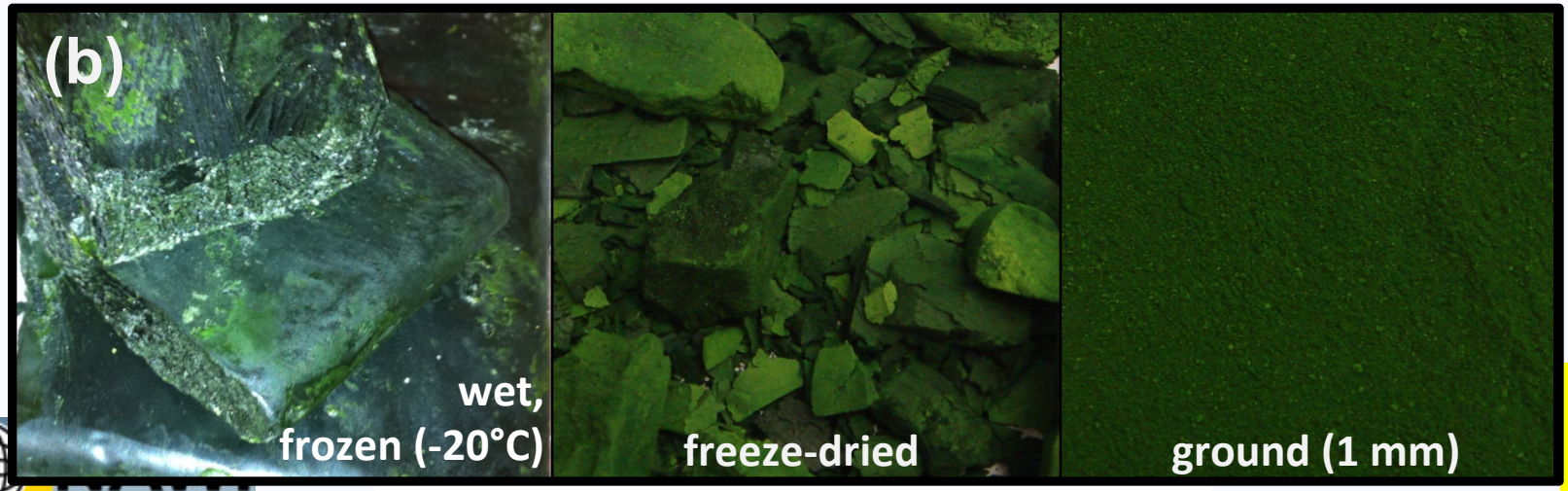

Ground area: 500m²
22 modules: 6 m height
Photoactive area: 75,000 m²
Photoactive volume: 90,000 l
Estimated biomass production: 15 t/a



Sample Material

- ***Nannochloropsis oculata*** from commercial supplier (Necton, Portugal)
  - (a) „Phytobloom Prof“ (PP)
  - (b) „Phytobloom Ice“ (PI)





Extraction Methods

scientific studies with (i) analytical and/or (ii) commercial focus

- „conventional method“ in analytics: $CHCl_3$/MeOH
  => not applicable in food industry

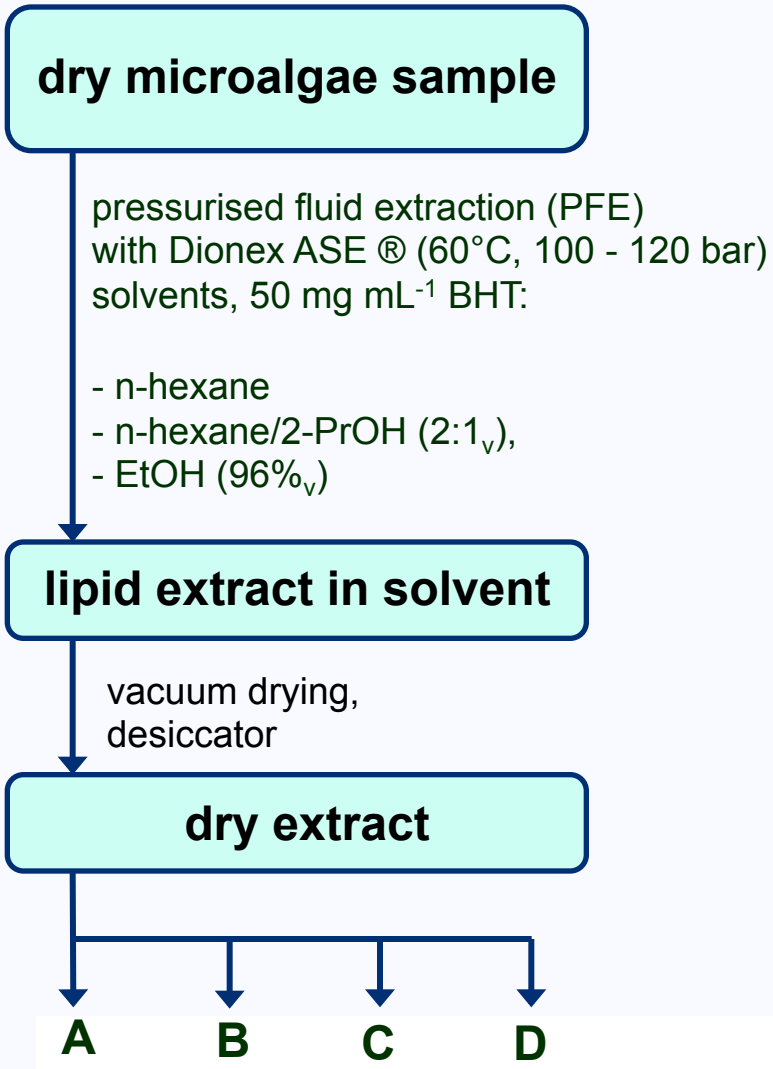
- commercial omega-3 fatty acid production from fish: supercritical CO_2
  => not applicable for microalgae (neutral vs. polar lipids!)

=> pressurised fluid / near critical fluid extraction

=> solvents suitable for food industry

=> solvent polarity

Experimental Methods



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Experimental Methods

A) Extraction yield

B) Qualitative characterisation

- HT-GC MS (DB5-HT column, COC)
- RP-UPLC/ESI-QTOF-MS (C18 column)

C) Total Fatty Acids ($\%_{area}$, $\%_{m,extract}$, $\%_{m,input}$)

- saponification and esterification with NaOH/MeOH and BF_3-MeOH → FAME
- internal quantification (C23:0-ME as ISTD)
- GC-FID (wax column, split)

D) Triacylglycerol / free sterols ($\%_{m,extract}$, $\%_{m,input}$)

- w/o extract purification with „Folch“ ($CHCl_3$/MeOH)
- quantification: C36:0-TAG as ISTD, cholesterol as ESTD)
- HT-GC-FID (DB5-HT column, COC)

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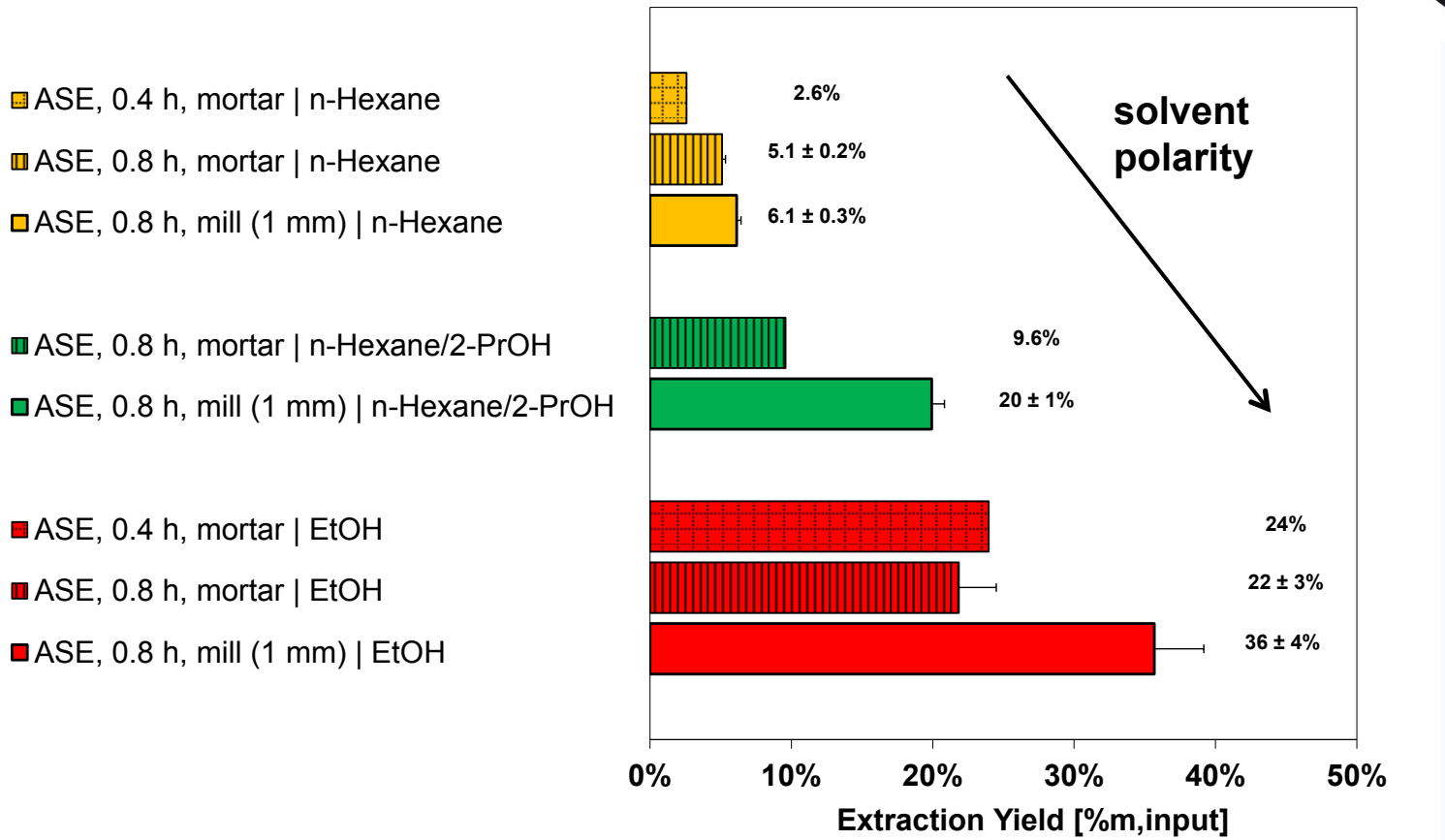
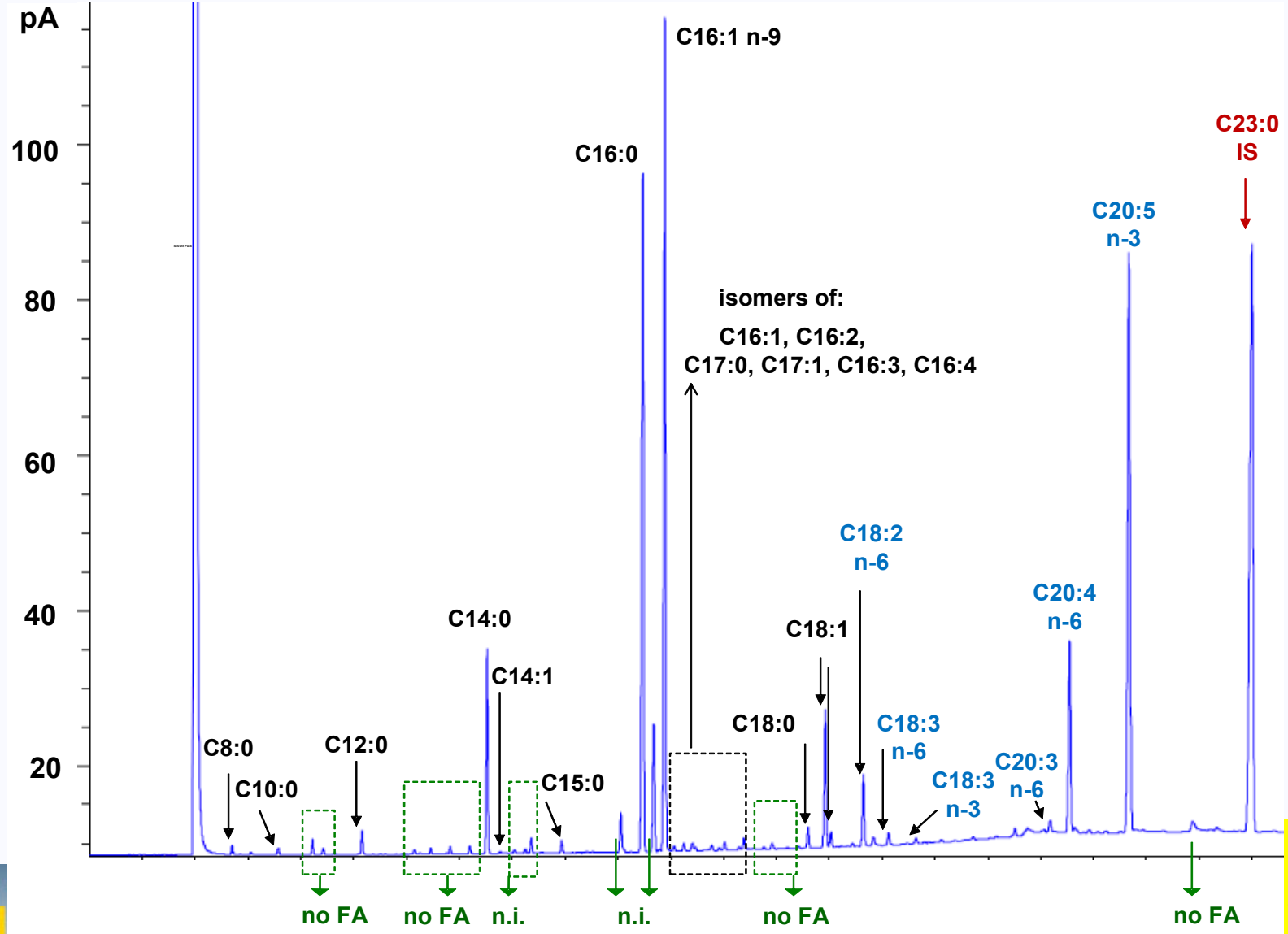
Extraction Yield

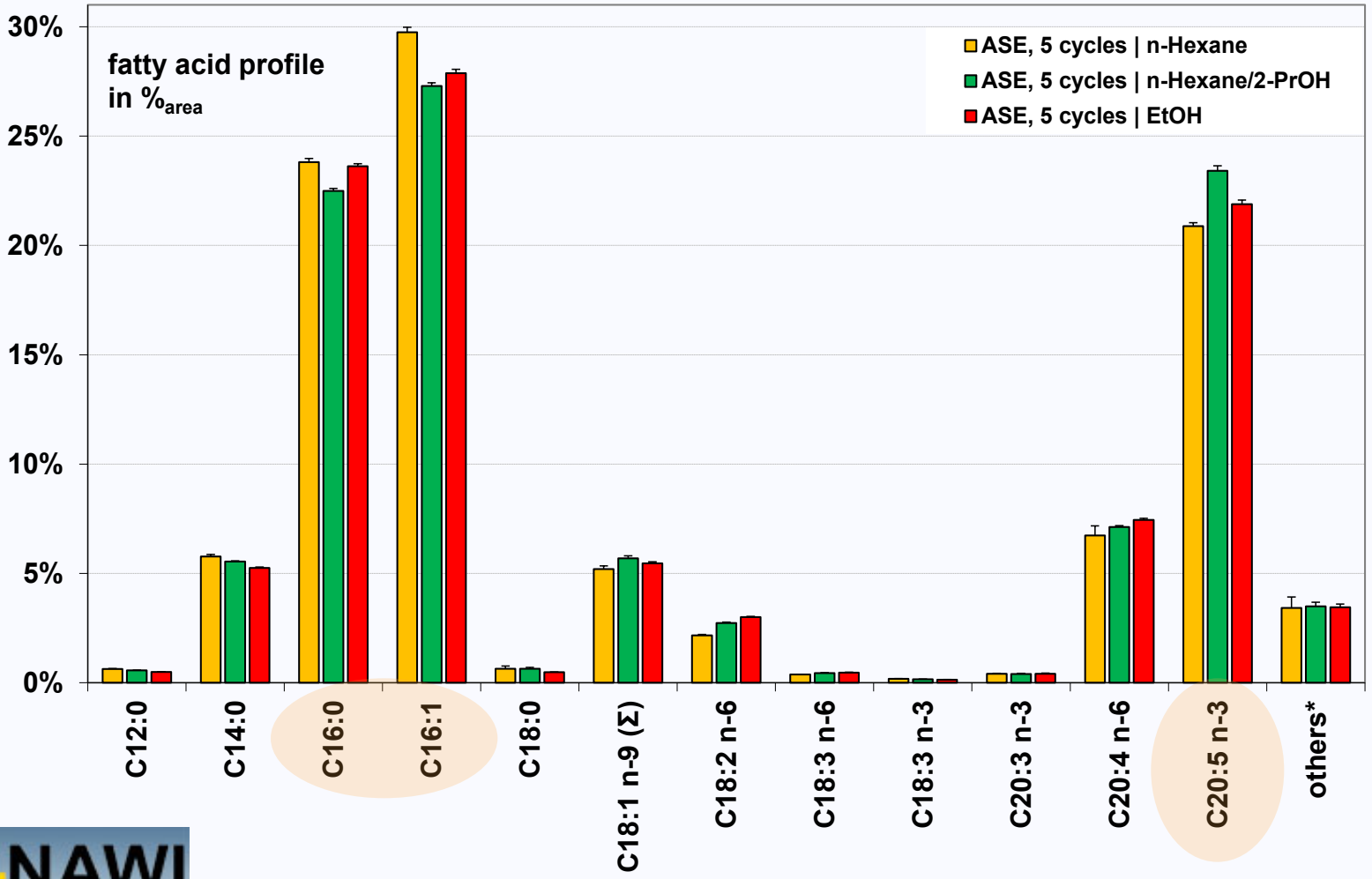
Variation of extraction time, sample homogenisation, solvent polarity



Fatty Acid Composition (1)



Fatty Acid Composition (2)



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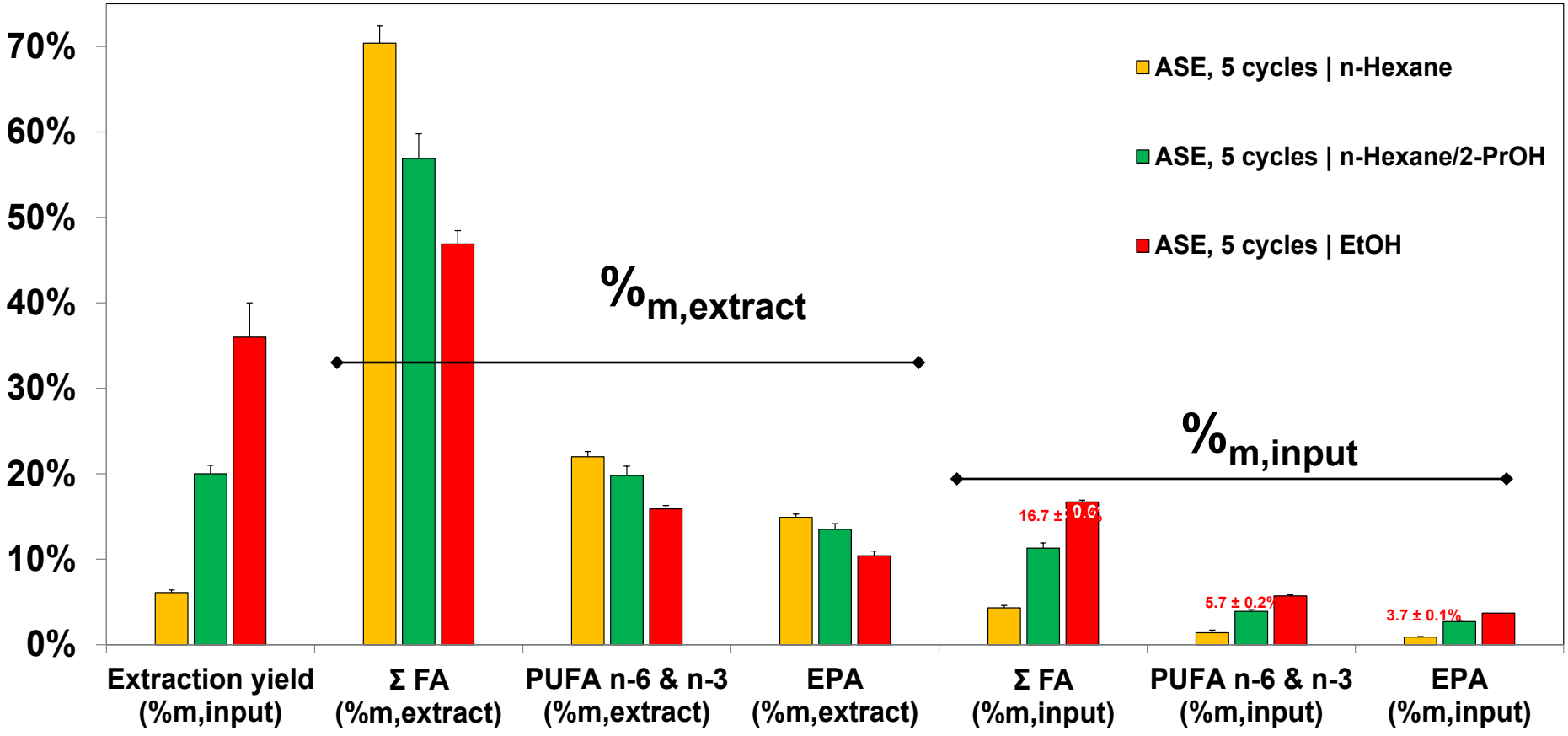
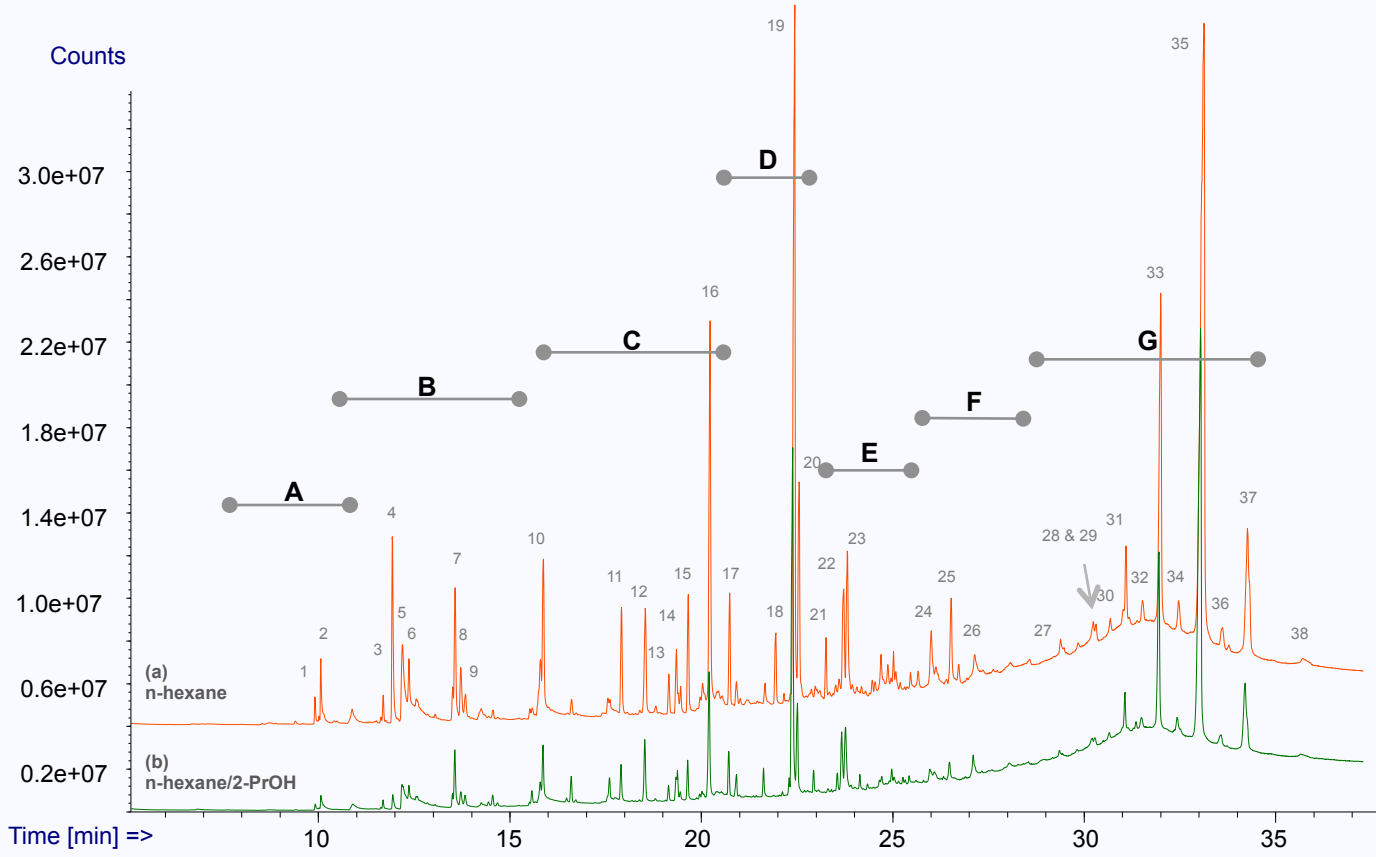
Extraction Yield and Total Fatty Acids



Qualitative Characterisation



A) alkanes, alkenes
(e.g. heptadecane, -decene)

B) FFA, FAME

C) alkanes, alkenes,
fatty alcohols, alkyl diols,
MAG

D) Free sterols (mainly
cholesterol),
Tocopherol

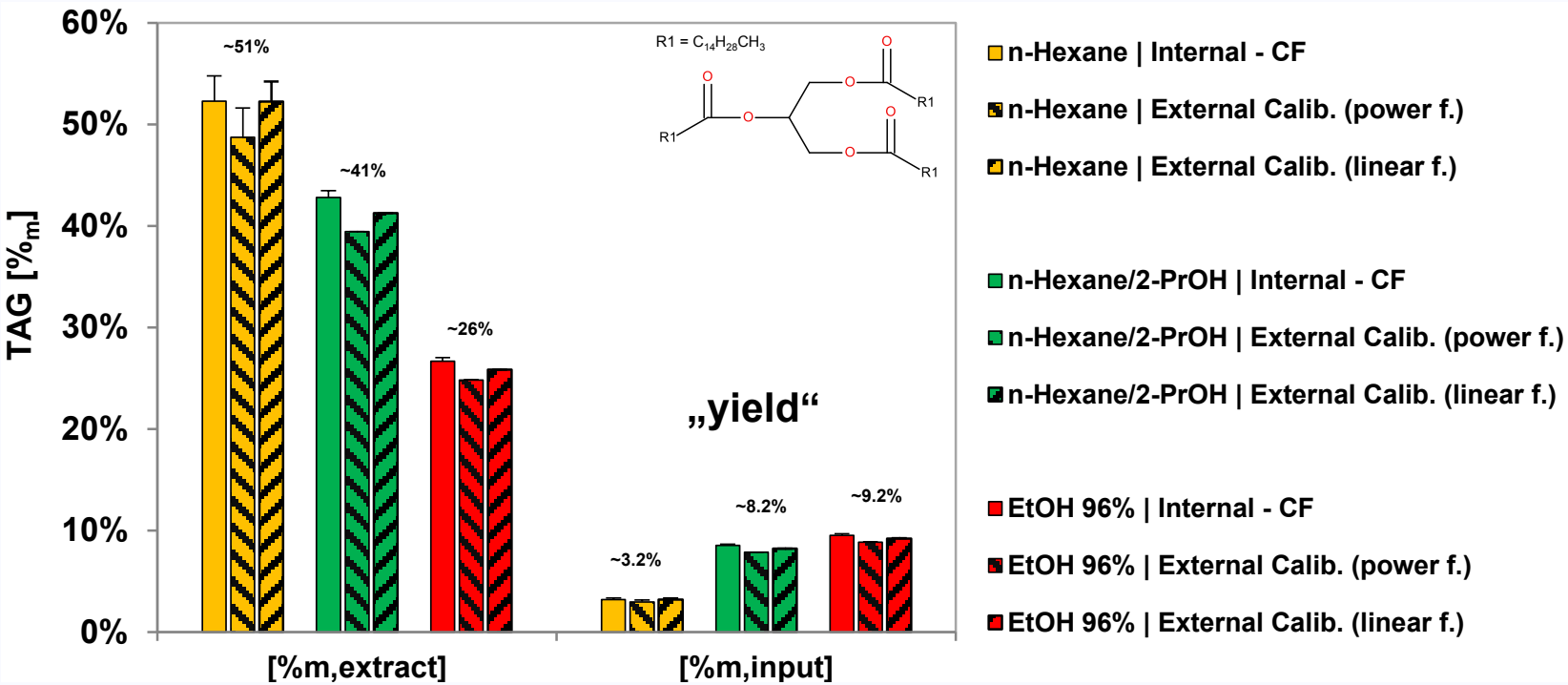
E) DAG, PL

F) TAGs or steryl glycosides

G) TAG (C44 – C52)

Triacylglycerol Content

Mainly C48 TAGs (C48:1 and C48:2 according to LC-MS measurments)



Folch „Recovery“: 96 – 100% for C48 TAGs

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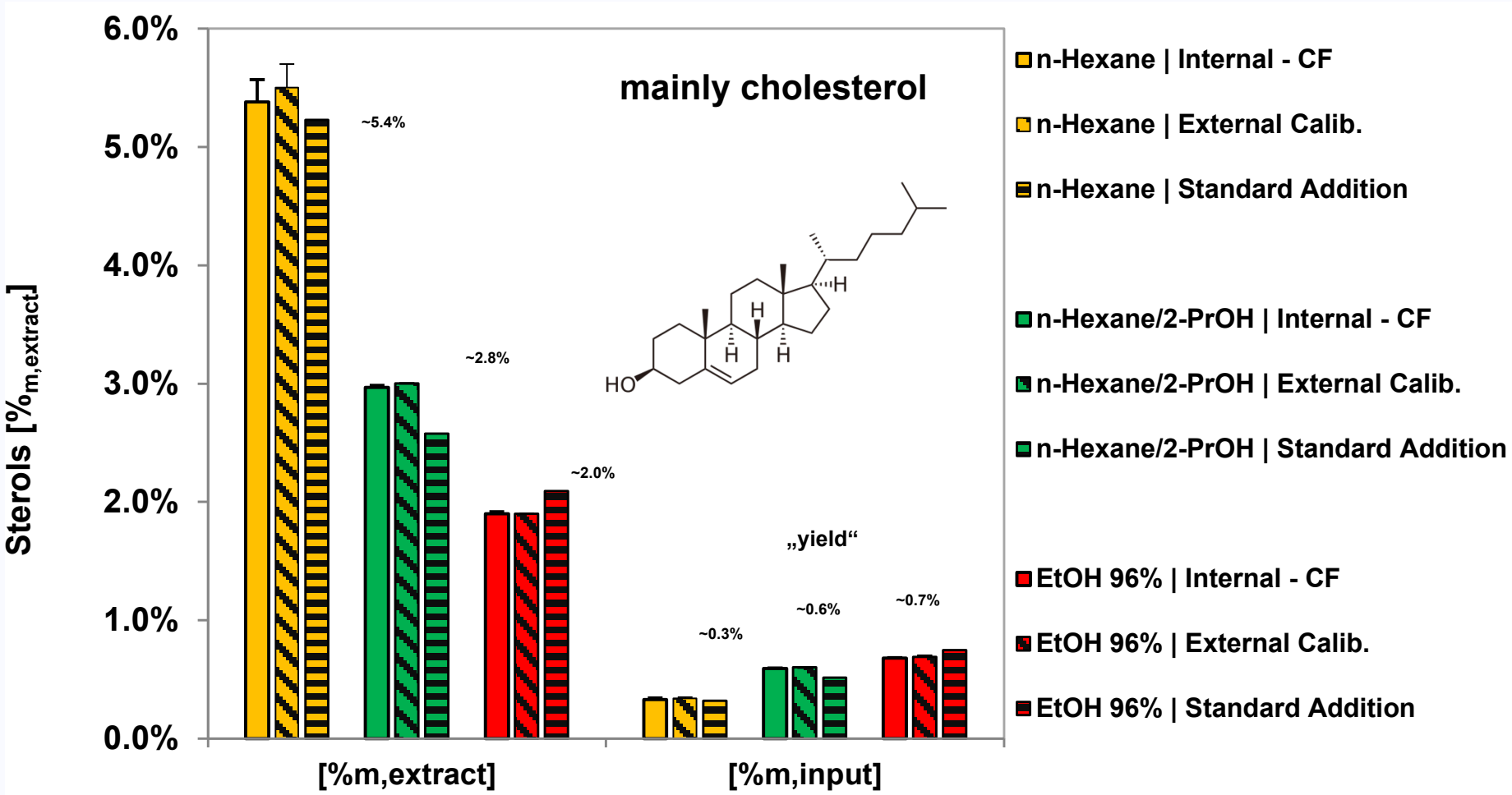
Free Sterol Content



Yields of Identified Compounds
(in relation to extract)

		n-Hexane	n-Hexane/2-PrOH (2:1$_v$)	EtOH 96%$_v$
Extraction Yield	%$_{m,input}$	6.1 ± 0.3	20 ± 1	36 ± 4
Σ FA$^{as\ ME}$	%$_{m,extract}$	70.4 ± 2.0	56.9 ± 2.9	46.9 ± 1.6
Σ PUFA n-6 & n-3$^{as\ ME}$	%$_{m,extract}$	22.0 ± 0.6	19.8 ± 1.1	15.9 ± 0.6
Σ EPA$^{as\ ME}$	%$_{m,extract}$	14.9 ± 0.4	13.5 ± 0.7	10.4 ± 0.4
TAG	%$_{m,extract}$	49 - 52	39 - 43	25 - 27
Free Sterols	%$_{m,extract}$	5.2 - 5.5	2.6 - 3.0	1.9 - 2.1
Σ PL based on P*	%$_{m,extract}$	~4.0 - 4.3	~8.1 - 8.5	~12.8 - 13.6
FFA*	%$_{m,extract}$	~4 - 5	~2	~1
Free Glycerol*	%$_{m,extract}$	-	~0.3	~0.3
USM	%$_{m,extract}$	35 ± 2	not analysed	13 ± 2

Yields of Identified Compounds
(in relation to dry biomass)

		n-hexane	n-hexane/2-propanol 2:1	ethanol
Σ FA$^{as\ ME}$	$\%_{m,input}$	4.3 ± 0.3	11.3 ± 0.6	16.7 ± 0.6
Σ PUFA n-6 & n-3$^{as\ ME}$	$\%_{m,input}$	1.4 ± 0.3	3.9 ± 0.2	5.7 ± 0.2
Σ EPA$^{as\ ME}$	$\%_{m,input}$	0.9 ± 0.1	2.7 ± 0.1	3.7 ± 0.1
TAG	$\%_{m,input}$	3.0 - 3.2	7.8 - 8.5	8.9 - 9.5
Free Sterols	$\%_{m,input}$	0.32 - 0.34	0.51 - 0.60	0.50 - 0.75
Σ PL based on P*	$\%_{m,input}$	~0.3	~1.5 - 1.6	~4.5 - 4.8
FFA*	$\%_{m,input}$	~0.2 - 0.3	~0.4	~0.4
Free Glycerol*	$\%_{m,input}$	-	~0.1	~0.1
USM	$\%_{m,input}$	1.8 ± 0.1	not analysed	2.8 ± 0.7

Summary (1)

- *Nannochloropsis oculata* is a good representative as feedstock for biorefineries, because of content of high added value products (omega-3 FA) as well as other lipids for energy production.

- Pressurized fluid extraction (60°C, 100 bar) of dried micro algae has proven to be superior over traditional extraction methods.

- Traditional solvents for industrial lipid extraction like hexane are less effective than more polar solvents like ethanol due to the polar nature of microalgal lipids.

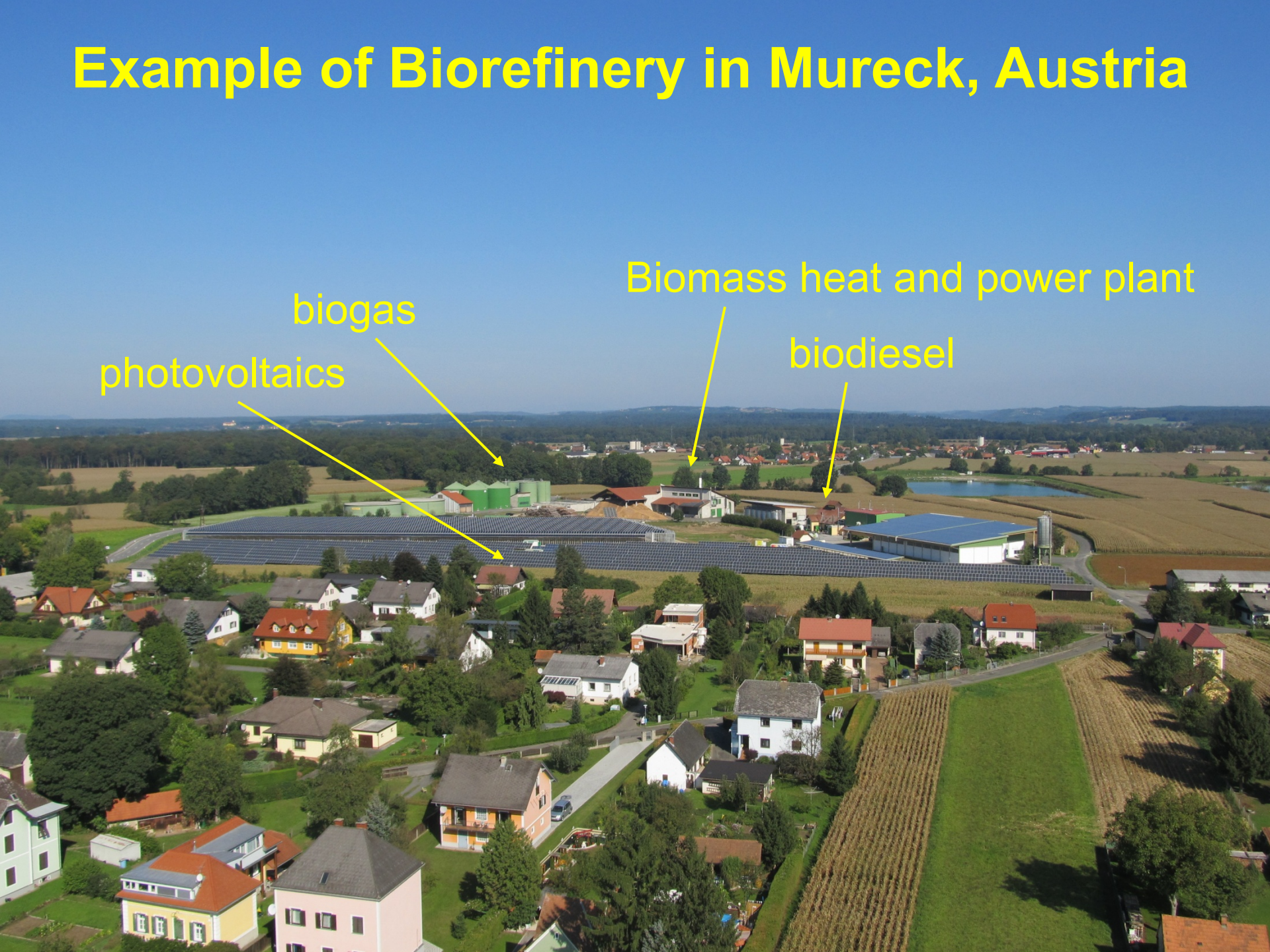
- About 17 % of total fatty acids could be obtained by extraction with ethanol, 9 % are bound in neutral lipids, and the rest is bound in polar phospholipids.

Summary (2)

- The total fatty acid content of 70%m based on the n-hexane extract and 47%m based on the EtOH extract are due to larger amounts of sterols and hydrocarbons (USM).
- Out of 17 % of total fatty acids from *Nannochloropsis oculata* 21 % could be identified as high value added EPA. The remaining FA could be used for energy production (biodiesel).
- New techniques for biodiesel production have to be developed, because only 53 % of FA are bound in neutral lipids.
- Detailed calculations for economical and ecological aspects have to be made in order to evaluate the potential for microalgae as feedstock for biorefineries.

Mittelbach et al.: Biomass & Bioenergy, 2012, online

Example of Biorefinery in Mureck, Austria



Thank You for Your Attention

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