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MICRO-ECOLOGICAL LIFE SUPPORT SYSTEM ALTERNATIVE

CREATING A CIRCULAR FUTURE

Production of high-quality edible biomass with high levels of antioxidants by genetic engineering of the photosynthetic microalga *Chlamydomonas reinhardtii*

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University of Verona
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Solar Energy Bio exploitation Lab

ASTAXANTHIN: ONE OF THE STRONGEST ANTIOXIDANT FOUND IN NATURE!

Revised from Nishida Y, et al., (2007). Carot Sci. 11: 16-20

3,30 -dihydroxy-β, β-carotene4,40 -dione

Astaxanthin

Comparing Astaxanthin In Different Sources

Salmon
5 ppm

Krill
120 ppm

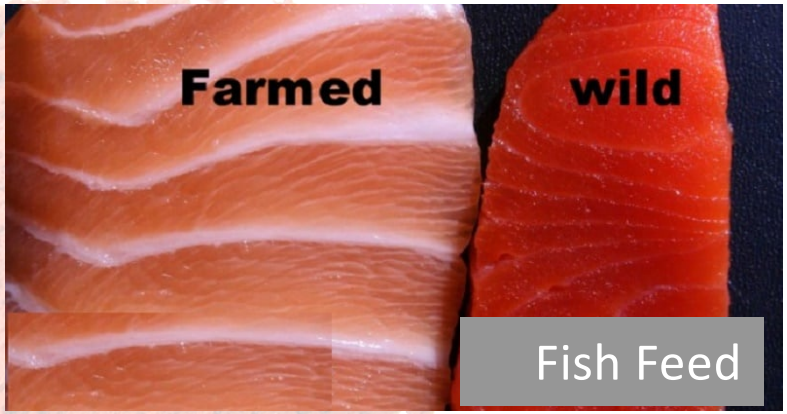
Artic Shrimp
(Pandalus Borealis)
1200 ppm

Green Algae
(Haematococcus pluvialis)
40000 ppm

*Astaxanthin Concentration (ppm)

3,30 -dihydroxy-β, β-carotene4,40 -dione

Astaxanthin



Fish Feed

Poultry Feed

Food colouring agent

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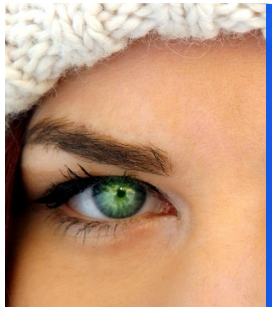
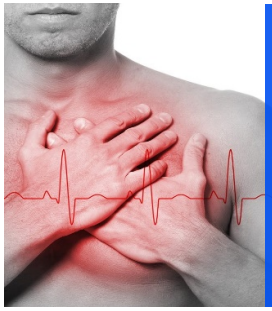
ASTAXANTHIN

Astaxanthin is employed as healthy food and many clinical studies revealed its possible role in human disease treatment

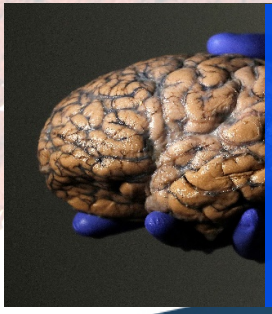


Eye Health



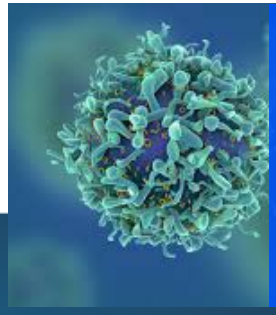
Cardiovascular support

Immune system modulation



Brain Health

Male Fertility



Anti-Aging & Cellular health

Joint, Tendon and muscle support

3,30 -dihydroxy-β, β-carotene4,40 -dione

Astaxanthin

Moreover:

- never becomes a prooxidant
- has anti-inflammatory properties
- suppresses a number of different inflammatory pathways

- Recommended dosage of astaxantin is in the rage of 4-12mg/day

FDA APPROVED

- Acceptable daily intake up to 0.2 mg/kg per day in adults

efsa
European Food Safety Authority

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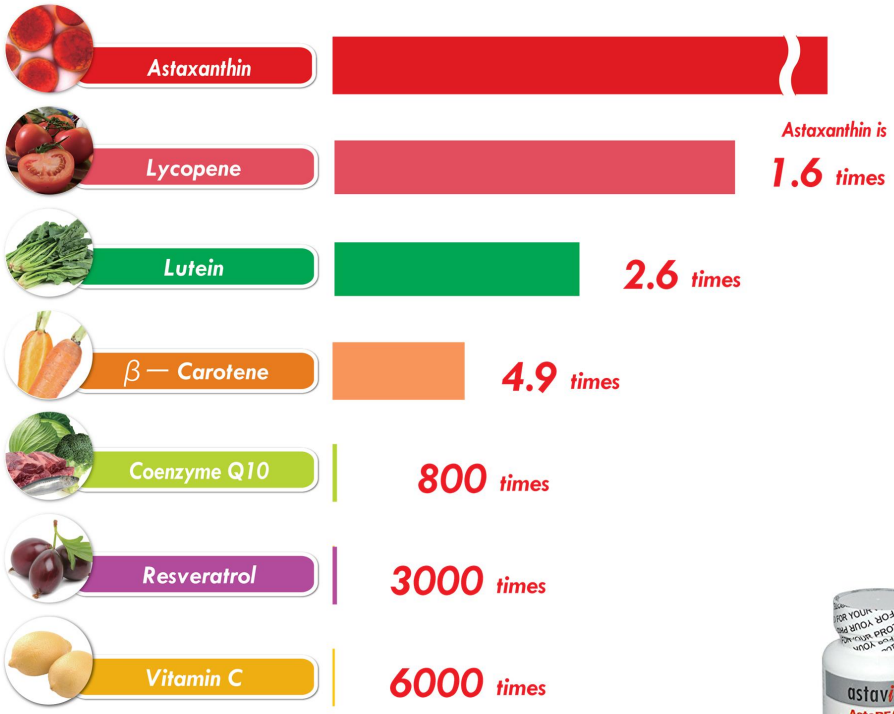
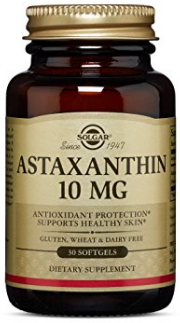


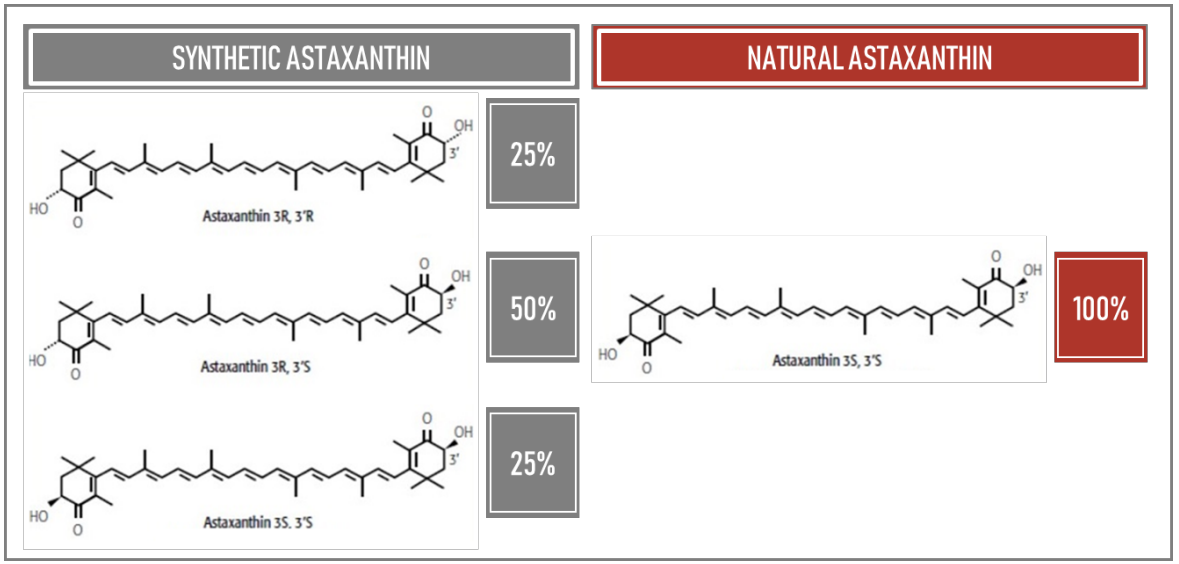
Revised from Nishida Y, et c

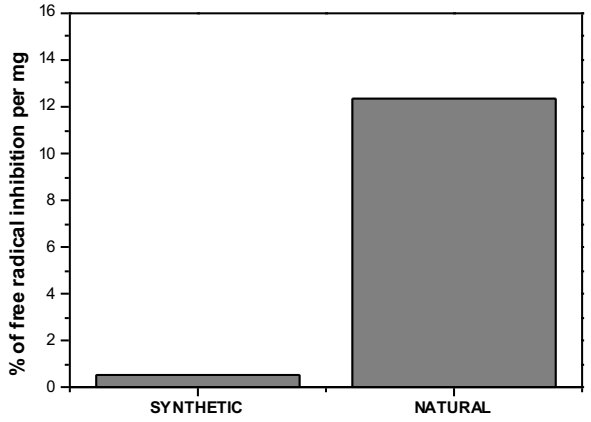
Cosmetics



GLOBAL MARKET IS DOMINATED BY SYNTHETIC ASTAXANTHIN

SYNTHETIC ASTAXANTHIN SHOWS 20 TIMES LESS ANTIOXIDANT ACTIVITY WITH RESPECT TO NATURAL ASTAXANTHIN





Adapted on Capelli et al. 2012

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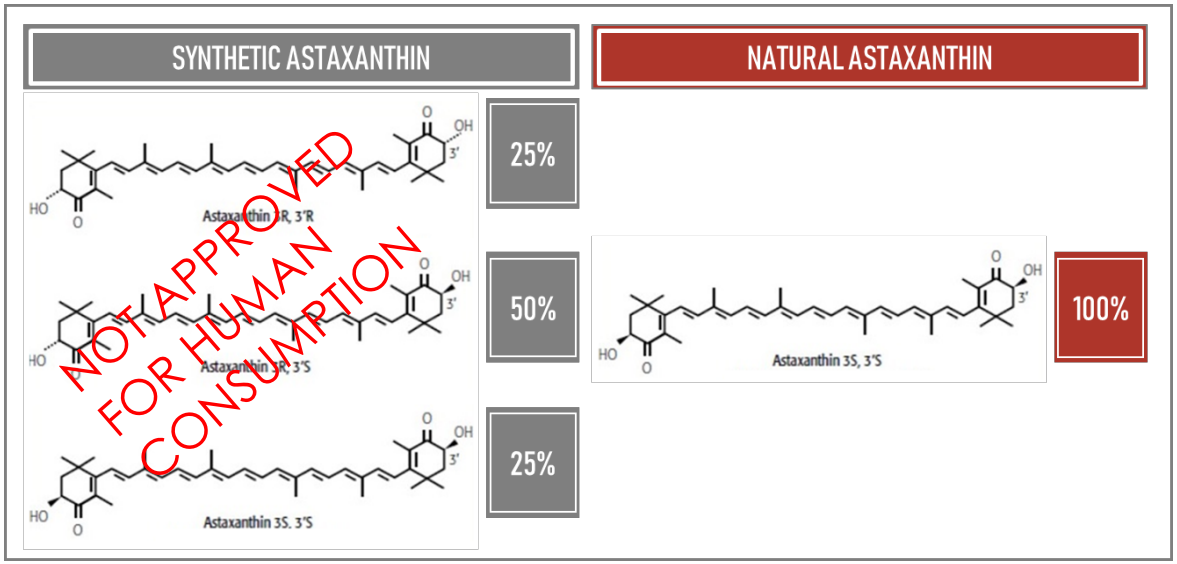

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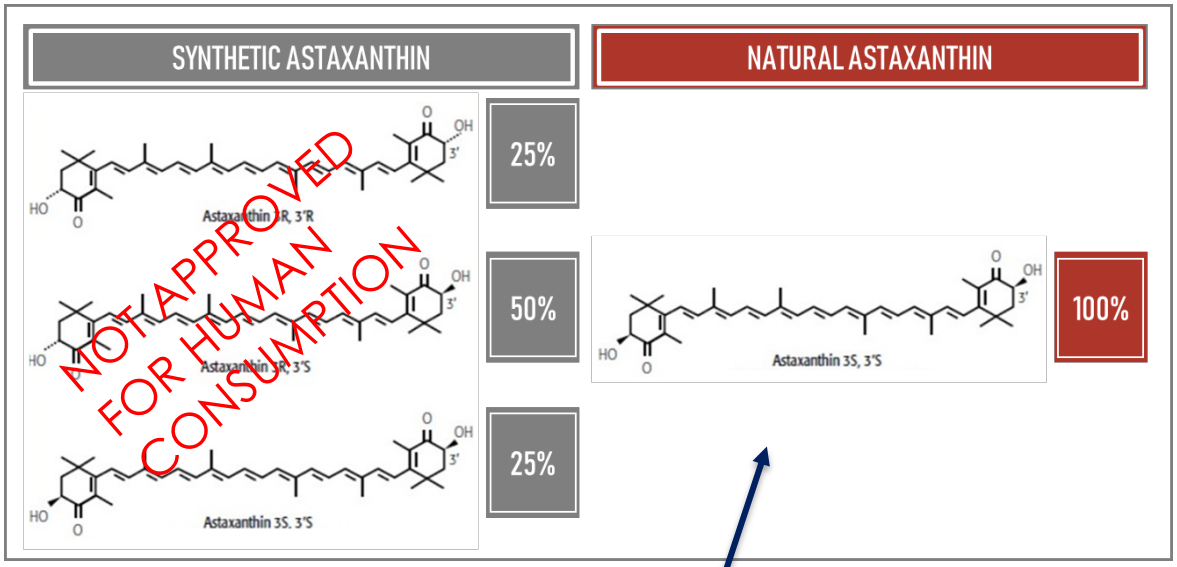

NOT FOUND IN HIGHER PLANTS!
NOT PRESENT IN CYANOBACTERIA!

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MAIN SOURCES OF NATURAL ASTAXANTHIN ARE EUKARYOTIC GREEN ALGAE

HAEMATOCUCCUS PLUVIALIS (or *LACUSTRIS*) HAS THE CAPACITY TO ACCUMULATE 4-5% DRY WEIGHT UPON STRESS CONDITIONS

BUT

SEVERAL CONSTRAINTS IN CULTIVATION AND EXTRACTION

CAN WE FIND OTHER SOURCES FOR ASTAXANTHIN?

THE CASE OF CHLAMYDOMONAS REINHARDTII:

- Model organisms for green algae
- Biotechnological tool available
- Strains with improved productivity already available

CAN WE FIND OTHER SOURCES FOR ASTAXANTHIN?

THE CASE OF CHLAMYDOMONAS REINHARDTII:

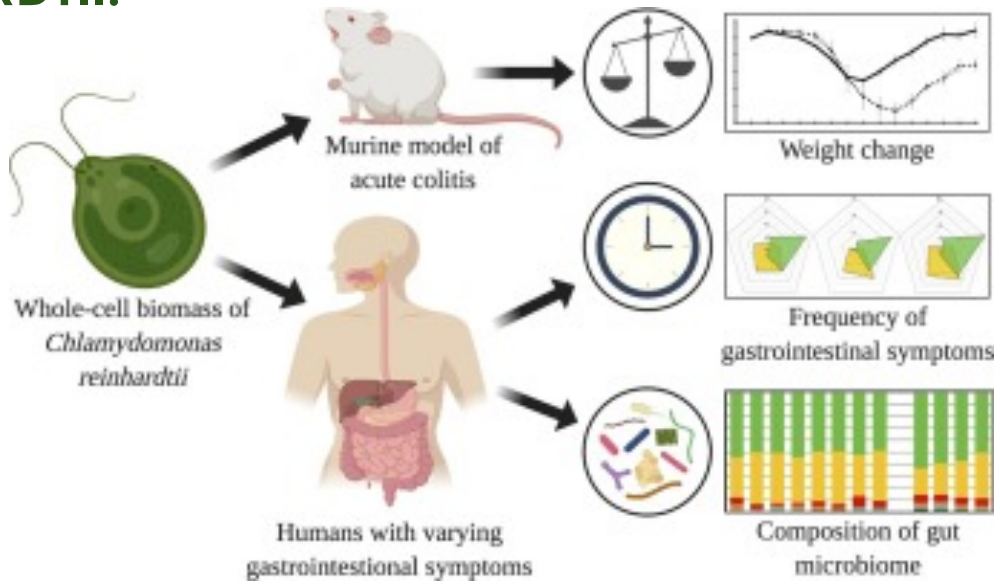
- **Protein content:** 40%
- **FAO/WHO values** (0.9–1.9)
- **Fatty acid predominantly unsaturated** (42% ALA)
- **Iron content** ~1mg/g of dry weight
- **Selenium content** ~10 µg/g DW

Darwish et al., Appl. Sci. **2020**, *10*(19), 6736; https://doi.org/10.3390/app10196736

CAN WE FIND OTHER SOURCES FOR ASTAXANTHIN?

THE CASE OF CHLAMYDOMONAS REINHARDTII:

- ***C. reinhardtii significantly mitigated weight loss in a murine model of acute colitis.***
- ***C. reinhardtii positively impacted gastrointestinal symptoms in humans.***
- ***C. reinhardtii had no adverse effect on the microbial composition of participants***



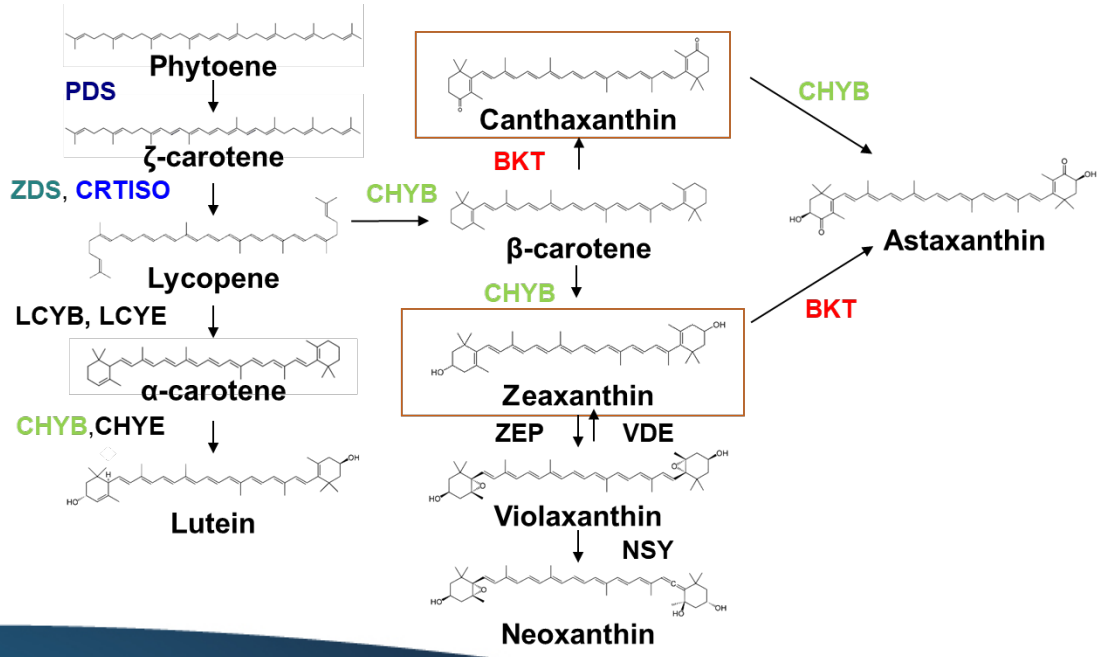
Fields et al. Journal of Functional Foods, 2020
https://doi.org/10.1016/j.jff.2019.103738

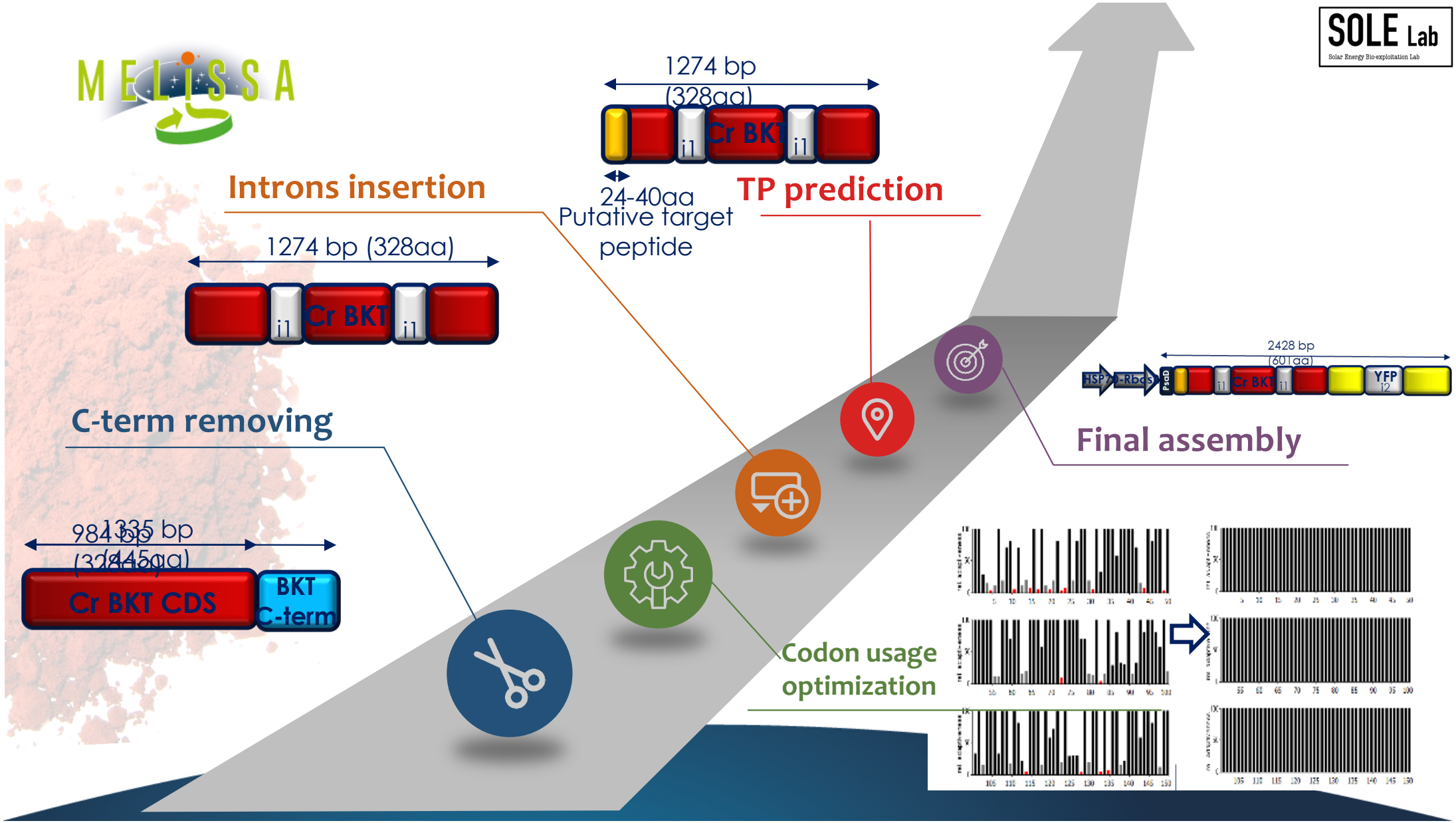
CAN WE FIND OTHER SOURCES FOR ASTAXANTHIN?

two different pathway for astaxanthin biosynthesis *in both cases BKT is the key enzyme required*

THE CASE OF CHLAMYDOMONAS REINHARDTII:

- BKT sequence is present in *C. reinhardtii*
- BKT sequence is poorly expressed
- **No astaxanthin** was ever found in *C. reinhardtii*

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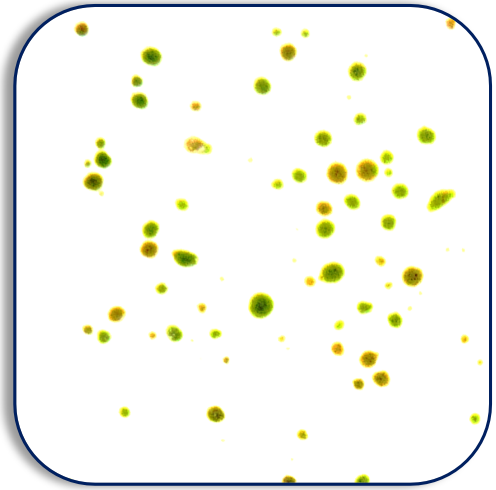
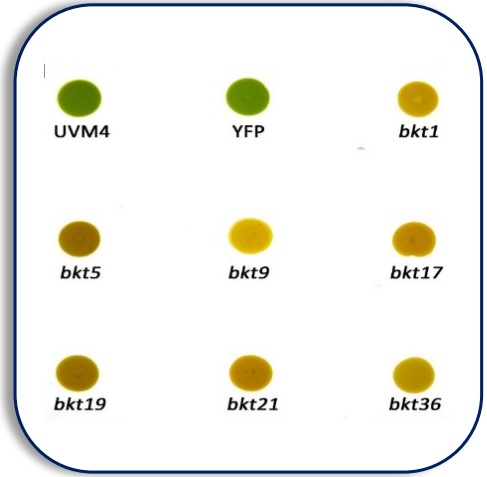
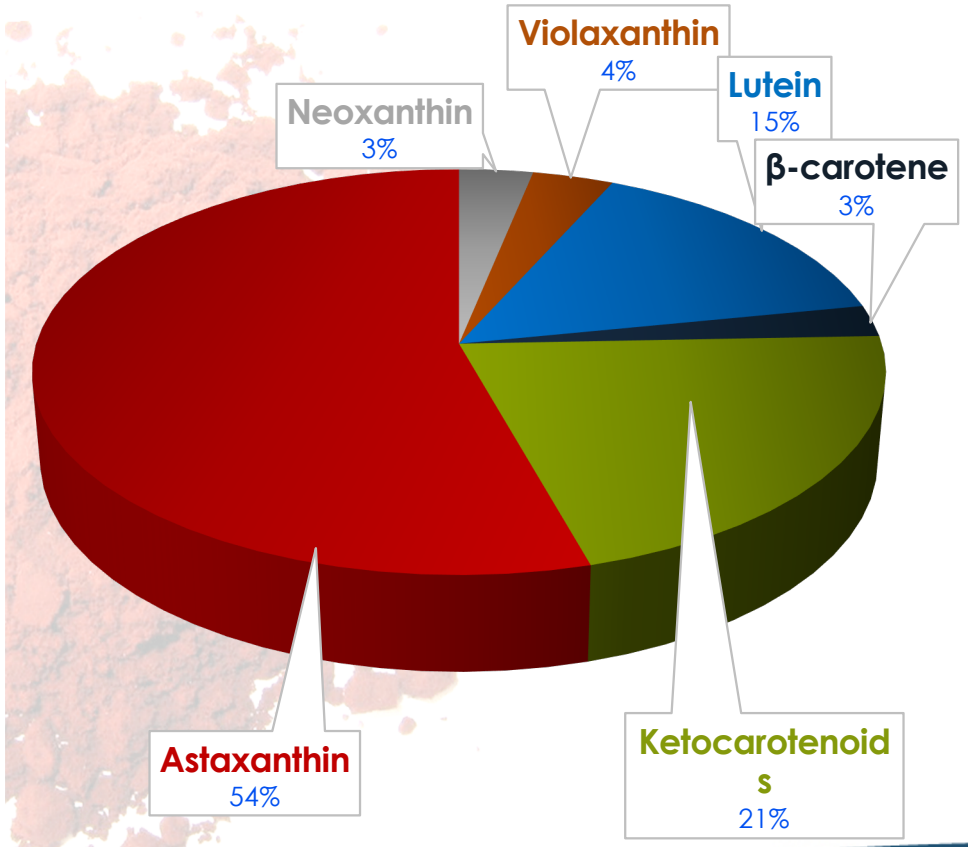
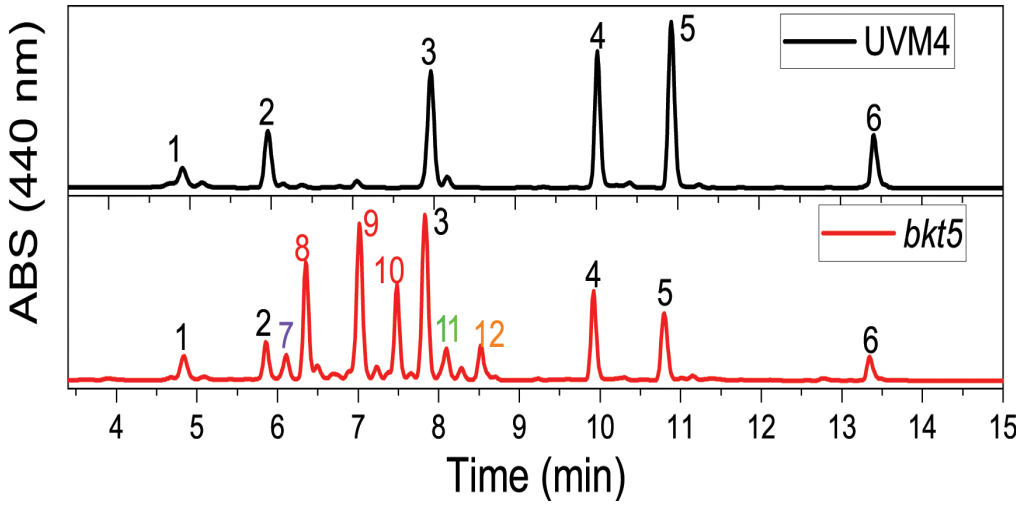
1274 bp
(328aa)
i1
Cr BKT
i1
24-40aa
Putative target peptide
Introns insertion
TP prediction
1274 bp (328aa)
i1
Cr BKT
i1
2428 bp
(601aa)
HSP70-Rbcs
PsaD
i1
Cr BKT
i1
YFP
i2
C-term removing
Final assembly
984 bp
(328aa)
1335 bp
(445aa)
Cr BKT CDS
BKT
C-term
Codon usage optimization





RED/ORANGE PHENOTYPE IS VISIBLE BY EYES





Astaxanthin

Canthaxanthin

Adonirubin

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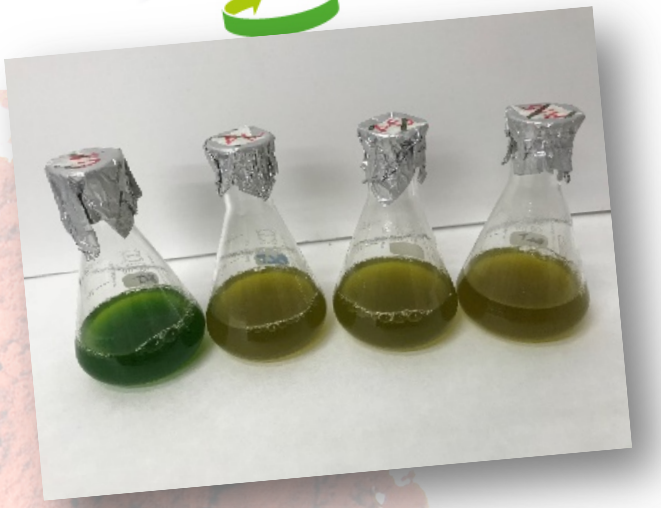
GROWTH ANALYSIS IN DIFFERENT CULTIVATION SYSTEM



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EVALUATION OF PRODUCTIVITY IN CLOSED PHOTOBIOREACTORS

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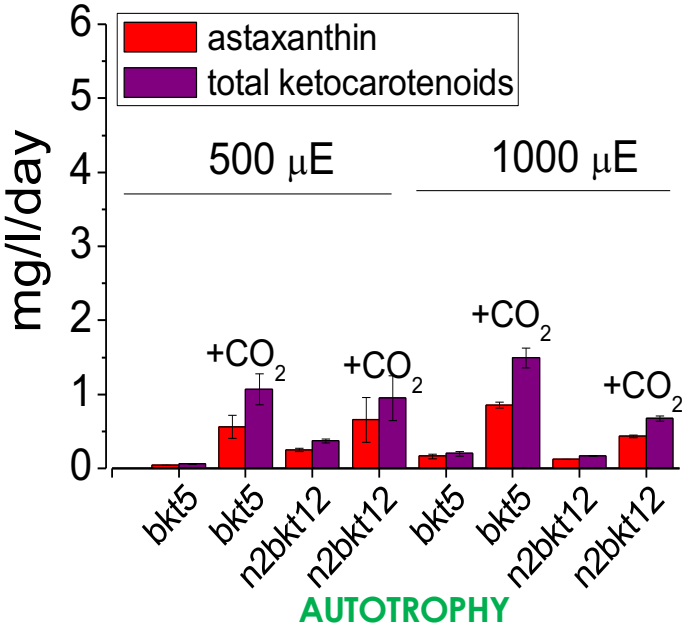
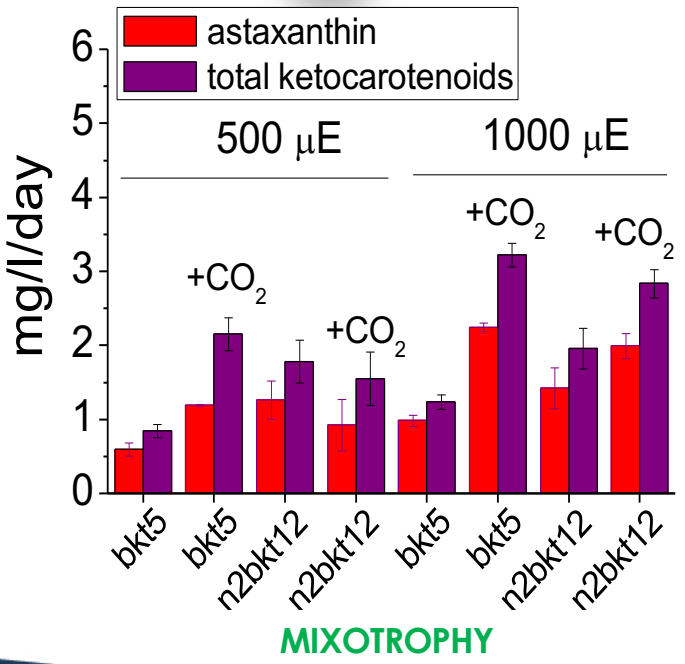
- **Light:** 500 or 1000 uE
- **Media:** HS or TAP
- **CO_2:** air or 3% CO_2

CO_2 INCREASES PRODUCTIVITY

ORGANIC CARBON POSITIVELY EFFECTS PRODUCTIVITY





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Light: 1000 uE– 3000 uE

Media: TAP

Stirring

SEMICONTINOUS CULTIVATION

PRODUCTION OF UP TO 3,1 mg/l/day ASTAXANTHIN

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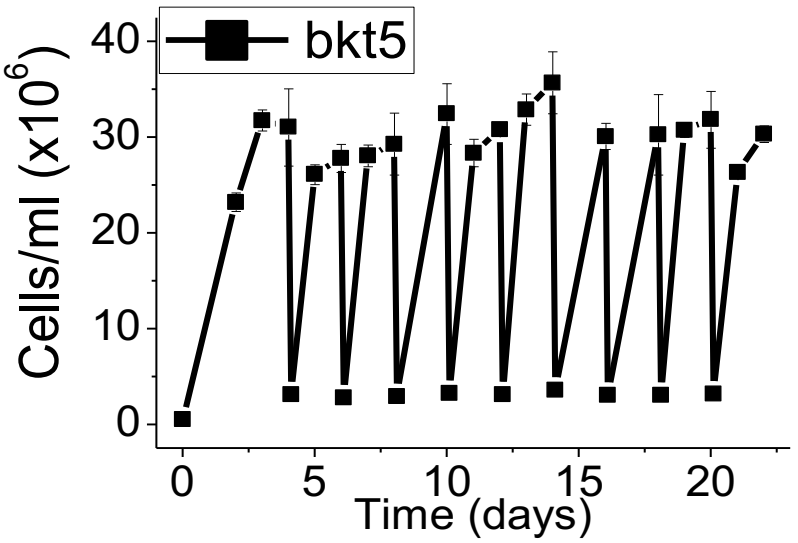
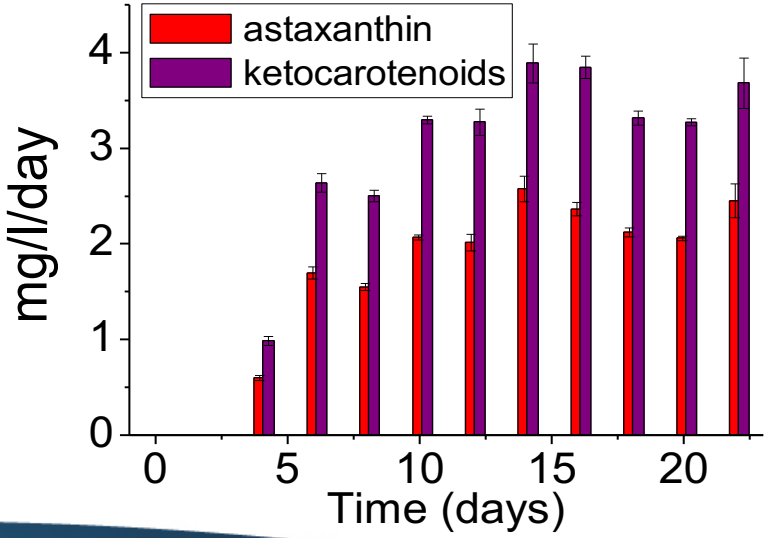
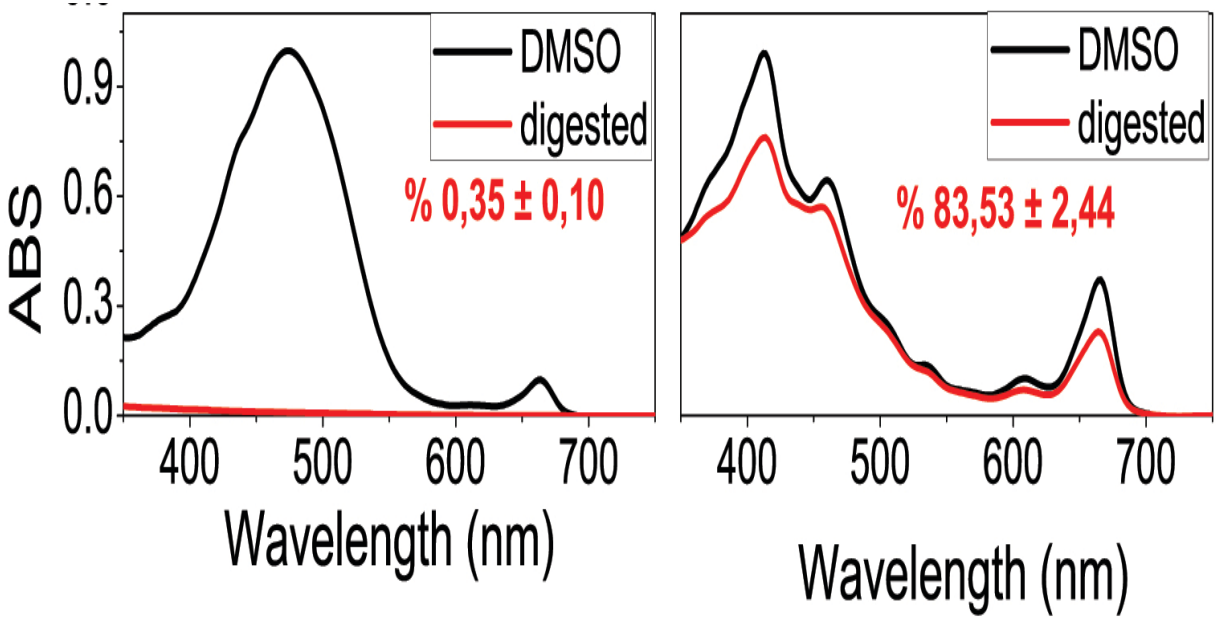




Extractability and bioavailability of astaxanthin



>85% of astaxanthin in *C. reinhardtii* can be extracted by *in vitro* digestion simulation vs. 0.35% in the case of *H. pluvialis*

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Chlamydomonas reinhardtii can be considered as potential superfood in closed systems

Astaxanthin production in *C. reinhardtii* is possible using endogenous bkt

High production yields can be obtained with extremely simple systems

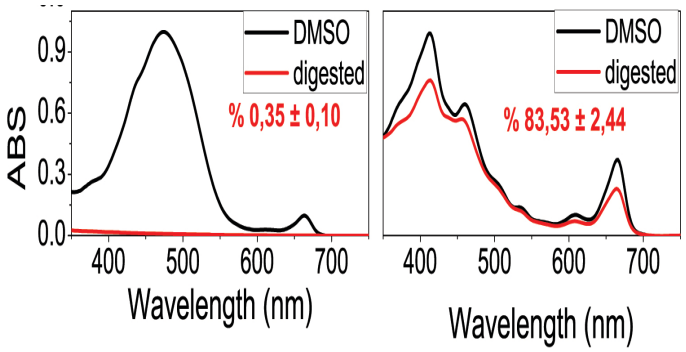
up to 1.75g/L/day

Biomass yield

3,1 mg/L/day

Astaxanthin yield

INCREASED BIOAVAILABILIITY



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Algae Biotechnology and Bioenergy Group
CeBiTec
Center for Biotechnology

Prof. Kruse Olaf
Wobbe Lutz
Baier Thomas
Lauersen Kyle J.

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THANK YOU.

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