Variability in nutritional value and safety of *Arthrosprira* and *Chlorella* biomass necessitates smart production of microalgae for human spaceflight

*Siegfried E. Vlaeminck, Sui Yixing, Pieter Vermeir & Maarten Muys*
Arthrosira (Spirulina) and Chlorella: key protein source in regenerative life support systems (RLSS)

E.g. MELiSSA concept (ESA)
Arthrosira upgrades nutrients to high-value dietary protein and produces oxygen

GOAL: Biomass production with optimal nutritional quality and safety!

BUT: Magnitude of variation in nutritional value and safety is largely unknown, as is the preference towards Arthrosira or Chlorella
Research goal:
- Quantification of spatial and temporal variation in nutritional quality and safety of microalgal biomass
- Indication on which common microalgae is favorable in terms of price-quality

Screening of:

<table>
<thead>
<tr>
<th>Spatial variability = from all over the world</th>
<th>Temporal variability = same brand/different batch</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 commercially available <em>Chlorella</em></td>
<td>5 commercially available <em>Chlorella</em>:</td>
</tr>
<tr>
<td>7 commercially available <em>Spirulina</em></td>
<td>5 commercially available <em>Spirulina</em>:</td>
</tr>
</tbody>
</table>
Origin of the microalgal samples

- **France**/Domaine Traverse
- **USA (California)**/Earthrise
- **Hawaii**/Cyanotech Nutrex
- **India**/Parry Nutraceuticals
- **China (Hainan)**/Pit-Pit
- **Japan (Ishigaki)**/Jarrow Formulas
- **Taiwan**/PingTung
- **South Korea**/Fresh Chlorella
- **Germany (Klötzle)**/Algomed
- **Mongolia**/Purasana
<table>
<thead>
<tr>
<th>Code</th>
<th>Brand</th>
<th>Country of origin</th>
<th>Retailer</th>
<th>Exp. date</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Purasana</td>
<td>Mongolia</td>
<td>Origin'O</td>
<td>31/05/18</td>
</tr>
<tr>
<td>C1a</td>
<td>Purasana</td>
<td>Mongolia</td>
<td>Origin'O</td>
<td>31/05/18</td>
</tr>
<tr>
<td>C1b</td>
<td>Purasana</td>
<td>Mongolia</td>
<td>Origin'O</td>
<td>31/05/18</td>
</tr>
<tr>
<td>C1c</td>
<td>Purasana</td>
<td>Mongolia</td>
<td>Bioplanet</td>
<td>30/03/19</td>
</tr>
<tr>
<td>C1d</td>
<td>Purasana</td>
<td>Mongolia</td>
<td>Bioplanet</td>
<td>30/04/19</td>
</tr>
<tr>
<td>C2</td>
<td>Febico</td>
<td>Taiwan Ping-Tung</td>
<td>Febico</td>
<td>04/03/19</td>
</tr>
<tr>
<td>C3</td>
<td>NA</td>
<td>China Hainan</td>
<td>pit-pit</td>
<td>16/09/18</td>
</tr>
<tr>
<td>C4</td>
<td>Jarrow formulas</td>
<td>Japan Ishigaki Island</td>
<td>Jarrow</td>
<td>07/17</td>
</tr>
<tr>
<td>C5</td>
<td>Clean chlorella</td>
<td>South Korea</td>
<td>Health Ranger Select</td>
<td>NA</td>
</tr>
<tr>
<td>C6</td>
<td>Algomed</td>
<td>Germany Klötze/Altmark</td>
<td>Algomed</td>
<td>14/12/18</td>
</tr>
<tr>
<td>C7</td>
<td>Iswari</td>
<td>China Hainan</td>
<td>Iswari</td>
<td>03/17</td>
</tr>
<tr>
<td>S1</td>
<td>Purasana</td>
<td>Mongolia</td>
<td>Origin'O</td>
<td>31/05/18</td>
</tr>
<tr>
<td>S1a</td>
<td>Purasana</td>
<td>Mongolia</td>
<td>Origin'O</td>
<td>30/04/19</td>
</tr>
<tr>
<td>S1b</td>
<td>Purasana</td>
<td>Mongolia</td>
<td>Bioplanet</td>
<td>30/04/19</td>
</tr>
<tr>
<td>S1c</td>
<td>Purasana</td>
<td>Mongolia</td>
<td>Bioplanet</td>
<td>30/04/19</td>
</tr>
<tr>
<td>S1d</td>
<td>Purasana</td>
<td>Mongolia</td>
<td>Origin'O</td>
<td>30/03/19</td>
</tr>
<tr>
<td>S2</td>
<td>Febico</td>
<td>Taiwan Ping-Tung</td>
<td>Febico</td>
<td>14/01/19</td>
</tr>
<tr>
<td>S3</td>
<td>NA</td>
<td>China Hainan</td>
<td>pit-pit</td>
<td>11/09/18</td>
</tr>
<tr>
<td>S4</td>
<td>Now foods</td>
<td>India Parry Nutraceutical</td>
<td>Now</td>
<td>11/17</td>
</tr>
<tr>
<td>S5</td>
<td>Nutrex Hawaii</td>
<td>Hawaii cyanotech nutrex</td>
<td>Nutrex</td>
<td>01/19</td>
</tr>
<tr>
<td>S6</td>
<td>Earthrise</td>
<td>USA California</td>
<td>Earthrise</td>
<td>01/19</td>
</tr>
<tr>
<td>S7</td>
<td>Domaine traverse*</td>
<td>France Toulon</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

- Huge difference in price for ‘the same’ product
- Chlorella biomass on average 18% more expensive than Spirulina biomass
- Per kg protein, Chlorella is 15% more expensive
Spatial and temporal variation in digestibility, VS/TS ratio and water content

**Spatial variability**

- Chlorella: 50% lower water content, slightly higher VS/TS ratio and a 20% lower mean digestibility
- Spatial variability in digestibility is larger for Chlorella (broken vs non-broken cell walls?)

**Temporal variability**

- Temporal variability in ash fraction up to 80% for Chlorella => not only spatial effect
- Up to 28% temporal difference in digestibility => significant effect of growing condition on digestibility
Spatial and temporal variation in protein content (Quantity)

Spatial variability

- Chlorella: 58% spatial difference in protein
- Spirulina: 23% spatial difference in protein
- Package protein content is based on KjN
- Up to 37% spatial difference in package match
- Markwell/KjN Chlorella > Spirulina => more non-protein N in Spirulina biomass

Temporal variability

- Both microalgae 20-25% temporal difference in protein content => room for improvement even within one company!
Spatial variability in normalized EAA profile

- Overall, Spirulina contains a higher amount of EAA and has a larger spatial variability in EAA
- Chlorella is mainly short in Lysine
- Spirulina is mainly short in Histidine

Temporal variability in normalized EAA profile

- E.g. Up to 100% difference in Lysine for both algae
- The temporal variability in EAA profile shows room for improvement in one company:
  - Overall, Spirulina contains a higher amount of EAA and has a larger spatial variability in EAA
  - Chlorella is mainly short in Lysine
  - Spirulina is mainly short in Histidine
Spatial and temporal variation in EAA content (Quality)

Essential amino acid index: \( \text{EAAI} = \sqrt[n]{\frac{\text{EAA}_1}{\text{EAA}_1^{\text{ref}}} \times \frac{\text{EAA}_2}{\text{EAA}_2^{\text{ref}}} \times \cdots \times \frac{\text{EAA}_n}{\text{EAA}_n^{\text{ref}}}} \)

\( \text{DEAAI} = \text{EAAI} \times \text{Digestibility (\%)} \)

- The EAAI differs between 13 and 29% spatial and between 12 and 20% temporal.
- Spirulina has a more favourable EAAI compared to Chlorella.
- Taking into account digestibility the DEAAI drops below 1, indicating an AA shortage compared to the reference protein (WHO).
Spatial and temporal variation in EAA content (Quality vs Quantity)

Essential amino acid index: \[ EAAI = \sqrt{\frac{EAA_{1_{scp}}}{EAA_{1_{ref}}} \cdot \frac{EAA_{2_{scp}}}{EAA_{2_{ref}}} \ldots \frac{EAA_{n_{scp}}}{EAA_{n_{ref}}}} \]

Spatial variability

\[ y = -0.0031x + 1.5214 \]
\[ y = 0.0028x + 1.0106 \]

Temporal variability

\[ y = -0.022x + 2.2959 \]
\[ y = 0.0141x + 0.4551 \]

High quantity ≠ high quality!
Both Chlorella and Spirulina have a spatial difference between 0 and 12% in lipid content. Chlorella lipid content is rather underestimated on the package, while the opposite is true for Spirulina.
Micro pollutants: PAH (Spatial variation)

Maximum levels in food supplements (Commission regulation (EU) 2015/1933 of 27 October 2015):

- Benzo(a)pyrene: **10 µg/kg wet weight**
- Sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene (‘PAH4’): **50 µg/kg**

For *Spirulina platensis* (Origin’O), slight violation in PAH4:

- Benzo(a)pyrene: **3.55 µg/kg**
- ‘PAH4’: **56.13 µg PAH4/kg**

For *Chlorella vulgaris* (Origin’O), strong violation Benzo(a)pyrene and PAH4:

- Benzo(a)pyrene: **538 µg/kg**
- ‘PAH4’: **2324 µg PAH4/kg**
Micro pollutants: PAH (Temporal variation)

Maximum levels in food supplements (Commission regulation (EU) 2015/1933 of 27 October 2015):
- Benzo(a)pyrene: 10 µg/kg wet weight
- Sum of benzo(a)pyrene. benz(a)anthracene. benzo(b)fluoranthene and chrysene (‘PAH4’): 50 µg/kg

1) **Chlorella**: Newer batches didn’t violate the limits for BaP and PAH4.
2) **Spirulina**: Newer batches didn’t violate the BaP limit but they all slightly violated the PAH4 limit

=> Violations are not constant trough time and are batch specific.
Micro pollutants: Heavy metals (Spatial variation)

Maximum levels in food supplements (Commission Regulation (EC) No 1881/2006):

Chlorella vulgaris

Spirulina platensis

Concentration (mg/kg)

No violations of heavy metal limits.
Conclusion

1) Spatial and temporal variation in nutritional composition of commercially available Chlorella and Spirulina is existing and not negligible.

2) Depending on the nutritional characteristic this variation can be large or rather limited.

3) The detected variation within one species of microalgae makes it likely that process conditions are essential in the production process of SCP. This should be further researched.

4) Safety of the product is not always guaranteed and should be monitored by the competent bodies.

5) From the digestible protein data available at this moment, Arthrospira is preferred for RLSS application above Chlorella.