MICROALGAE BISCUITS
Sensory, physical and chemical properties, antioxidant activity and in vitro digestibility

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Microalgae in Food - previous studies in ISA

Since 2003 – Research and development of microalgae derived food products

Oil-in-water emulsions (vegetarian “mayonnaise”)

Gelled Vegetarian Desserts

Biscuits

Pasta
Fradique et al (2013) LWT 50:312

Poster 46 – Raymundo et al
“Development of bread with Chlorella vulgaris addition: a rheological approach”
Microalgae in Food – current approach (ISA)

Main Focus:

- Development of novel microalgae-based food products in direct collaboration with Food industries, namely through LEAF connection and advisory to national agro-industrial clusters.

- Use of commercial Premium quality microalgal biomass produced in Europe (Food Grade)

- Optimization of new product development based on physical and rheological techniques. By understanding algae specific features and compatibility with other food ingredients it will be possible to add techno-functional and nutritional value, namely through fat mimetic, gluten replacement or gel forming in tailor-made food prototypes.

- Investigation of bioactive properties (e.g. antimicrobial, anti-inflammatory) and their resistance to thermal treatment
Context and aim of the present work

**AIM:**
Develop microalgae-based biscuits with significantly higher concentrations than the ones found in commercial products (typically below 1% w/w), in order to provide higher level of bioactive compounds, whilst not compromising the sensorial acceptability and digestibility issues.
Microalgae strains

**Arthrospira platensis F&M-C256**
- Zarrouk medium (Zarrouk, 1966)
- Biomass was washed with saline solution to remove excess bicarbonate before being frozen
- Does not require EU Novel Food approval (Reg EC 258/97)

**Tetraselmis suecica F&M-M33**
- F medium (Guillard & Ryther, 1962)
- Requires EU Novel Food approval (Reg EC 258/97)
- *T. chuii* novel food application by Fitoplancton Marino has recently been approved by EFSA

**Phaeodactylum tricornutum F&M-M40**
- F medium (Guillard & Ryther, 1962)
- Requires EU Novel Food approval (Reg EC 258/97)

**Chlorella vulgaris**
- BG11 medium (Rippka et al., 1979)
- Does not require EU Novel Food approval (Reg EC 258/97)
Biscuits

Convenient nutrient dense snack food, widely consumed on a daily basis by European citizens from all age groups.

The aim of this work was to study this baked food matrix as a relevant vehicle for algal functional ingredients, especially regarding higher incorporation levels.

**Base Recipe**
- Wheat flour
- Sugar
- Margarine
- Water
- Baking Powder

**Kneading**
(Food processor, 15 s)

2% (w/w) microalgal biomass
6% (w/w) microalgal biomass

- *A. platensis* (Ap)
- *C. vulgaris* (Cv)
- *T. suecica* (Ts)
- *P. tricornutum* (Pt)

**Moulding**
(Ø 48 mm, h 4 mm)

**Baking**
(40min/120ºC)
Biscuit preparation
Microalgae Biscuits

- Control
- *Arthrospira platensis*
- *Chlorella vulgaris*
- *Tetraselmis suecica*
- *Phaeodactylum tricornutum*
## Colour stability – ΔE*

\[
\Delta E^* = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2}
\]

\(\Delta E^* < 5\) : total colour differences are not distinguishable by human eye

<table>
<thead>
<tr>
<th></th>
<th>week 1 vs. week 0</th>
<th>week 2 vs. week 0</th>
<th>week 3 vs. week 0</th>
<th>week 4 vs. week 0</th>
<th>week 8 vs. week 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.84</td>
<td>0.86</td>
<td>1.23</td>
<td>1.55</td>
<td>1.89</td>
</tr>
<tr>
<td><em>A. platensis</em></td>
<td>2%</td>
<td>0.60</td>
<td>0.66</td>
<td>1.16</td>
<td>1.63</td>
</tr>
<tr>
<td></td>
<td>6%</td>
<td>0.73</td>
<td>0.89</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td><em>C. vulgaris</em></td>
<td>2%</td>
<td>0.70</td>
<td>1.17</td>
<td>0.96</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>6%</td>
<td>0.75</td>
<td>1.26</td>
<td>1.11</td>
<td>1.32</td>
</tr>
<tr>
<td><em>T. suecica</em></td>
<td>2%</td>
<td>1.02</td>
<td>1.73</td>
<td>2.43</td>
<td>2.49</td>
</tr>
<tr>
<td></td>
<td>6%</td>
<td>1.83</td>
<td>2.12</td>
<td>2.40</td>
<td>3.80</td>
</tr>
<tr>
<td><em>P. tricornutum</em></td>
<td>2%</td>
<td>1.50</td>
<td>2.03</td>
<td>2.48</td>
<td>2.37</td>
</tr>
<tr>
<td></td>
<td>6%</td>
<td>1.31</td>
<td>2.57</td>
<td>2.37</td>
<td>3.35</td>
</tr>
</tbody>
</table>
Texture analysis

Biscuits with 6% microalgae biomass show a significant structural reinforcement (p<0.05) in relation to the control, which is most evident for *A. platensis*.
Water activity ($a_w$)

Water Activity, $a_w$

A$_w$ < 0.5  ➔  Low % of free water for microbial proliferation  ➔  High stability

Aw meter (HygroPalm HP23-AW)
### Chemical Composition

<table>
<thead>
<tr>
<th></th>
<th>Moisture (g/100g)</th>
<th>Total Ash (g/100g)</th>
<th>Crude Fat (g/100g)</th>
<th>Crude Protein (g/100g)</th>
<th>Dietary Fiber (g/100g)</th>
<th>Carbohydr.* (g/100g)</th>
<th>Energy Value (kcal/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>3.8 ± 0.2 ab</td>
<td>2.7 ± 0.2 a</td>
<td>16.1 ± 0.1 a</td>
<td>4.9 ± 0.5 a</td>
<td>3.2 ± 0.003 a</td>
<td>69.4</td>
<td>448</td>
</tr>
<tr>
<td><strong>A. platensis</strong></td>
<td>2% 3.8 ± 0.1 ab</td>
<td>2.6 ± 0.4 a</td>
<td>16.1 ± 0.5 a</td>
<td>6.1 ± 0.2 abc</td>
<td>6.7 ± 0.3 ab</td>
<td>64.7</td>
<td>441</td>
</tr>
<tr>
<td></td>
<td>6% 5.0 ± 0.2 d</td>
<td>2.3 ± 0.1 a</td>
<td>16.1 ± 0.1 a</td>
<td>7.8 ± 0.3 de</td>
<td>8.5 ± 0.1 b</td>
<td>60.2</td>
<td>434</td>
</tr>
<tr>
<td><strong>C. vulgaris</strong></td>
<td>2% 3.2 ± 0.1 a</td>
<td>2.3 ± 0.1 a</td>
<td>16.3 ± 0.2 a</td>
<td>5.9 ± 0.5 abc</td>
<td>6.2 ± 0.4 ab</td>
<td>66.1</td>
<td>447</td>
</tr>
<tr>
<td></td>
<td>6% 4.8 ± 0.3 cd</td>
<td>2.6 ± 0.1 a</td>
<td>16.9 ± 0.4 a</td>
<td>8.0 ± 0.6 e</td>
<td>8.2 ± 0.2 b</td>
<td>59.5</td>
<td>439</td>
</tr>
<tr>
<td><strong>T. suecica</strong></td>
<td>2% 3.4 ± 0.2 ab</td>
<td>2.4 ± 0.2 a</td>
<td>16.1 ± 0.1 a</td>
<td>5.2 ± 0.1 a</td>
<td>6.1 ± 0.4 ab</td>
<td>66.9</td>
<td>445</td>
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<td>3.2 ± 0.1 a</td>
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<td>6.9 ± 0.4 cd</td>
<td>7.0 ± 0.7 ab</td>
<td>63.4</td>
<td>442</td>
</tr>
<tr>
<td><strong>P. tricornutum</strong></td>
<td>2% 3.9 ± 0.1 ab</td>
<td>2.3 ± 0.2 a</td>
<td>16.1 ± 0.1 a</td>
<td>5.1 ± 0.2 ab</td>
<td>7.5 ± 1.3 ab</td>
<td>65.2</td>
<td>441</td>
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<td>6% 4.3 ± 0.2 bc</td>
<td>3.0 ± 0.1 a</td>
<td>16.2 ± 0.1 a</td>
<td>6.6 ± 0.4 bc</td>
<td>7.4 ± 0.4 ab</td>
<td>62.5</td>
<td>437</td>
</tr>
</tbody>
</table>

All microalgae biscuits, either with 2% or 6%, can be associated to the **nutritional claim “high fiber”**, according to Regulation (EC) 1924/2006, which requires “a claim that a food is high in fibre, and any claim likely to have the same meaning for the consumer, may only be made where the product contains at least 6 g of fibre per 100 g or at least 3 g of fibre per 100 kcal.”

The inclusion of microalgae enabled the supplementation of soluble dietary fiber (SDF).
The addition of microalgae results in an effective supplementation of phenolic compounds, which are practically absent in the control biscuit.

*A. platensis* 6% biscuit presented the highest phenolic content (0.90 mg GAE/g), followed by *P. tricornutum* 6% cookie (0.62 mg GAE/g).
Antioxidant activity

FRAP Method (Ferric Reducing Antioxidant Power)

For all the microalgae studied, a significant $(p<0.05)$ increase in antioxidant capacity was observed when increasing biomass concentration from 2% to 6%.

Biscuits with 2% alga showed values around 7.0 and 9.5 mmol TEAC/kg (+65% to +125% in relation to the control) while 6% cookies showed values around 11.8 to 15.4 mmol TEAC/kg (+178% to +272% in relation to the control).
In vitro digestibility

The *in vitro* analysis reproduces the chemical-enzymatic catalysis that occurs in the proximal tract of the mammalian digestive system. (Method Boison & Fernández, 1997)

No significant difference (p<0.05) in *in vitro digestibility* between microalgae biscuits and the control (IVD 95%) was found.
Sensory Analysis – *A. platensis* and *C. vulgaris*

Responses of the sensory analysis panel tasters (n=40) regarding *A. platensis* and *C. vulgaris* cookies. 0 – very unpleasant; 1 – unpleasant; 2 – slightly unpleasant; 3 – slightly pleasant; 4 – pleasant; 5 – very pleasant.

**Colour** – *C. vulgaris* 2%

**Smell** – *A. platensis* 2% and 6%

**Taste** – *A. platensis* 2%

**Texture** – *A. platensis* 2% and *C. vulgaris* 6%

**Global appreciation**: 2% *A. platensis*

Unpreferred: 6% de *C. vulgaris*. 
Sensory Analysis – buying intention

*A. platensis* 2% – 68% “certainly” + “probably” would buy

*C. vulgaris* 2% – 44% “certainly” + “probably” would buy

*A. platensis* 6% – 66% “probably” + “don’t know if” would buy

*C. vulgaris* 6% – 73% “certainly” + “probably” wouldn’t buy
Sensory Analysis – broader and simpler surveys

Ficha de prova “GREENCOOKIES”

<table>
<thead>
<tr>
<th>Idade:</th>
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<tbody>
<tr>
<td>Género:</td>
<td>( ) Masculino</td>
</tr>
</tbody>
</table>

Código da amostra:

- [ ] Gosto
- [ ] Não Gosto
- [ ] Compraria
- [ ] Não Compraria

Observações:

Estoril Municipality Event promoting Ecological Sustainability directed to the general public
Tasting session after giving a lecture on Microalgae

TV studio
No previous information on the benefits of microalgae
Conclusions

• The addition of microalgal biomass as natural ingredient resulted in biscuits with an attractive and innovative appearance, presenting **colour, texture and aw stability** along 8 weeks storage.

• A significant (p<0.05) **structuring effect**, in terms of biscuit texture and dough viscoelastic properties, was observed when microalgae was added at 6%, particularly for *A. platensis*.

• The inclusion of microalgae in biscuits, particularly *A. platensis* and *C. vulgaris*, can be regarded as an effective nutritional fortification in terms of protein and fibre, contributing to a healthier diet. **All the microalgae biscuits developed are eligible for “high fiber” nutritional claim in the EU.**

• A significant (p<0.05) increase in total phenolic compounds and antioxidant capacity was observed with microalgae incorporation in relation to the control and with increasing algae concentration.

• **No significant differences (p<0.05) were found in in vitro digestibility** in relation to the control.

• *A. platensis* cookies presented the highest sensory scores.

• There are prospects for considering microalgae-based cookies functional nutrient foods, which could be widely consumed on a daily basis by European citizens from all age groups.
Future Work

Salted cookies

Microalgae savoury foods might be more easily accepted by the consumer due to their characteristic organoleptic properties, resembling a marine flavour. Moreover, the incorporation of algae might enable a reduction of refined salt addition with positive health impact.

STSM (Short-term Scientific Mission)
AP Batista (Early Career Investigator)
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Aknowledgements

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THANK YOU FOR YOUR ATTENTION !!!

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