Evaluation of the risk-benefit associated to the consumption of seaweeds based on the bioaccessibility of antioxidant compounds and contaminants

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Seaweeds consumption has increased worldwide due to the growing awareness of its several benefits.
Overview

- **Seaweeds (SW) contains valuable biomolecules:**
  - Antioxidants;
  - Essential amino acids;
  - Essential elements;
  - Polyunsaturated fatty acids;
  - Carbohydrates

Due to the increasing levels of pollution in the oceans, SW also tend to bioaccumulate contaminants that can compromise their consumption.
Knowledge is of great importance to consumers, producers and health authorities.

- Seaweed consumption is increasing.
- Risk-benefit assessment
- Human exposure to heavy metals
- Bioaccessibility of compounds (simulating the human digestive process)
WHY Bioaccessibility???

The total amount of nutrient, does not reflect the amount that is bioaccessible and bioavailable.

**Bioaccessibility:** the fraction of a compound that is released from its matrix in the gastrointestinal tract, and thus becomes available for intestinal absorption.
Objectives

The main objective of this work was to assess the benefit/risk associated to the consumption of a brown SW (Fucus spiralis) based on the bioaccessibility of antioxidant compounds and contaminants (simulating the human digestive process)
Methods - Seaweed
Methods - Seaweed

Washed and selected

Freeze-dried
Methods - *In vitro* digestion

3 steps that simulate digestive process in.....

Seaweed

- Mouth
  - $\alpha$-amilase
- Stomach
  - Pepsin
- Small intestine
  - Trypsin;
  - Chymotrypsin;
  - Lipase;
  - Pancreatin;
  - Bile salts.

Composition of digestive juices was used on the method described by Versantvoort et al. (2005) and Afonso et al. (2015).
Methods - Quantification

- **Contaminants were evaluated by ICP-MS**
  - Arsenic (As)
  - Cadmium (Cd)
  - Lead (Pb)
  - Mercury (Hg)

Total mercury was determined by atomic absorption spectrometry

A minimum of two replicate analyses were performed for each sample and analysis.
Methods - Quantification

Antioxidant Potential:

- Methanolic Extract:
  - DPPH (1,1-Diphenyl-2-Picryl-Hydrazyl) radical scavenging activity;
  - Total phenolic content (TPC), mg equivalents (eq.) gallic acid/g Seaweed (SW);
  - Ferric reducing antioxidant power (FRAP), μMoles eq. of ascorbic acid (AA)/g SW;
  - Oxygen Radical Absorbance Capacity (ORAC), mMoles eq. Trolox/g SW.

Due to the presence of different bioactive components with anti-oxidative potential in the crude extracts of the samples 4 methods were considered.
Methods - *Calculation of bioaccessible*

The percentage (%) of antioxidants and contaminants in the bioaccessible was estimated as follows:

\[
\%NC_{\text{bioaccessible}} = \frac{[NC]_{\text{bioaccessible}}}{[S]} \times 100
\]

Where \([NC]\) is the antioxidant or contaminant concentration. \([S]\) is the \([NC]\) in the bioaccessible fraction plus \([NC]\) in the non-digested fraction.
Results - Antioxidants

High range of antioxidant compounds, including polyphenols

DPPH: the bioaccessible fraction of the seaweed product only had 30% of DPPH reduction, making it impossible to determine IC50.

<table>
<thead>
<tr>
<th></th>
<th>TPC</th>
<th>FRAP</th>
<th>ORAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg Eq. GA/g SW)</td>
<td>(mMoles Eq. AA/g SW)</td>
<td>(mMoles eq. Trolox/g SW)</td>
</tr>
<tr>
<td>SW</td>
<td>Inicial</td>
<td>Bioaccessible</td>
<td>Inicial</td>
</tr>
<tr>
<td>FD</td>
<td>8.357 ± 0.919</td>
<td>1.923 ± 0.085</td>
<td>31.466 ± 0.521</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. All treatments were statistically different (P < 0.05).
It can be verified that there is a significant reduction of the antioxidants activity after *in vitro* digestion.
Results - Antioxidants

• Benefits???

Recommended values of ingestion needed

• Dietary Reference Intakes (DRI) - polyphenols or other antioxidant compounds
## Results - Contaminants

<table>
<thead>
<tr>
<th></th>
<th>As</th>
<th>Cd</th>
<th>Pb</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ug/g)</td>
<td>(ng/g)</td>
<td>(ng/g)</td>
<td>(ng/g)</td>
</tr>
<tr>
<td>Initial</td>
<td>24.36 ± 2.04*</td>
<td>15.20 ± 1.20*</td>
<td>543.7 ± 33.8*</td>
<td>470.0 ± 34.6*</td>
</tr>
<tr>
<td>Bio</td>
<td>15.20 ± 1.20*</td>
<td>543.7 ± 33.8*</td>
<td>470.0 ± 34.6*</td>
<td>130.3 ± 6.6*</td>
</tr>
<tr>
<td>Initial</td>
<td>13.6 ± 6.6</td>
<td>13.6 ± 6.6</td>
<td>13.6 ± 6.6</td>
<td>13.6 ± 6.6</td>
</tr>
</tbody>
</table>

Peniche’s coastal waters, near the sampling site, can be claimed to be unpolluted, for all the studied contaminants lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As).

Values are presented as mean ± SD. (*) Represents values statistically different between initial and bioaccessible fraction \((p<0.05)\).
Results - Contaminants

There is a reduction of the contaminants’ concentration after *in vitro* digestion.

The Hg concentration after digestion was below the LOD, thus the bioaccessibility of this contaminant metal from SW was not possible to determine.
Results - Risk???

Preliminary approach

• Provisional tolerable intake
  • Weekly (PTWI) - World Health Organization
  • Daily consume of 3g of SW by an adult, 65Kg

<table>
<thead>
<tr>
<th></th>
<th>Maximum Dose*</th>
<th>Initial</th>
<th>Bio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd (month)</td>
<td>25</td>
<td>0.75</td>
<td>0.65</td>
</tr>
<tr>
<td>Pb (week)*</td>
<td>25</td>
<td>0.042</td>
<td>0.037</td>
</tr>
<tr>
<td>As (week)*</td>
<td>15</td>
<td>7.87</td>
<td>4.91</td>
</tr>
</tbody>
</table>

*JECFA (Joint FAO/WHO Expert Committee on Food Additives). Units: µg/kg

F. spiralis did not raise any serious concern regarding human health for the contaminants in study.
Conclusions

*F. spiralis* antioxidants can resist to the stress provoked by the digestion, and thus being available to be absorbed by the intestine cells.

The antioxidants detected by the FRAP method were the most bioaccessible.

The very low contaminant levels in this SW are a clear indicator of a low degree of pollution of the Portuguese shoreline and, particularly, of the coastal waters in Peniche.

It seems that *F. spiralis* consumption poses no hazard to humans concerning the elements assessed.

On the other hand, the high levels of bioaccessibility also determined for the contaminants make the consumption of this SW species from very polluted areas unadvisable.
Thank you!

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