1. Introduction

- Traditional non polar solvents like alkanes are obtained from non-renewable sources. The idea of Green Chemistry has its roots in sustainable development. Green Analytical Chemistry emerged from Green Chemistry in 2000 [1]. The principles of Green Analytical Chemistry emphasize the importance of using reagents obtained from renewable sources, eliminate toxic reagents and increase safety of the operator.
- The use of limonene as non-polar extracting agent in order to replace hexane has been slightly studied. Limonene is obtained from citrus peel residues, being the main

2. Objective

To optimize a fast and green method for the isolation of high value lipids from aquatic microorganisms using Pressurized Liquid Extraction (PLE) with food grade solvents (as limonene), as an alternative to traditional hexane extraction.

3. Work flow

- **Limonene vs Hexane**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Total extraction yield (%, w/w)</th>
<th>Lipids % in the extract (w/w)</th>
<th>GLnA % in the extract (w/w)</th>
<th>Lipid enrichment</th>
<th>Total recovery</th>
<th>GLnA recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limonene</td>
<td>4.1 ± 0.6b</td>
<td>11.4 ± 2.2</td>
<td>8.3 ± 0.6</td>
<td>3.7 ± 0.3</td>
<td>29.6 ± 2.1</td>
<td>68.4 ± 2.7</td>
</tr>
<tr>
<td>Limonene:Ethanol (1:1, v/v)</td>
<td>14.4 ± 1.0</td>
<td>34.7 ± 2.5</td>
<td>7.7 ± 0.5</td>
<td>4.0 ± 0.3</td>
<td>58.1 ± 4.1</td>
<td>63.0 ± 4.5</td>
</tr>
<tr>
<td>Hexane</td>
<td>5.6 ± 0.4</td>
<td>15.8 ± 2.5</td>
<td>8.9 ± 0.6</td>
<td>4.2 ± 0.3</td>
<td>23.3 ± 1.6</td>
<td>28.5 ± 2.0</td>
</tr>
<tr>
<td>Hexane:Ethanol (1:1, v/v)</td>
<td>13.2 ± 0.9</td>
<td>38.9 ± 2.8</td>
<td>9.0 ± 0.6</td>
<td>4.5 ± 0.3</td>
<td>60.1 ± 4.3</td>
<td>67.8 ± 4.8</td>
</tr>
</tbody>
</table>

Yield expressed as g of dry extract/100 g Spirulina (w/w); *Values relative to untreated Spirulina content of lipids (8.6%, w/w) and GLnA (1.8%, w/w) *N=3. (n+2) in each column, means with different letters are significantly different (p<0.05).

![Figure 1. Chromatograms of the fatty acid profile of Spirulina extracted with different solvents using PLE at 20.7 MPa, 180 ºC and 15 min.](image1)

Table 1. Changes in the extraction yield, lipid concentration and concentration of γ-linolenic acid (GLnA) in Spirulina applying pressurized liquid extraction at 20.7 MPa pressure, 180 ºC temperature and 15 min extraction time using different solvents.

- **Optimization of extraction process with Spirulina**

![Figure 2. Changes in the fatty acid profile of Spirulina extracted at different temperatures compared to untreated Spirulina (C16:0: Palmitic acid; C16:1: Palmitoleic acid; C18:0: Stearic acid; C18:1: Oleic acid; C18:2: Linoleic acid; C18:3: γ-Linolenic acid or GLnA).](image2)

The best conditions to obtain valuable lipidic (rich in polyunsaturated fatty acids) extracts from Spirulina within the ranges tested were: limonene:ethanol (1:1, v/v) as solvent, 200 ºC of extraction temperature; 20.7 MPa as extraction pressure and a total of 15 min as extraction time.

4. Results and discussion

5. Conclusions

- The proposed method (limonene:ethanol (1:1, v/v) as solvent, 200 ºC of extraction temperature; 20.7 MPa as extraction pressure and a total of 15 min as extraction time) can be an interesting option to be used both for sample preparation and for lipid extraction production in short time.
- Extracts obtained with this method are not only useful in Analytical Chemistry, they can be labeled as green extracts and directly used in food, pharmaceutical or cosmetic preparations.