

Extraction of lipophilic bioactive compounds from *Alaria esculenta* using novel extraction techniques and solvents

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Introduction: Over the last 10 years there has been an increase in seaweeds cultivated for human consumption in Europe. *Alaria esculenta*, an edible seaweed commonly known as ‘dabberlocks’, presents a commercial brown seaweed of cold waters and belongs to the order *Laminariales*, from the family *Alariaceae* (Afonso et al.,2021).

To design biorefinery process for the extraction of valuable compounds from brown seaweed, it is important to assess food safety and nutritional quality of the biomass. Extraction of lipophilic bioactive compounds can be considered before the extraction of laminarin, alginate, mannitol and protein in cascade manner that was already proposed by Zhu et al. (2023). The aim of the study is to test novel extraction techniques and solvents including pressurized liquid extraction (PLE) and natural deep eutectic solvents (NADES) to recover lipophilic extracts from brown seaweed.

Materials and Methods:

Dried seaweed biomass was obtained from Ireland (BMRS) in 2022 and stored for 2 years before the analysis at room temperature in dark.



PLE Extraction: Dionex ASE 350 device (Thermo Scientific) 3 g sample was mixed with diatomaceous earth in 33 mL extraction cells, the extraction was performed with ethanol (90% v/v) and acetone (90% v/v), extraction temperature 160 °C; static extraction time 15 minutes; washing the cell with solvent at 60% volume level, number of cycles 2.

Acetone extraction: Three grams of seaweed were homogenized using a pestle and mortar with 90% acetone and then diluted to a final volume of 25 mL.

NADES assisted acetone extraction: The same as acetone extraction after the addition of three grams of C10:Menthol (1:2) NADES.

Soxlet extraction: Five grams of seaweed were extracted with petroleum ether for 2h.

Chemical composition was done as reported in Mišan et al. (2017), fatty acid profile as reported in Popović et al. (2023), DPPH scavenging activity and NADES preparation as reported by Božović et al. (2024) and carotenoid profile as reported by Stupar et al. (2021).

Results:

Tab 1. Fatty acid profile (in % of total fatty acids) of the studied seaweed species *Alaria esculenta*

Fatty acid	% total fatty acids
C10:0	0.034±0.009
C12:0	0.203±0.008
C14:0	7.768±0.019
C15:0	0.604±0.008
C16:0	25.400±0.093
C16:1	2.873±0.015
C17:0	0.798±0.017
C18:0	1.646±0.017
C18:1n9c	18.360±0.128
C18:2n6c	7.655±0.017
C20:0	0.666±0.001
C18:3n6	0.492±0.003
C18:3n3	7.366±0.028
C18:4n3	7.921±0.053
C20:3n6	0.294±0.036
C20:4n6	8.628±0.006
C20:5n3	9.262±0.009
Total fatty acids	100.000
Σ SFA	37.120±0.142
Σ MUFA	21.230±0.113
Σ PUFA	41.620±0.081
Σ ω-3	24.549±0.037
Σ ω-6	17.060±0.045
Σ ω-3/Σ ω-6	1.439±0.000

Values are presented as average ± standard deviation.

Tab 2. Mineral composition of the studied seaweed species *Alaria esculenta*

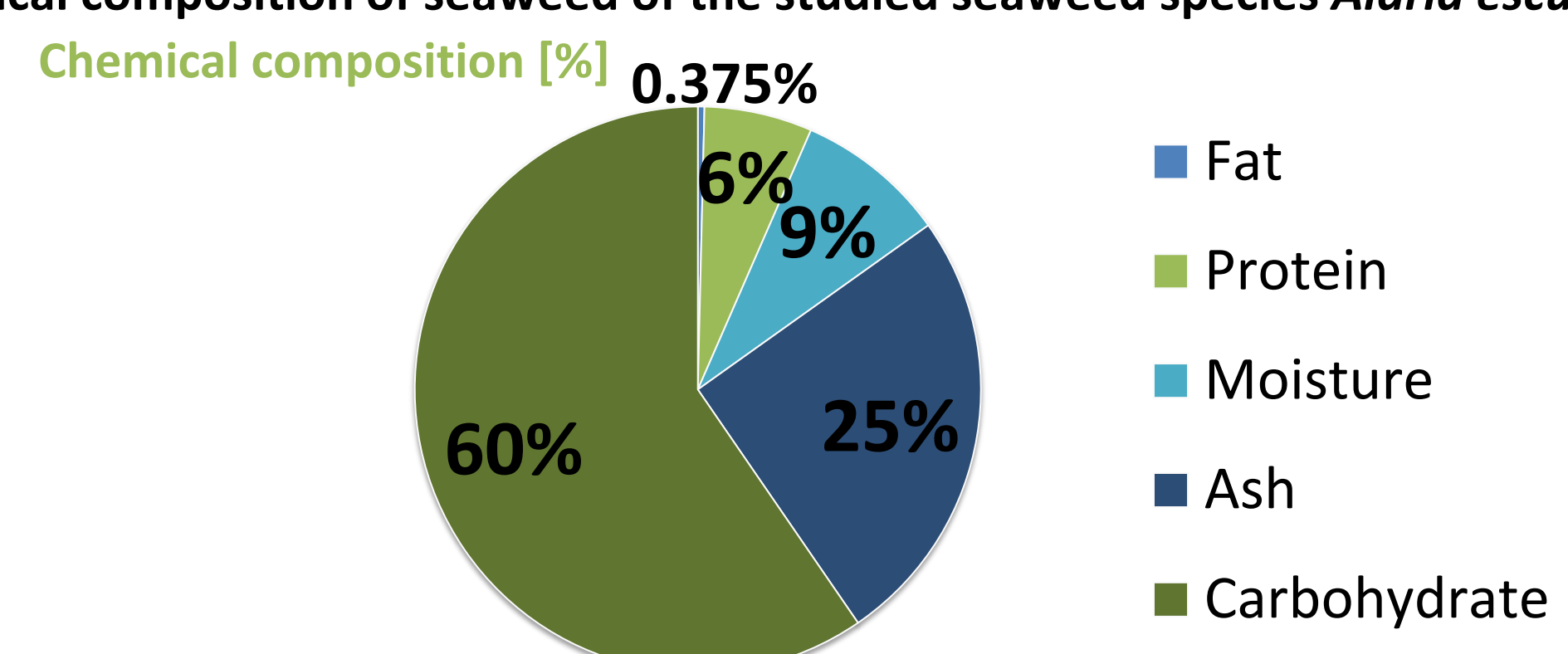
	Content [mg/kg]
Hg	<0.001
Pb	0.16 ± 0.05
Cd	0.26 ± 0.01
Zn	39.95 ± 6.16
Cu	0.55 ± 0.08
Fe	204.32 ± 31.51
K	39850 ± 6543
Mg	5824 ± 902.1
Ca	12507 ± 1937
Mn	31.48 ± 5.52
As	<0.1
Cr	<0.1

Tab 3. DPPH-radical-scavenging activity and fucoxanthin content of extracts of the studied seaweed species *Alaria esculenta*

	DPPH [μM/g]	fucoxanthin [μg/g]
PLE-Acetone	7.106±2.830	0.700±0.045
PLE-EtOH	3.731±0.748	1.160±0.272
Soxlet	0.093±0.022	0.075±0.006
NADES-Acetone	3.478±0.986	0.034±0.007
Acetone	2.805±0.619	0.848±0.012

Values are presented as average ± standard deviation.

Chemical composition of seaweed of the studied seaweed species *Alaria esculenta*



Conclusions:

- The examined sample contained lower lipid and protein content compared to the literature data, which can be attributed to the location and method of cultivation, seasonal variations, post-harvest treatments and a long time of storage before the analysis.
- Although the content of lipids was low, the ω-3/ω-6 ratio reached 1.44. The FAs 18:4 ω-3 (stearidonic acid, SDA) and 20:5 ω-3 (eicosapentaenoic acid, EPA) were the main contributors to the relative ω-3 PUFA richness in *A. esculenta*. Given the importance of EPA, it must be highlighted that its content reached 9.262±0.009%.

- Regarding elemental composition, the contents of Pb and Cd are worth a special attention.
- Given the low content of lipophilic compounds, low contents of fucoxanthin and weak antioxidant activity were expected. However, PLE-EtOH extraction is a promising technique for the extraction of fucoxanthin and PLE-Acetone for the extraction of antioxidants.

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