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Nannochloropsis sp. extract as a potential functional ingredient for food applications

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INTRODUCTION & AIM

Microalgae, particularly *Nannochloropsis* sp., have gained attention due to their rich composition in lipids, carbohydrates, proteins and bioactive compounds, such as polyunsaturated fatty acids (PUFAs) carotenoids, and phenolic compounds. These molecules are associated with heath promoting effects including antioxidant, anti-inflammatory, antimicrobial properties and prevention of coronary heart disease, making them promising candidates for nutraceutical and functional food applications.

The extraction of bioactive compounds from *Nannocloropsis* sp. using green technologies allows a sustainable up-scale to use this extract as a functional food ingredient.

Exploring *Nannochloropsis* sp. bioactive rich-extract can contribute not only to sustainable food innovation but also to the development of bioactive ingredients capable of supporting human health and disease prevention.

METHODS

- *Nannochloropsis* sp. freeze-dried microalga biomass was used (Fig. 1).
- The extraction was performed using an ultrasound-assisted extraction (USAE), 90% ethanol (20 kHz, 30 s pulses, 10 min) (Figs. 2 and 3).

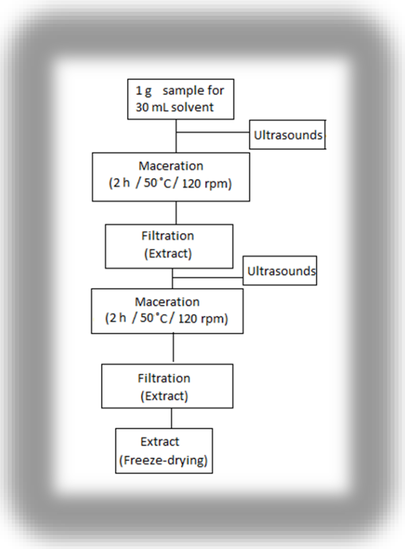

Figure 2. USAE methodology.

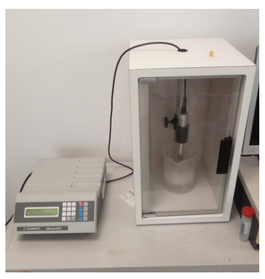

Figure 3. USAE equipment.

Figure 1. *Nannocloropsis* sp. Freeze-dried biomass.

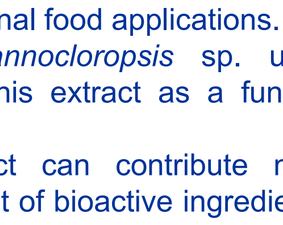

Figure 4. *Nannocloropsis* sp. bioactive-rich extract.

- GC-FID was performed to determine the fatty acids profile (Saturated Fatty Acids and Unsaturated Fatty Acids, Monounsaturated fatty acids and Polyunsaturated fatty acids)

- Determination of Total Phenolic Content and Antioxidant Acrivity
  - ✓ Folin Ciocalteu
  - ✓ ABTS assay
  - ✓ DPPH assay
  - ✓ ORAC assay

- Determination of Antimicrobial activity
  - ✓ Minimal Concentration Inhibition (MIC)
  - ✓ Minimal Bactericidal Concentration (MBC)

Gram negative species tested were:
- ➢ *Escherichia coli* ATCC 25922,
- ➢ *Yersinia enterocolitica* NCTC 10406, and
- ➢ *Salmonella enterica* serovar Enteritidis ATCC 13076

Gram positive species tested were:
- ➢ *Staphylococcus aureus* ATCC 6538
- ➢ *Bacillus cereus* NCTC 2599
- ➢ *Listeria monocytogenes* NCTC 10357

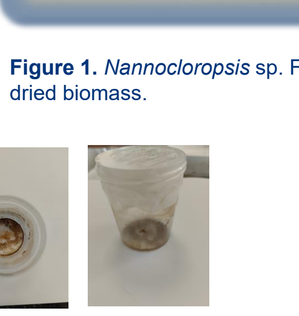

Figure 5. *Nannocloropsis* sp. bioactive-rich extract tested for ABTS performed in microplate 96 wells.

Figure 6. *Nannocloropsis* sp. bioactive-rich extract tested for MIC and MBC performed in microplate 96 wells.

RESULTS & DISCUSSION

Main fatty acids in the extracts:

- **Palmitic acid – intake is 20-30 g/day** that corresponds to **8-10% of total calories** (energy).
  SFA 310.7 ±2 1.03 mg/g
- **Myristic acid - intake** recommendation is around **0.5 to 2% of total calories**.
  SFA 67.45 ± 2.82 mg/g)
- **Palmitoleic acid** - MUFA present in some foods but also converted in the human organism from palmitic acid; **nutritional guidelines don´t mention its recommended daily intake**.
  USFA MUFA 235.03 ± 11.25 mg/g
- **Eicosapentaenoic acid** - WHO recommends a **daily intake of 200 to 500 mg of EPA+ DHA**; American Heart Association recommends 500 mg/day as a preventive measure; and EFSA recommends 250 mg/day of EPA + DHA intake for adults.
  USFA PUFA 165.34 ± 5.82 mg/g

Table 1. Fatty acids quantification.

Fatty acids	Chain lenghth	Quantity (mg/g)
Caprylic acid	C8	0.409 ± 0.073
Capric acid	C10	0.378 ± 0.049
Lauric acid	C12	2.724 ± 0.112
Myristic acid	C14	67.45 ± 2.837
Myristoleic acid	C14:1	0.886 ± 0.003
Pentadecylic acid	C15	5.314 ± 0.229
cis-10-Pentadecenoic Acid	C15:1 *c*10	4.754 ± 1.090
Palmitic acid	C16	310.7 ± 21.03
Palmitoleic acid	C16:1 *c*7	0.531 ± 0.098
Palmitoleic acid	C16:1 *c*9	235.03 ± 11.25
Margaric acid	C17	2.587 ± 0.148
cis-10-Heptadecenoic acid	C17:1 *c*10	0.953 ± 0.108
Stearic acid	C18	4.061 ± 3.551
Elaidic acid	C18:1 *t*9	0.165 ± 0.008
Oleic acid	C18:1*c*9	70.175 ± 4.630
Linoleic acid (ω-6)	C18:2 *c*6	32.105 ±1.914
Arachidic acid	C20	1.913 ± 0.457
γ-linolenic acid (ω-6)	γ C18:3	0.206 ± 0.012
Gondoic acid	C20:1	0.196 ± 0.011
α-linolenic acid (ω-3)	α C18:3	1.374 ± 0.084
Heneicosylic acid	C21	0.442 ± 0.029
Eicosadienic acid	C22:2 *c*11 *c*14	0.626 ± 0.008
Behenic acid	C22	0.429 ± 0.057
Dihomo-γ-linolenic acid (ω-6)	C20:3 *c*8 *c*11 *c*14	64.788 ± 3.21
Erucic acid	C22:1 *c*13	0.520 ± 0.105
Eicosatrienoic acid (ω-3)	C20:3 *c*11 *c*14 *c*17	0.852 ± 0.043
Arachidonic acid	C20:4	1.513 ± 0.012
Eicosapentaenoic acid (ω-3)	C20:5	165.34 ± 5.82
Tricosylic acid	C23	0.463 ± 0.052
Docosadienoic acid	C22:2 *c*13 *c*16	0.202 ± 0.006
Lignoceric acid	C24	0.078 ± 0.005
Nervoic acid	C24:1	0.122 ± 0.045
Docosahexaenoic acid	C22:6	0.505 ±0.012
Σ fat acids		978.265 ± 55.495
Σ SFA		580.706 ± 28.182
Σ MUFA		313.179 ± 17.128
Σ PUFA		265.149 ± 11.040
Σ Mufa + Σ PUFA		578.329 ± 28.168
Σ PUFA ω-3		230.636 ± 9.027
Σ PUFA ω-6		33.139 ± 1.929

Table 2. Nutrition quality of *Nannochloropsis* sp. USAE extract.

Nutrition parameters	Quantity (mg/g)
Index of Atherogenicity (AI)	1.01 ± 0.007
Index of Thrombogenicity (TI)	0.43 ± 0.009
Hypocholesterolemic/Hypercholesterolemic Ratio (HH)	0.88 ± 0.013
Health-Promoting Index (HPI)	0.99 ± 0.007

Table 3. Bioactivity of *Nannochloropsis* sp. USAE extract.

Bioactive-rich Extracts	TPC (mg GAE/g DW)	ABTS (µmol TE/g DW)	DPPH (µmol TE/g DW)	ORAC (µmol TE/g DW)
***Nannochloropsis* sp.**	5.7 ± 1.0	13.5 ± 2.7	2.5 ± 0.6	75.3 ± 6.8

- *Nannochloropsis sp.* **ethanolic extracts** are **more effective against Gram+** than Gram- because Gram- bacteria have an outer membrane (lipopolysaccharides + proteins), which acts as a permeability barrier [18]. **Gram+ bacteria lack this barrier**. **Ethanol extracts pull out more lipophilic compounds** (fatty acids, chlorophylls, carotenoids), and these **compounds have membrane-disrupting effects**, which can sometimes **enhance activity against Gram- bacteria compared with aqueous extracts**, but still Gram+ bacteria are more sensitive overall [18].

Table 4. Antimicrobial properties of *Nonnochloropsis* sp. USAE extract.

Bacteria	MIC	MBC
Escherichia coli	1.25	>5
Yersinia enterocolitica	0.63	>5
***Salmonella enterica Serovar* Enteritis**	1.25	>5
Staphylococcus aureus	1.25	>5
Bacillus cereus	>5	>5
Listeria monocytogenes	1.25	>5

CONCLUSIONS

The USAE allows a *Nannochloropsis* sp. bioactive-rich extract with balanced fatty acids composition and moderate antioxidant potential, while antimicrobial activity remains limited. The extract shows a promise potential as functional ingredient for food formulations aimed at cardiovascular health. However, at therapeutic doses, the SFA contribution must be considered to ensure dietary safety.

FUTURE WORK

Future work should focus on purifying EPA-rich fractions and improving bioactivity through micro/nanoencapsulation for enhanced antioxidant and antimicrobial effects. Optimization of extraction, stabilization and packaging will be crucial to preserve bioactive integrity. Additionally, toxicological and clinical validation are needed before commercial application as a functional ingredient or nutraceutical.