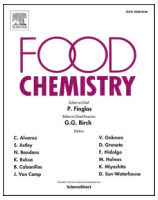
Food Chemistry 405 (2023) 134885

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Effects of pressurized liquid extraction with dimethyl sulfoxide on the recovery of carotenoids and other dietary valuable compounds from the microalgae *Spirulina*, *Chlorella* and *Phaeodactylum tricornutum*

Min Wang [a,b,1], Ángeles Morón-Ortiz [c,1], Jianjun Zhou [a,b], Ana Benítez-González [c], Paula Mapelli-Brahm [c], Antonio J. Meléndez-Martínez [c,*], Francisco J. Barba [a]

[a] *Preventive Medicine and Public Health, Food Science, Toxicology and Forensic Medicine Department, Faculty of Pharmacy, Universitat de València, Avda. Vicent Andrés Estellés, s/n, 46100 Burjassot, Valencia, Spain*
[b] *Institute of Agrochemistry and Food Technology, National Research Council (IATA-CSIC), Valencia, Spain*
[c] *Food Colour and Quality Laboratory, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain*

ARTICLE INFO

Keywords:
Carotenoids
Chlorophylls
Minerals
Polyphenols
Proteins
Sustainability

ABSTRACT

The impact of pressurized liquid extraction (PLE) and DMSO concentration (0, 30, 50, 100 %) on the yield of antioxidants and minerals from *Chlorella* were investigated. The results showed that PLE increased the antioxidant yield. Water extracted more proteins, while with 100 % DMSO more polyphenols, chlorophylls, and carotenoids were obtained. The efficiency coefficient (K_{PLE}) results showed that PLE + 100 % DMSO was more suitable for the recovery of antioxidants and pigments from *Chlorella* (polyphenols 10.465 mg/g, chlorophyll *a* 6.206 mg/g, chlorophyll *b* 3.003 mg/g, carotenoids 0.971 mg/g). Thus, PLE + 100 % DMSO was used for recovery studies on *Spirulina*, *Chlorella,* and *Phaeodactylum tricornutum*. Fucoxanthin, β-carotene, zeaxanthin, and lutein were the major carotenoids in *P. tricornutum*, *Spirulina,* and *Chlorella*, respectively. Regarding the extraction of minerals, Relative Nutrient Values results were calculated based on Recommended Dietary Allowances. The results indicated that the extracts could be used as a mineral source for different populations.

1. Introduction

The global shortage of food resources, the increase in demand for healthy food brought about by Covid-19, and the war between Russia and Ukraine, make it necessary for the food industry to make full use of the existing natural food resources as a solution. From the perspective of environmentally friendly strategies and nutritional value, marine resources show a great potential as edible resources, especially marine microalgae. On the one hand, marine microalgae can use light and sequestrate CO_2 to synthesize high-value nutrients, such as proteins, polysaccharides, pigments, etc. (Markou & Nerantzis, 2013). On the other hand, the cultivation of marine microalgae does not compete with traditional agriculture for land space, which allows marine microalgae to play a huge potential advantage in helping humans cope with food resource crises (Vaz et al., 2016). Moreover, microalgae are rich in bioactive compounds such as carotenoids, polyphenols, or polysaccharides, that are thought to be involved in health-promoting biological actions such as antioxidant, anti-inflammatory, anti-cardiac, or anti-diabetic actions or even in the regulation of gut microbiota (Laamanen et al., 2021).

Extraction with solvents is a key process to recover microalgae valuable compounds. For instance, most of marine microalgae contain hard cell walls, with a great range of thicknesses depending on the microalgae species. Overcoming the cell wall barrier of microalgae to dissolve cytoplasmic nutrients into the extraction solution is currently an important issue (Zhang et al., 2022). Traditional techniques such as Soxhlet, Folch, and hot water extractions have been used to extract valuable compounds from marine microalgae. Although these techniques can yield important quantities of such compounds, they have been gradually replaced by new efficient extraction techniques due to their disadvantages such as long extraction time and the use of large amounts of organic solvents (Zhou, Wang, Saraiva, Martins, Pinto, Prieto, Simal-Gandara, et al., 2022). Pressurized liquid extraction (PLE) is a highly efficient extraction technique compared to traditional

* Corresponding author.
E-mail address: ajmelendez@us.es (A.J. Meléndez-Martínez).
[1] Min Wang and Ángeles Morón-Ortiz contributed equally to this work.

https://doi.org/10.1016/j.foodchem.2022.134885
Received 18 August 2022; Received in revised form 21 October 2022; Accepted 4 November 2022
Available online 8 November 2022

extraction techniques. The PLE process is proceeding in a tightly closed stainless-steel cell, in order to be submitted to high temperature and pressure (Hoff & Pizzolato, 2018). During the extraction process, the solvent is kept below the critical point of the liquid phase. The pressure and temperature conditions are selected to increase the mass transfer rate by reducing the surface tension and viscosity of the solvent and increasing the solubility of the components, thus facilitating the penetration of the solvent into the matrix of the microalgae (Hoff & Pizzolato, 2018). Briefly, the PLE facilitates the process of matrix-solvent interaction during the extraction process and greatly shortens the extraction time. Moreover, PLE has additional advantages such as small sample and solvent usage or automatic extraction of multiple samples. Currently, it is widely used in the extraction of bioactive components (Zhou, Wang, Berrada, Zhu, Grimi, & Barba, 2022).

Although the duration of the high-temperature phase has been greatly shortened in the PLE process compared to traditional extraction techniques, this process is still not conducive to the protection of the properties of heat-sensitive bioactive components which may lead to the degradation of substances such as carotenoids, chlorophylls, or proteins, etc. Therefore, PLE combined with a suitable extraction solvent to reduce the dependence on high-temperature extraction conditions is an issue worth exploring. Compared with conventional extraction solvents (i.e., ethanol, methanol, or acetone), DMSO has the following advantages: (A) extraction is easy and fast because multiple grinding and centrifuging are not required. (B) high stability of microalgae bioactive components (chlorophyll, carotenoids, etc.) in DMSO solvents (Nikolopoulos et al., 2008). (C) low toxicity, high fluidity, and good selectivity at room temperature and pressure (Liu, Zhao, et al., 2021). The above advantages indicate that with DMSO as the solvent, PLE may perform the extraction of microalgae bioactive compounds at mild temperature conditions, although this is rarely reported. In this study, PLE combined with different concentrations of DMSO was used to recover dietary valuable compounds (proteins, polyphenols, chlorophylls, carotenoids, Mg, Ca, P, Fe, Zn, Se) at room temperature from *Chlorella*, *Spirulina,* and *Phaeodactylum tricornutum*. Principal component analysis was conducted to analyze the intrinsic correlation of bioactive components and antioxidant properties in the extracts, and the relative nutrient values of minerals in the extracts were assessed to provide a reference for the edible value of marine microalgae.

2. Materials and methods

2.1. Chemicals and reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS (2,2′-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid), AAPH (2,2′-Azobis(2-methylpropionamidine) dihydrochloride), fluorescein sodium salt, Folin-Ciocalteu, gallic acid, D-glucose, phenol, potassium persulfate and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (Steinheim, Baden-Württemberg, Germany). Sodium carbonate was acquired from VWR (Saint-Prix, France). Sodium hydroxide, bicinchoninic acid (BCA) kit, glacial acetic acid, and sulfuric acid were supplied by Fisher Scientific (Madrid, Spain). Deionized water (resistivity >18 MΩ cm^{-1}) was produced by a Milli-Q SP® Reagent Water System (Millipore Corporation, Bedford, MA, USA). β-Carotene (≥95.0 % purity), lutein (≥96.0 % purity), zeaxanthin (≥95.0 % purity), and fucoxanthin (≥95.0 % purity) were from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

2.2. Samples

Chlorella from Hainan Island (China) was produced in open raceway ponds, where the average temperatures varied from 21 °C to 33 °C and precipitations were 1600 mm per year. At the time of harvesting, biomass was washed and spray-dried at 160–180 °C. The final product, a green powder with a characteristic smell and taste, was stored at −20 °C until the experimental analysis. *Spirulina* biomass came from *Arthrospira platensis* species, strain paracas 15016, being Paracas the lake where it was originally isolated (Lima, Peru). Cultivation took place at EcoSpirulina company (Serra, Valencia, Spain) in raceway ponds using a greenhouse under natural sunlight. The day-time temperature was 32 °C on average, while the temperature decreased to 24 °C at night. The biomass was used for further experiments after freeze-drying. *Phaeodactylum tricornutum* was produced in four 800 L GemTube (LGEM, Rotterdam, The Netherlands) photobioreactors at the National Algae pilot plant in Mongstad (NAM), Norway. The photobioreactors were in a greenhouse exposed to natural light and additionally equipped with artificial illumination (EAX 170 W LED lights, Evolys AS, Oslo, Norway) with an average incident artificial light of 200 μmol m^{-2} s^{-1}. The culture temperature of *Phaeodactylum tricornutum* was maintained between ~15–35 °C by heating the greenhouse or spraying the reactors with water to cool them down, and the biomass after freezing dried was used for further experiments.

2.3. PLE extraction process

The PLE extraction was carried out based on a previous study (Moret et al., 2014) with slight modifications. Microalgae and diatomaceous earth were thoroughly mixed (0.5 g: 1.5 g) in a mortar and then placed into the PLE extraction tank. An ASE-200 Accelerated Solvent Extractor (Sunnyvale, CA, USA) was used to perform the extraction, and the operating conditions were referred to our previous study: preheating time of 1 min, heating time of 5 min, flush volume 60 %, nitrogen purge 60 s, extraction pressure of 103.4 bars, extraction temperature of 40 °C, extraction time of 15 min. Different proportions of DMSO (0, 30, 50, and 100 %) were used for PLE extraction to evaluate the effect of DMSO concentration on the extraction yield. For 0.5 g microalgae (dw), the volume of the final extracts was near 20 mL. According to the PLE extracts volume, the control experiment was carried out as 0.5 g algae powder/20 mL solvent (0, 30, 50, and 100 %) stirred at 40 °C for 15 min. The samples were centrifuged (2504*xg*, 4 °C, 15 min), and the supernatants were stored at −20 °C for further analyses.

2.4. Analysis of dietary valuable compounds

2.4.1. Protein yield

The bicinchoninic acid (BCA) method was used to analyze the protein content of the microalgae extracts (Al Khawli et al., 2021). Specifically, 10 μL of samples or BSA and 200 μL of BCA working solution were added to a 96-well plate, mixed well, and incubated at 37 °C for 30 min. Finally, the absorbances were measured at 562 nm. The protein content was determined using a calibration curve (0 ~ 2000 mg/L) with bovine serum albumin (BSA) as a standard.

2.4.2. Pigments (chlorophylls and carotenoids) and polyphenols yield

The concentration of pigments and polyphenols in the microalgae extracts were analyzed following the procedures previously reported by Zhou et al. (2021). The absorbance values and formulas used to analyze the DMSO and 50 % DMSO extracts were as follows (Zhou et al., 2021):

$$C_a = 12.47 \times Abs665.1\ nm - 3.62 \times Abs649.1\ nm \quad (1)$$

$$C_b = 25.06 \times Abs649.1\ nm - 6.5 \times\ Abs665.1\ nm \quad (2)$$

$$C_{Carotenoids} = (100 \times Abs480\ nm\ -\ 1.29 \times C_a -\ 53.78 \times C_b)/220$$

$$C_{Total\ chlorophylls} = C_a + C_b$$

Total phenolic content was determined by the Folin-Ciocalteu assay. For instance, 0.2 mL of extracts, 1 mL of Folin-Ciocalteu (diluted with water at a ratio of 1:10, v/v), and 0.8 mL of Na_2CO_3 (75 g/L) were mixed and incubated in a water bath at 50 °C for 10 min. Then, the absorbances were measured at 750 nm using a spectrophotometer. Gallic acid was

used as a standard to prepare the calibration curve to quantify the content of total polyphenols in the microalgae extracts.

2.4.3. Extraction of carotenoids and HPLC profile

For the extraction of carotenoids, 3 mL of trichloromethane were added to 1 mL of *Chlorella*, *Spirulina,* and *Phaeodactylum tricornutum* (PLE + 100 % DMSO extracts). The samples were vortexed for 2 min and then centrifuged at 20 °C (to avoid DMSO freezing) for 3 min. The supernatant was removed. After washing the solution with 4 mL of 10 % NaCl, the samples were evaporated to dryness in a rotary evaporator (<30 °C). Finally, the carotenoid extracts were dissolved in 200 μL of ethyl acetate and injected to identify the carotenoid profile by HPLC.

The HPLC analysis was performed on an Agilent 1260 system (Agilent, Palo Alto, CA). The carotenoids were separated on a YMC C_{30} column (5 μm, 250 × 4.6 mm) (YMC, Wilmington, NC), which was kept at 20 °C. The mobile phase, which comprised methanol, methyl *tert*-butyl ether, and water, was pumped at 1 mL/min. The linear gradient elution used is described elsewhere (Stinco et al., 2012).

The chromatograms were monitored for absorbance at 450 nm. The identification of carotenoids was carried out by comparison of their chromatographic and UV/vis spectroscopic characteristics with those of standards. The carotenoid content was measured using the calibration curves described by Stinco et al. (2019) and a calibration curve performed with a fucoxanthin standard. The quantification of the carotenoids for which standards were not available was carried out using the calibration curves of carotenoids with a similar spectrum. Thus, myxoxanthophyll and diatoxanthin were quantified using the calibration curves of lycopene and zeaxanthin, respectively. The total carotenoid content was calculated as the sum of the content of the individual compounds.

2.4.4. Dynamic proportion and PLE efficiency coefficient (K_{PLE})

In order to characterize the impact of PLE on the extraction efficiency, the PLE efficiency coefficient (Parniakov, Barba, et al., 2015) was evaluated, being K_{PLE} defined as the yields of the extracts obtained for PLE and control extraction procedures according to the Eq. (9):

$$K_{PLE} = Y_{compounds}(PLE)/Y_{compounds}(control)$$

where $Y_{compounds}$ was the yield of specific bioactive compounds (protein, polyphenol, chlorophyll *a*, chlorophyll *b*, or carotenoids) in PLE or control extracts.

2.4.5. Antioxidant properties

The antioxidant capacity of the microalgae extracts was evaluated through the oxygen radical antioxidant capacity (ORAC) and the Trolox equivalent antioxidant capacity (TEAC) assays (Zhou et al., 2021). For the ORAC assay, Trolox, and 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) were used as antioxidants and oxygen free radicals, respectively, and phosphate buffer was used as a blank control. Specifically, 50 μL of extract and 50 μL of the fluorescein sodium salt solution were added to a 96-well plate and incubated in a microplate reader at 37 °C for 10 min, then 25 μL AAPH solution was added, and the absorbance was recorded at 520 nm. For the TEAC assay, 25 mL of 7 mM ABTS were mixed with 440 μL of 140 mM potassium thiosulfate solution and incubated under darkness at room temperature for 12–16 h to obtain the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) working solution. During the measurement, the ABTS working solution was diluted with 96 % ethanol to obtain an absorbance value of 0.700 ± 0.020 at 734 nm. Then, 0.1 mL of the extracts or Trolox solution and 2 mL of the working solution were mixed, and after reacting for 3 min in a dark room, the absorbance at 734 nm was recorded.

2.4.6. ICP-MS mineral determination and Nutrient Relative Value (NRV) analysis

The contents of Mg, Ca, P, Fe, Zn, and Se in the extracts were evaluated considering methodologies described elsewhere (de la Fuente et al., 2019). Specifically, 1 mL extracts were mixed with 1 mL of concentrated nitric acid (HNO_3, 69 %) and 250 μL H_2O_2, then placed in a microwave furnace for digestion with a maximum temperature of 180 °C. After that, the volume was adjusted to 5 mL with ultrapure water, a 100 μL-aliquot was taken, and the volume was adjusted to 10 mL with ultrapure water. Finally, the mineral content was analyzed using a ICP-MS (7990 ICP-MS, Agilent Technologies, CA, USA). Furthermore, the contribution of minerals in extracts from 100 g microalgae dry basics (Nutrient Relative Value, NRV) towards DRI was calculated as Eq. (10) (Jalali & Fakhri, 2021):

$$NRV = (X/R) \times 100 \quad (10)$$

where X and R corresponded to the mineral content in PLE extracts (DMSO as a solvent) from 100 g microalgae dry powder and Recommended Dietary Allowances (RDAs, as shown in Supplementary Table 1), respectively.

3. Statistical analysis

The statistical analysis was performed by analysis of variance (ANOVA), using SPSS19.0 analysis software. The means were compared by Duncan's multiple range test ($p < 0.05$).

4. Results and discussion

4.1. Evaluation of Chlorella extraction process: yields, antioxidant properties, and efficiency coefficient

4.1.1. Extraction yields

The extraction effect of DMSO was evaluated with *Chlorella* as the raw material and the extraction conditions were determined, which were subsequently used for the recovery of the bioactive components of *Spirulina* and *Phaeodactylum tricornutum.* The effects of PLE and DMSO concentrations on the yield of *Chlorella* valuable compounds are shown in Fig. 1. PLE extraction increased the protein yield of *Chlorella* samples compared to the control group. The highest yield was obtained when water was used as the extraction solvent and decreased as the DMSO concentration increased, thus indicating that high concentrations of DMSO are not suitable for protein recovery from *Chlorella* samples. In this line, a previous study showed the negative impact of DMSO on protein recovery from *Nannochloropsis*, which was attributed to protein precipitation promoted when high concentrations of DMSO are used (Parniakov, Apicella, et al., 2015).

On the other hand, the effect of PLE on the extraction yield of polyphenols was affected by the concentration of DMSO. The polyphenol contents of the PLE extracts were higher than the control group when the concentration of DMSO was higher than 30 %. The polyphenol yield augmented with the increase of DMSO concentration until reaching about 10 mg/g dw at 100 % DMSO, thus indicating that DMSO concentration over 30 % could increase the polyphenol yield.

Up to a DMSO concentration of 50 %, no significant differences were found between the PLE and the control group in the values of the dynamic curves of pigments, including chlorophyll *a*, chlorophyll *b*, and carotenoids. Concentrations of DMSO higher than 50 %, resulted in a significant increase in the pigment yield of the PLE extracts compared to the control group. It should be also noted that when DMSO at 100 % was used as the extraction solvent, the contents of chlorophyll *a*, chlorophyll *b*, total chlorophylls, and carotenoids of the PLE extracts reached values up to 6.3 ± 0.1 mg/g dw, 3.0 ± 0.1 mg/g dw, 9.3 ± 0.2 mg/g dw, and 1.08 ± 0.03 mg/g dw, respectively. Chlorophyll *a*, chlorophyll *b*, and carotenoids are fat-soluble pigments, whereas DMSO has amphiphilic properties and can be used to dissolve polar and non-polar compounds in various solvent systems, thus promoting the increase of the extraction yield of those pigments when DMSO concentrations higher than 50 %

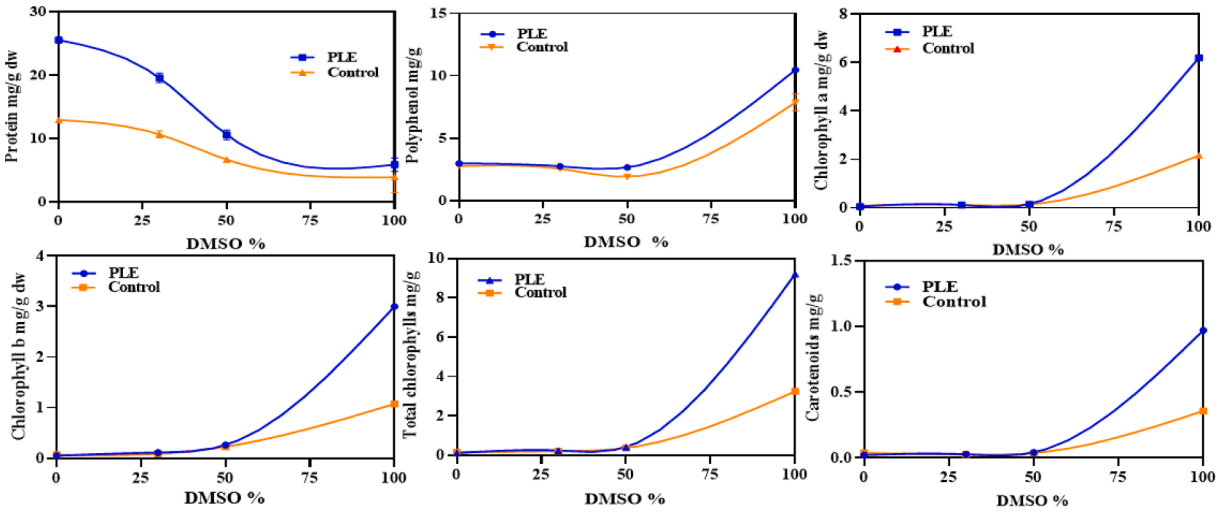


Fig. 1. Effects of pressurized liquid extraction (PLE) and dimethyl sulfoxide (DMSO) concentration on *Chlorella* valuable compounds yield (mg/g dw).

are used (Selvakumar et al., 2021). Similar to this study, Parniakov, Barba, et al. (2015) used the combination of pulsed electric field-DMSO to recover about 20 mg/g dw and 10 mg/g dw of total chlorophylls and carotenoids, respectively, from *Nannochloropsis*. The results were attributed to the good solubility of pigments in DMSO (Parniakov, Barba, et al., 2015).

4.1.2. *Efficiency coefficient*

To evaluate the effect of PLE on the yields, the PLE efficiency coefficient (K_{PLE}) was evaluated. The K_{PLE} value was calculated according to Eq. (9), and the results are shown in Fig. 2. The K_{PLE} values of proteins and polyphenols both exceeded 1, corresponding to their maximum K_{PLE} values of 2.0 (water as the extraction solution) and 1.25 (50 % or 100 % DMSO as the extraction solution), respectively. When the DMSO concentration was 0 %, 30 % and 50 %, the K_{PLE} values of the pigments were all close to 1, while when the DMSO concentration was 100 %, the K_{PLE} values of chlorophyll *a*, chlorophyll *b*, and total chlorophylls were close to 3.0, while the K_{PLE} values of carotenoids were close to 2.7. From the overall K_{PLE} results observed in Fig. 2, it can be depicted that PLE combined with 100 % DMSO appeared to be more suitable for the

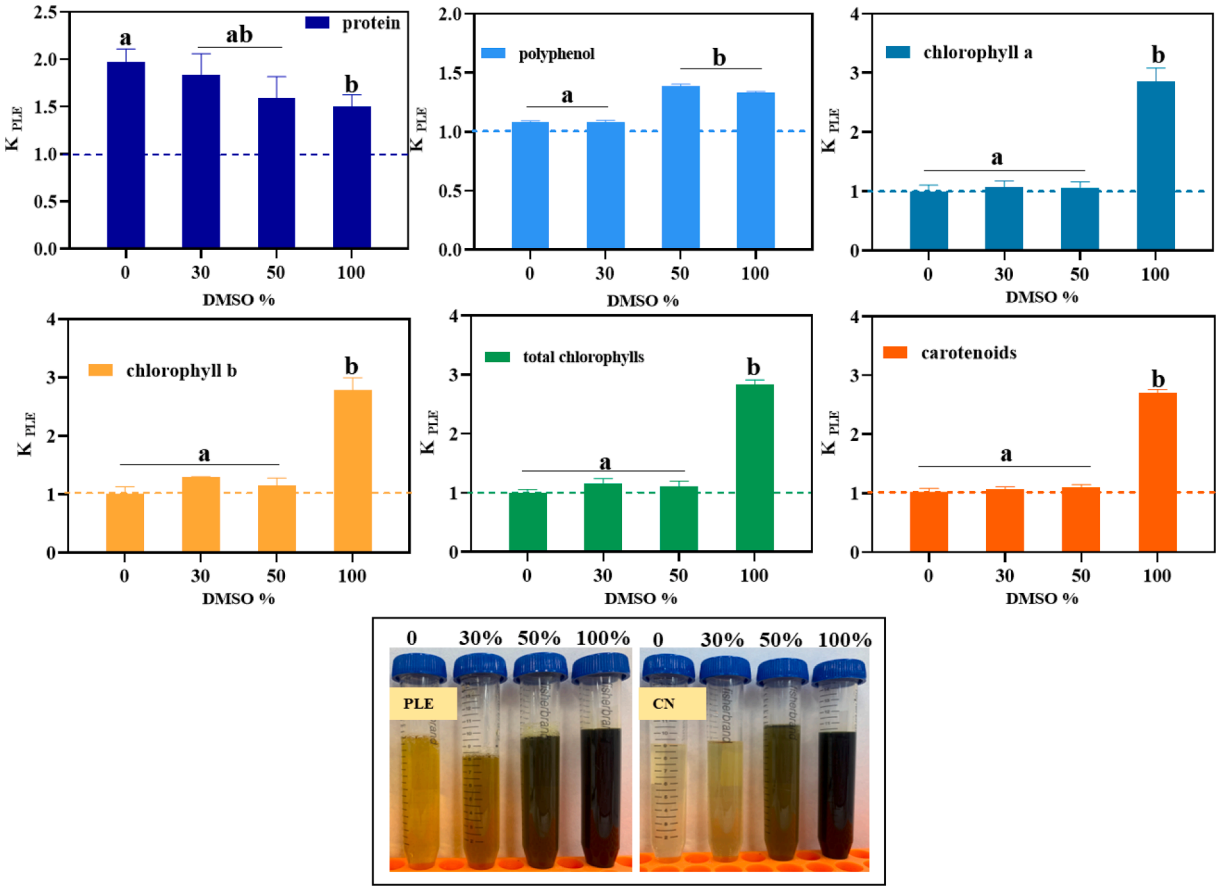


Fig. 2. Effects of dimethyl sulfoxide (DMSO) concentration on efficiency coefficient of pressurized liquid extraction (PLE) (K_{PLE}). CN, control extraction.

recovery of chlorophylls, carotenoids, and proteins from *Chlorella*. The microalgae mechanical disruption treatment process was not involved in this study, so the extraction of bioactive compounds is mainly attributed to the behavior of the solvent diffusion into the interior of microalgal cells (Nikolopoulos et al., 2008). Moreover, the pressurization process of PLE can reduce the strong interaction of the solute with the matrix caused by van der Waals forces or hydrogen bonds in the system (Cao et al., 2021), thus facilitating the extraction process, resulting in K_{PLE} values of 2 or even 3.

4.1.3. Antioxidant properties and principal component analysis (PCA)

Oxygen radical antioxidant capacity (ORAC) and Trolox equivalent antioxidant capacity (TEAC) were used to analyze the antioxidant properties of *Chlorella* extracts. The results are shown in Fig. 3**a-b**. The ORAC results (Fig. 3**a**) showed that the *in vitro* antioxidant capacity of the PLE extracts was higher than that of the control group. At DMSO concentrations lower than 30 % the main difference between PLE and control extracts was a higher content of proteins in the former (Fig. 1). Whether this higher antioxidant capacity was due to the original proteins or derivatives that may have formed requires further investigation. When the concentration of DMSO was higher than 50 %, the increase in the antioxidant capacity of the PLE extracts was correlated with the increased content of polyphenols, chlorophylls, and carotenoids. Contrastingly, the TEAC results (Fig. 3**b**) showed that the antioxidant capacity of the PLE and control extracts were very similar at concentrations of DMSO over 50 %, but higher in the PLE extracts below such concentration. The inconsistency between the ORAC and TEAC results could be attributed to the different oxidizing agents and mechanisms of both methodologies. Overall, methods for measuring antioxidant capacity could be divided into two categories: methods based on hydrogen atom transfer (HAT) or electron transfer (ET) (Huang et al., 2005). The assay principle of HAT followed that antioxidants and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds (such as ORAC), while the assay of ET measures the ability of antioxidants to reduce oxidants, such as TEAC (scavenging ABTS cationic radicals) (Barba et al., 2013). Therefore, in complex extracts containing different kinds of antioxidants (polyphenols, carotenoids, etc.), the relevance of the ORAC and TEAC methods could be reduced due to different kinetics and reaction mechanisms. To further explain the association of bioactive components with antioxidant activity, a principal component analysis (PCA) was carried out (Fig. 3**c**). The results showed that 100 %-PLE and 100 %-Control were on the same side of the first principal component (PC1), while the other extraction conditions were on the other side (PC1). Moreover, 100 %-PLE, 100 %-Control, polyphenols, carotenoids, chlorophyll *a*, chlorophyll *b*, ORAC, and TEAC were distributed on the same side of PC1, thus indicating that DMSO had a more significant effect on the yield and antioxidant activity of bioactive compounds than the technology used (PLE). ORAC and TEAC were distributed in the same quadrant (the first quadrant), thus indicating a positive correlation between these assays. More specifically, ORAC was closely distributed with carotenoids and chlorophyll *a*, while TEAC was more closely distributed with polyphenols, which may be the reason for the difference in the results observed for the ORAC and TEAC assays. Overall, the extracts with

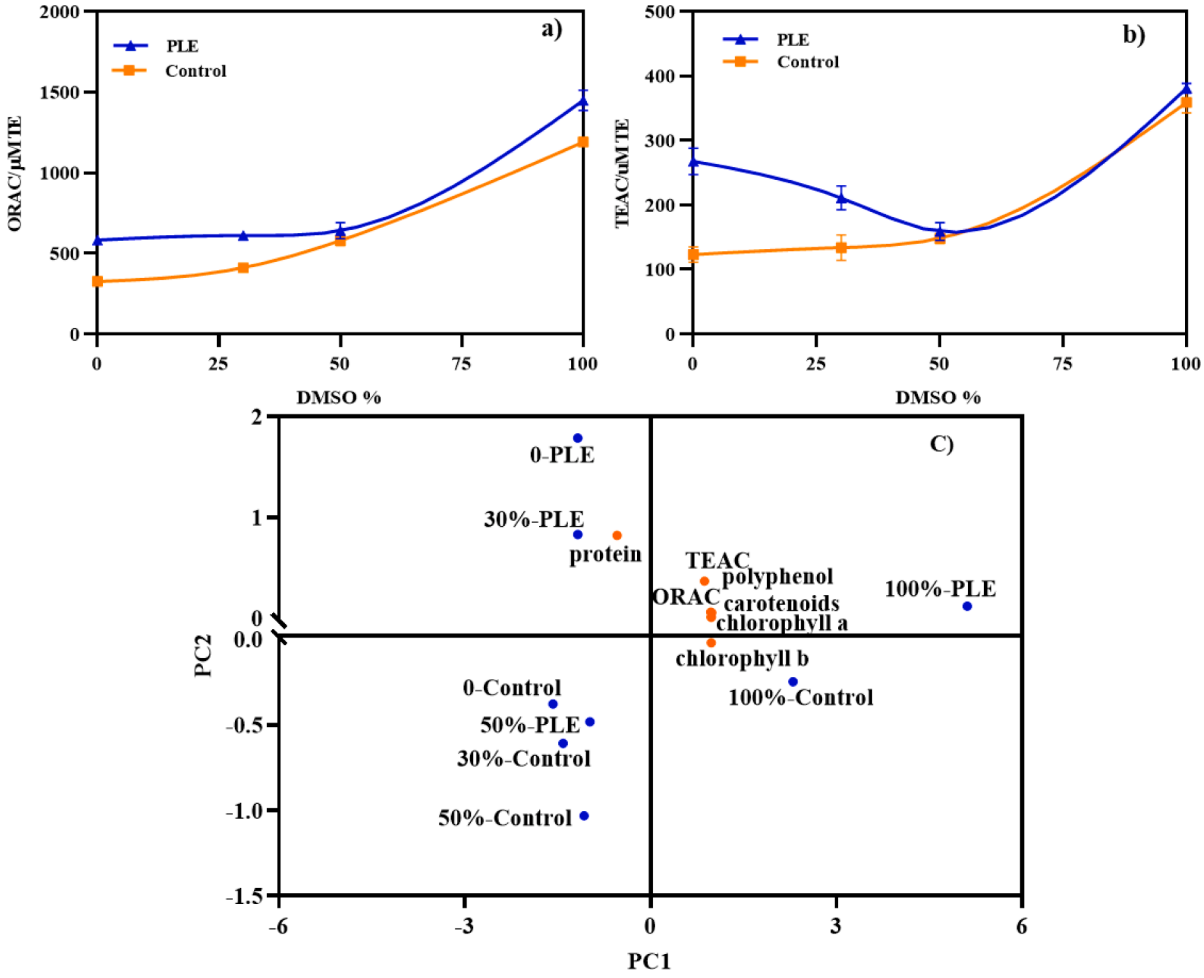


Fig. 3. Effects of pressurized liquid extraction (PLE) and dimethyl sulfoxide (DMSO) concentration on the *in vitro* antioxidant capacity of *Chlorella* extracts. Fig. 3a). Oxygen radical antioxidant capacity (ORAC), Fig. 3b). Trolox equivalent antioxidant capacity (TEAC), Fig. 3c). Principle component analysis (PCA).

higher content of polyphenols, chlorophyll, and carotenoids as well as higher antioxidant properties were recovered when 100 % DMSO was used as the extraction solvent. Therefore, 100 % DMSO can be selected as the PLE extraction solvent for the recovery of these compounds from *Spirulina* and *Phaeodactylum tricornutum*.

4.2. Comparison of extraction effect on Spirulina, Chlorella, and Phaeodactylum tricornutum

4.2.1. Yields, antioxidant properties, and efficiency coefficient

The results of the extractions with 100 % DMSO are shown in Fig. 4. Under these conditions, the highest yields for the recovery of protein (45 mg/g dw), polyphenols (12.5 mg/g dw), chlorophyll *a* (8.0 mg/g dw), chlorophyll *b* (3.0 mg/g dw), and carotenoids (1.9 mg/g dw) from *Spirulina* were found. In the case of *Phaeodactylum tricornutum*, the highest yields of protein (33 mg/g dw), polyphenols (11.5 mg/g dw), chlorophyll *a* (5.7 mg/g dw), chlorophyll *b* (1.5 mg/g dw) and carotenoids (1.6 mg/g dw) were lower. When the yields of protein and other valuable compounds of the three microalgae extracts were compared it was found that protein, polyphenols, carotenoids, and antioxidant capacity yield of the extracts obtained from *Spirulina* and *P. tricornutum* were significantly higher than those of *Chlorella* ($P < 0.05$), while the chlorophylls *of Chlorella* extracts were significantly higher than that of *P. tricornutum* ($P < 0.05$), which might be attributed not only to differences in the biosynthesis of the compounds across species but also to distinct characteristics, such as cell wall thickness, cell particle size, etc. (Zhou, Wang, Saraiva, Martins, Pinto, Prieto, Simal-Gandara, et al., 2022).

Fig. 4 shows that all K_{PLE} values exceeded the value of 1. Specifically, for *Spirulina* (S—P) the K_{PLE} values of protein, polyphenols, chlorophyll *a*, chlorophyll *b*, and carotenoids were 8.2, 3.5, 4.0, 8.0, and 5.5, respectively. In the case of *P. tricornutum* (P—P), the K_{PLE} values of protein, polyphenols, chlorophyll *a*, chlorophyll *b*, and carotenoids were 4.8, 2.0, 3.0, 1.5, and 3.7, respectively. The results indicate that PLE enables the efficient recovery of the compounds studied when 100 % DMSO was used as the solvent. A recent study compared the extraction efficiency of pigments from mixed microalgae consortium using DMSO, methanol, chloroform, and acetone. The maximum chlorophyll *a* (4.62 μg/ml), chlorophyll *b* (4.78 μg/ml) and total carotenoid (1.76 μg/ml) contents were obtained when DMSO was used as the extraction solvent for 16 h (Jain et al., 2021). Compared with the extraction time used in the study of Jain et al. (2021), the whole PLE extraction process of our study took 15 min, so it could be concluded that the combination of PLE + 100 % DMSO greatly improved the extraction efficiency of microalgae pigments. Overall, the pressurized process of PLE extraction and the superior diffusivity of DMSO synergistically improved the extraction yield of microalgae biomass, while similar studies were rarely reported and deserve further exploration.

4.2.2. Carotenoid content

Carotenoids are isoprenoids biosynthesized by all photosynthetic organisms (plants, algae, and cyanobacteria) as well as in some fungi, bacteria, and arthropods. They are important as natural colorants, precursors of vitamin A, antioxidants, and for health promotion, as evidence has accumulated that they can contribute reducing the risk of developing cancer, cardiovascular diseases, eye conditions, metabolic diseases, and other conditions (Meléndez-Martínez, 2019; Rodriguez-Concepcion et al., 2018). Being present in high amounts in microalgae, these matrices are important in the context of healthy diets and the development of innovative products, including functional foods or nutricosmetics, among others (Meléndez-Martínez et al., 2021). Previous studies have shown that *Spirulina* is an industrial-scale source of carotenoids (0.5 mg to 2 g β-carotene/kg dry matter), with β-carotene being the main carotenoid in this matrix (Santos Assunção et al., 2021).

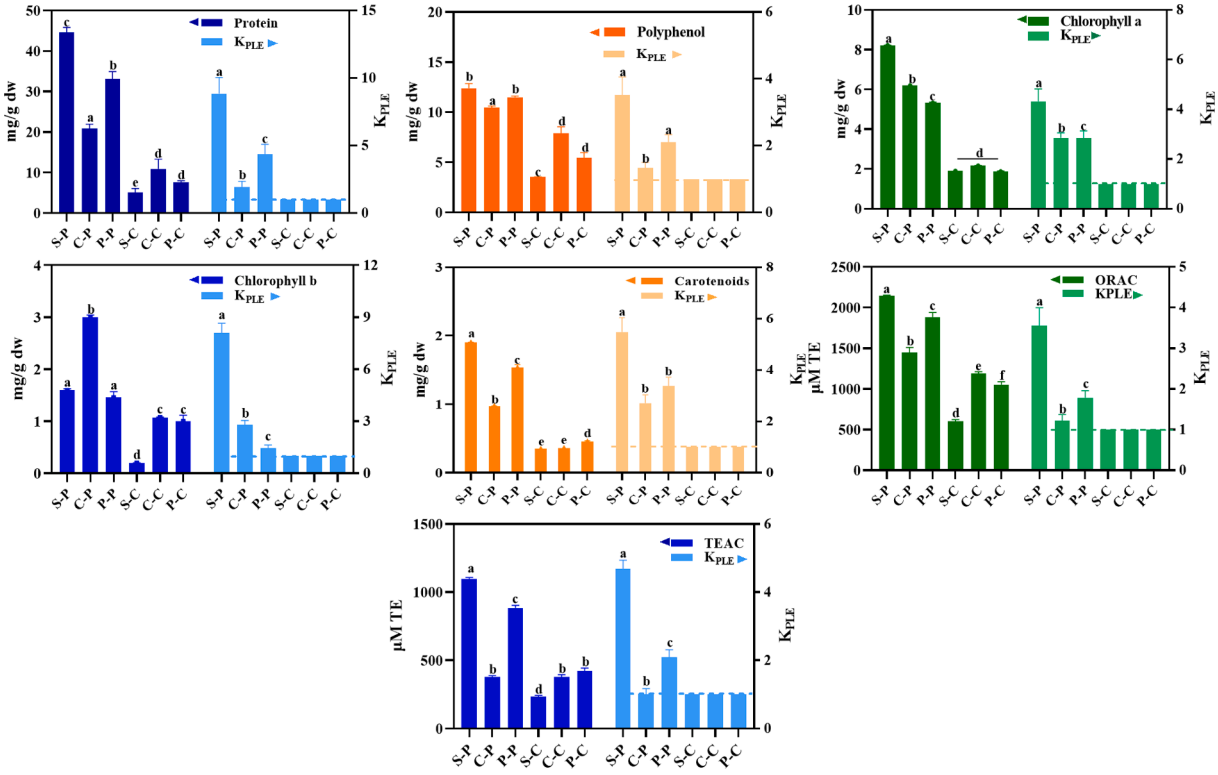


Fig. 4. Effects of pressurized liquid extraction (PLE) and dimethyl sulfoxide (DMSO) concentration on the yield, antioxidant properties, and efficient coefficient (K_{PLE}) of *Spirulina*, *Chlorella*, and *Phaeodactylum tricornutum* extracts. Different lowercase letters indicate significant differences ($p < 0.05$), same lowercase letters indicate no significant differences ($p > 0.05$). S—P, *Spirulina*-PLE; C—P, *Chlorella*-PLE; P—P, *Phaeodactylum tricornutum*-PLE; S—C, *Spirulina*-Control; C—C, *Chlorella*-Control; P—C, *Phaeodactylum tricornutum*-Control.

Similarly, *Chlorella* is also considered a good source of carotenoids, including β-carotene, α-carotene, lutein, and zeaxanthin, being a genus commonly used for large-scale production of carotenoids (Liu, Hu, et al., 2021). Other studies have shown that *Phaeodactylum tricornutum* is a good source of fucoxanthin (Gille et al., 2019).

The *Phaeodactylum* samples contained high amounts of a carotenoid with a UV/vis spectrum like that of zeaxanthin, although it eluted at a different time. That carotenoid was identified as diatoxanthin on a chemosystematics basis, as this is a general major carotenoid in diatoms. Myxoxanthophyll, which was found in *Spirulina*, was tentatively identified likewise as it is a common carotenoid in cyanobacteria (Liaaen-Jensen, 1998). The total carotenoid content of *Spirulina* was considerably higher relative to *P. tricornutum* and *C. vulgaris* (~1.5- and 15-fold, respectively) (Fig. 5). Fucoxanthin, all-*trans*-β-carotene, diatoxanthin, lutein, and (9*Z*)-β-carotene, in decreasing order of abundance, were the predominant in *P. tricornutum*. β-Carotene, zeaxanthin, (9*Z*)-β-carotene, and myxoxanthophyll, in decreasing order of abundance, were the main carotenoids in *S. maxima*. Lutein, α-carotene, β-carotene, and (9*Z*)-β-carotene, in decreasing order of abundance, were the predominant carotenoids in *C. vulgaris*. The lowest total carotenoid levels were found in the biomass of these species.

Ultrasound, microwave, and supercritical extraction have been the most studied techniques for extracting carotenoids from plant tissues and microalgae (Elik et al., 2020). In this study, different types of carotenoids were obtained from microalgae using PLE extraction with DMSO as a solvent, which provided a new idea for the recovery of carotenoids from microalgae. Overall, the present results showed that microalgae extracts were rich in carotenoids, and studies have shown that the carotenoid release could be limited by the cell wall when the complete microalgae were used as a diet, resulting in low carotenoid bioavailability (Fernandes et al., 2021).

4.2.3. Minerals and nutrient reference value (NRV)

The application of innovative and efficient extraction technologies to obtain macromolecular substances such as lipids, polysaccharides, and proteins from microalgae has been widely studied (Zhou, Wang, Saraiva, Martins, Pinto, Prieto, Simal-gandara, et al., 2022). However, the information regarding the efficient recovery of mineral elements from microalgae is more limited. Some previous studies have shown that the mineral content of *Spirulina* mainly consists of Mg (383.5 mg/100 g dw), P (752.5 mg/100 g dw), Ca (798 mg/100 g dw), Fe (96.8 mg/100 g dw), Zn (2.73 mg/100 g dw), and Se (0.11 mg/100 g dw), while the main minerals found in *Chlorella* are Mg (344.3 mg/100 g dw), P (1761.5 mg/100 g dw), Ca (593.7 mg/100 g dw), Fe (259.1 mg/100 g), Zn (1.19 mg/100 g), and Se (0.07 mg/100 g) (Tokuşoglu & Ünal, 2003), and the mineral content of *Phaeodactylum tricornutum* is mainly Mg (555 mg/100 g dw), P (269 mg/100 g dw), Ca (1910 mg/100 g dw), and Zn (373 mg/100 g dw) (Rebolloso-Fuentes et al., 2000). At this stage of development, there is a need for efficient tools to recover these minerals, therefore, in this study, the potential of the PLE process to recover Mg, Ca, P, Fe, Zn, and Se was evaluated (Table 1). When the total mineral content of the different microalgae was compared, it was observed that the Zn content was relatively higher in *Spirulina* (44 ± 1 mg/kg dw), being the extraction rate close to 100 %. On the other hand, the P content was relatively higher in *Chlorella* (10647 ± 248 mg/kg dw), being the extraction rate of ~ 14 %. Moreover, the contents of Mg, Ca, and Fe were relatively higher in *Phaeodactylum tricornutum*, with values of 6206 ± 99 mg/kg dw, 27333 ± 2401 mg/kg dw, and 2194 ± 6 mg/kg dw, respectively, which corresponded to extraction rates of 12.4 %, 0.6 %, and 4.8 %, respectively.

The minerals evaluated in this study (Mg, Ca, P, Fe, and Zn) are of great significance to human health. Specifically, Mg is an indispensable mineral in the human diet for the processing of ATP (adenosine triphosphate) and for the bones, P is an important component of bones and cells, and Ca is the most abundant mineral in the body and is essential for muscles, bones, teeth, the health of the heart and the digestive system, and the synthesis and function of blood. Fe plays an important role in DNA synthesis and repair, ATP production, and oxygen transport and Zn is a key component of many protein functions and also an essential micronutrient required for many cellular processes and the development of the immune system. Moreover, the lack of Fe and Zn in the human body can trigger related diseases (Eggleston et al., 2022; Ho et al., 2022). Therefore, it is meaningful to discuss the mineral contents found in microalgae extracts to meet the needs of human health. Based on this, we further calculated the Nutrient Relative Values (NRV, %) of minerals in the extract (from 100 g of microalgae dw) taking into account the Recommended Dietary Allowances (RDAs) (Table 1). From the NRV results in Table 1, *Spirulina* and *Chlorella* extracts can be considered as suitable sources of Zn and P, respectively, while *Phaeodactylum tricornutum* is an interesting source of Mg and Fe. More specifically, *P. tricornutum* extract could satisfy 100, 96.5, 59.4, and 18.4–32.2 % of the Mg requirements for breastfeeding (19–30 years), babies (6–12 months), children (1–3 years), and other populations (>4 years), respectively. *Chlorella* extract can cover 54.7, 32.7, 30.1, and 12.0–21.5 % of the P requirements for breastfeeding (19–30 years), babies (6–12 months), children (1–3 years), and other populations (>4 years old), respectively. Compared with other minerals, microalgae extracts can only provide a limited amount of calcium for the human body, i.e., <7 %.

Phaeodactylum tricornutum and *Spirulina* can meet the Fe requirements of the human body to a large extent, among which *Phaeodactylum tricornutum* extract could meet the total Fe requirements of babies (6–12 months), children (1–8 years), males (>14 years), and females (31–70 years), whereas it could cover above 58.9 % of that of other populations (except for pregnant (19–30 years), meeting 3.4 % of its Fe requirement). For Zn, *Spirulina* extract could satisfy 146.7, 146.7,

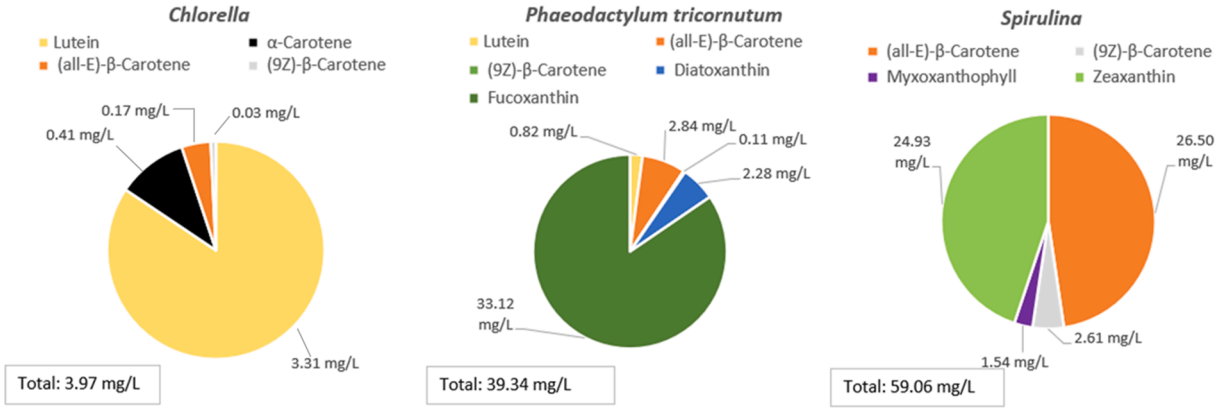


Fig. 5. Carotenoids profile determination in PLE-100% DMSO extracts of *Spirulina*, *Chlorella,* and *Phaeodactylum tricornutum*.

Table 1
Mg, P, Ca, Fe, Zn, Se (Nd) yield and Nutrient Relative Value (NRV) analysis.

Mineral	Mg			P			Ca			Fe			Zn		
Algae	S-	C-	P-	S-	C-	P-	S-	C-	P-	S-	C-	P-	S-	C-	P-
Total (mg/kg dw)	3239 ± 89	3605 ± 55	6206 ± 99	9312 ± 868	10647 ± 248	6753 ± 158	790 ± 6	1574 ± 20	27333 ± 2401	290 ± 13	674 ± 5	2194 ± 6	44 ± 1	22 ± 1	27 ± 2
Extracts (mg/kg dw)	595 ± 2	324 ± 6	772 ± 8	1120 ± 72	1504 ± 28	309 ± 8	109 ± 4	36 ± 2	169 ± 5	63 ± 1	20 ± 1	106 ± 1	44 ± 1	8 ± 1	18 ± 1

NRV: contribution of minerals in extracts (extract from 100 g microalgae dry matter) towards DRI

	Mg			P			Ca			Fe			Zn		
Life stages	S-	C-	P-	S-	C-	P-	S-	C-	P-	S-	C-	P-	S-	C-	P-
Baby (6 ~ 12 Months)	74.4	40.5	96.5	24.3	32.7	6.7	1.6	0.5	2.4	90.0	28.6	151.4	146.7	26.7	60.0
Children (1 ~ 3 Years)	45.8	24.9	59.4	22.4	30.1	6.2	1.1	0.4	1.7	63.0	20.0	106.0	88.0	16.0	36.0
Children (4 ~ 8 Years)	24.8	13.5	32.2	9.0	12.0	2.5	0.8	0.3	1.3	78.8	25.0	132.5	55.0	10.0	22.5
Males (9 ~ 13 Years)	14.5	7.9	18.8	9.0	12.0	2.5	0.8	0.3	1.3	57.3	18.2	96.4	40.0	7.3	16.4
Males (14 ~ 18 Years)	14.9	8.1	19.3	16.0	21.5	4.4	1.1	0.4	1.7	78.8	25.0	132.5	40.0	7.3	16.4
Males (19 ~ 30 Years)	14.2	7.7	18.4	16.0	21.5	4.4	1.1	0.4	1.7	78.8	25.0	132.5	40.0	7.3	16.4
Males (31 ~ 50 Years)	14.2	7.7	18.4	16.0	21.5	4.4	1.1	0.4	1.7	78.8	25.0	132.5	40.0	7.3	16.4
Males (51 ~ 70 Years)	14.2	7.7	18.4	16.0	21.5	4.4	0.9	0.3	1.4	78.8	25.0	132.5	40.0	7.3	16.4
Males (>70 Years)	24.8	13.5	32.2	9.0	12.0	2.5	0.8	0.3	1.3	78.8	25.0	132.5	55.0	10.0	22.5
Females (9 ~ 13 Years)	16.5	9.0	21.4	9.0	12.0	2.5	0.8	0.3	1.3	42.0	13.3	70.7	48.9	8.9	20.0
Females (14 ~ 18 Years)	19.2	10.5	24.9	16.0	21.5	4.4	1.1	0.4	1.7	35.0	11.1	58.9	55.0	10.0	22.5
Females (19 ~ 30 Years)	18.6	10.1	24.1	16.0	21.5	4.4	1.1	0.4	1.7	35.0	11.1	58.9	55.0	10.0	22.5
Females (31 ~ 50 Years)	18.6	10.1	24.1	16.0	21.5	4.4	0.9	0.3	1.4	78.8	25.0	132.5	55.0	10.0	22.5
Females (51 ~ 70 Years)	18.6	10.1	24.1	16.0	21.5	4.4	0.9	0.3	1.4	78.8	25.0	132.5	55.0	10.0	22.5
Females (>70 Years)	17.0	9.3	22.1	16.0	21.5	4.4	1.1	0.4	1.7	23.3	7.4	39.3	40.0	7.3	16.4
Pregnant (19 ~ 30 Years)	19.2	10.5	24.9	16.0	21.5	4.4	1.1	0.4	1.7	2.0	0.6	3.4	36.7	6.7	15.0
Breastfeed (19 ~ 30 Years)	79.3	43.2	102.9	40.7	54.7	11.2	4.2	1.4	6.5	57.3	18.2	96.4	146.7	26.7	60.0

Note. The Nutrient Relative Value (NRV)– contribution of minerals in extracts (extract from 100 g microalgae dry matter) towards DRI was calculated as: NRV = X/R 100 %, Where X and R corresponded to the mineral content in microalgae extracts (form 100 g microalgae dry matter) and Recommended Dietary Allowances (RDAs) respectively. S-, C-, P- corresponds to *Spirulina*, *Chlorella* and *Phaeodactylum tricornutum* respectively, Nd-not detected.

88.0, and 36.7–55 % of this mineral requirement for breastfeeding (19–30 years), babies (6–12 months), children (1–3 years), and other populations (>4 years), respectively.

From the NRV results, in addition to Ca, microalgae extract can meet the body's mineral requirements to a large extent. It should be noted that in this study, we selected DMSO as the extraction solvent based on the yield, so the extract cannot be directly ingested. Nowadays, DMSO can be completely removed by methods such as oil pump vacuum distillation (60 °C) in the laboratory, which provides the possibility for the further preparation of safe microalgae mineral supplements.

5. Conclusions

The total carotenoid content of *Spirulina* biomass was considerably higher relative to *P. tricornutum* and *C. vulgaris* (~1.5- and 15-fold, respectively). Fucoxanthin, all-*trans*-β-carotene, diatoxanthin, lutein, and (9*Z*)-β-carotene were the major carotenoids in *P. tricornutum*. β-Carotene, zeaxanthin, (9*Z*)-β-carotene, and myxoxanthophyll in *S. maxima* and lutein, α-carotene, β-carotene, and (9*Z*)-β-carotene in *C. vulgaris*. PLE + H_2O can be considered as a useful strategy to recover proteins from microalgae, while PLE + DMSO is an interesting tool to recover lipophilic pigments, observing an increase when the DMSO concentration was augmented, especially over 30 %. The NRV value based on the mineral content and DRIs indicated that the PLE microalgae extracts could be used after drying as Mg, P, Ca, Fe, and Zn supplements for different populations. Similar studies have rarely been reported so far.

Funding

This research was funded by the University of Valencia through the projects OTR2021-21736INVES and OTR2021-21570INVES supported by the University of Vigo. Moreover, it was partially funded by the EU Commission and BBI-JU Horizon H2020, through the AQUABIOPRO-FIT

project (aquaculture and agriculture biomass side stream proteins and bioactive for feed, fitness, and health-promoting nutritional supplements) Grant No 790956. JZ and MW were supported by a Ph.D. fellowship from the China Scholarship Council (CSC) (No. 201908420246 and No. 201908420245, respectively). PMB was supported by a posdoc fellowship from Consejería de Transformación Económica, Industria, Conocimiento y Universidades de la Junta de Andalucía (PAIDI 2020). AJMM and AMO acknowledge grant PID2019-110438RB-C21 (NEWCARFOODS), funded by MCIN/AEI/https://doi.org/10.13039/501100011033.

CRediT authorship contribution statement

Min Wang: Investigation, Formal analysis, Visualization, Writing – original draft. **Ángeles Morón-Ortiz:** Investigation, Formal analysis, Visualization, Writing – original draft. **Jianjun Zhou:** Investigation, Formal analysis, Visualization, Writing – original draft. **Ana Benítez-González:** Conceptualization, Methodology, Formal analysis, Supervision, Writing – review & editing. **Paula Mapelli-Brahm:** Conceptualization, Methodology, Formal analysis, Supervision, Writing – review & editing. **Antonio J. Meléndez-Martínez:** Conceptualization, Methodology, Resources, Visualization, Supervision, Writing – review & editing. **Francisco J. Barba:** Conceptualization, Methodology, Resources, Visualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Upon the authors' agreement data can be made available on request

Acknowledgments

The authors would like to acknowledge Dr. María Carmen Collado (IATA-CSIC) and her team for the help provided as well as Dr. Dámaso Hornero-Méndez (IG-CSIC) for his support with the identification of carotenoids. Moreover, the authors are thankful to Nicolas Mazurier (Ecospirulina company) for providing the Spirulina and Chlorella samples to carry out the experiments as well as Katerina Kousoulaki, Marielena Kokkali, Dorinde Kleingris and her team at the National Algae pilot plant in Mongstad (NAM) in Norway for cultivating and providing the *P. tricornutum* biomass samples to carry out the experiments. Finally, the authors also acknowledge the Research Council of Norway, which provided funding through the A2F (Algae 2 Future) project (Project No. 267872/E50) for the production of the microalgae biomass used in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134885.

References

Al Khawli, F., Martí-Quijal, F. J., Pallarés, N., Barba, F. J., & Ferrer, E. (2021). Ultrasound extraction mediated recovery of nutrients and antioxidant bioactive compounds from *Phaeodactylum tricornutum* microalgae. *Applied Sciences, 11*(4), 1–19. https://doi.org/10.3390/app11041701

Barba, F. J., Esteve, M. J., Tedeschi, P., Brandolini, V., & Frígola, A. (2013). A comparative study of the analysis of antioxidant activities of liquid foods employing spectrophotometric, fluorometric, and chemiluminescent methods. *Food Analytical Methods, 6*(1), 317–327.

Cao, Y., Liu, W., Gong, X., Yu, J., Tu, P., Li, J., & Song, Y. (2021). Online pressurized liquid extraction enables directly chemical analysis of herbal medicines: A mini review. *Journal of Pharmaceutical and Biomedical Analysis, 205*, Article 114332. https://doi.org/10.1016/j.jpba.2021.114332

de la Fuente, B., López-García, G., Máñez, V., Alegría, A. C. A., & Barberá, R. (2019). Evaluation of the bioaccessibility of antioxidant bioactive compounds and minerals of four genotypes of brassicaceae microgreens. *Foods, 8*(7), 250. https://doi.org/10.3390/foods8070250

Eggleston, G., Triplett, A., Bett-Garber, K., Boue, S., & Bechtel, P. (2022). Macronutrient and mineral contents in sweet sorghum syrups compared to other commercial syrup sweeteners. *Journal of Agriculture and Food Research, 7*, Article 100276. https://doi.org/10.1016/j.jafr.2022.100276

Elik, A., Yanık, D. K., & Göğüş, F. (2020). Microwave-assisted extraction of carotenoids from carrot juice processing waste using flaxseed oil as a solvent. *LWT, 123*. https://doi.org/10.1016/j.lwt.2020.109100

Fernandes, A. S., Nascimento, T. C., Pinheiro, P. N., Vendruscolo, R. G., Wagner, R., de Rosso, V. V., … Zepka, L. Q. (2021). Bioaccessibility of microalgae-based carotenoids and their association with the lipid matrix. *Food Research International, 148*, Article 110596. https://doi.org/10.1016/J.FOODRES.2021.110596

Gille, A., Hollenbach, R., Trautmann, A., Posten, C., & Briviba, K. (2019). Effect of sonication on bioaccessibility and cellular uptake of carotenoids from preparations of photoautotrophic *Phaeodactylum tricornutum*. *Food Research International, 118*, 40–48. https://doi.org/10.1016/j.foodres.2017.12.040

Ho, E., Wong, C. P., & King, J. C. (2022). Impact of zinc on DNA integrity and age-related inflammation. *Free Radical Biology and Medicine, 178*, 391–397. https://doi.org/10.1016/j.freeradbiomed.2021.12.256

Hoff, R. B., & Pizzolato, T. M. (2018). Combining extraction and purification steps in sample preparation for environmental matrices: A review of matrix solid phase dispersion (MSPD) and pressurized liquid extraction (PLE) applications. *TrAC - Trends in Analytical Chemistry, 109*, 83–96. https://doi.org/10.1016/j.trac.2018.10.002

Huang, D. jian, Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, *53*(6), 1841–1856. 10.1021/jf030723c.

Jain, A., Behera, B., & Paramasivan, B. (2021). Evaluation of physicochemical procedures for pigment extraction from mixed microalgal consortium. *Bioresource Technology Reports, 15*, Article 100775. https://doi.org/10.1016/j.biteb.2021.100775

Jalali, M., & Fakhri, R. (2021). Evaluation of macro and trace elements content of wild edible Iranian plants and their contribution to dietary reference intakes. *Journal of Food Composition and Analysis, 102*, Article 104049. https://doi.org/10.1016/j.jfca.2021.104049

Laamanen, C. A., Desjardins, S. M., Senhorinho, G. N. A., & Scott, J. A. (2021). Harvesting microalgae for health beneficial dietary supplements. *Algal Research, 54*, Article 102189. https://doi.org/10.1016/j.algal.2021.102189

Liaaen-Jensen, S. (1998). Carotenoids in chemosystematics. In G. Britton, S. Liaaen-Jensen, & H. Pfander (Eds.), *Carotenoids volume 3: Biosynthesis and metabolism* (pp. 217–248). Birkhauser.

Liu, C., Hu, B., Cheng, Y., Guo, Y., Yao, W., & Qian, H. (2021). Carotenoids from fungi and microalgae: A review on their recent production, extraction, and developments. *Bioresource Technology, 337*(April), Article 125398. https://doi.org/10.1016/j.biortech.2021.125398

Liu, C., Zhao, D., Zhang, H., Wang, Y., Bai, H., & Liu, Q. (2021). Cumene extraction separation from alkanes using DMSO: Influence of the alkane structure. *Fluid Phase Equilibria, 550*, Article 113236. https://doi.org/10.1016/j.fluid.2021.113236

Markou, G., & Nerantzis, E. (2013). Microalgae for high-value compounds and biofuels production: A review with focus on cultivation under stress conditions. *Biotechnology Advances, 31*(8), 1532–1542. https://doi.org/10.1016/j.biotechadv.2013.07.011

Meléndez-Martínez, A. J., Böhm, V., Borge, G. I., Cano, M. P., Fikselová, M., Gruskiene, R., … O'Brien, N. (2021). Carotenoids: Considerations for their use in functional foods, nutraceuticals, nutricosmetics, supplements, botanicals and novel foods in the context of sustainability, circular economy and climate change. *Annual Review of Food Science and Technology, 12*, 433–460.

Meléndez-Martínez, A. J. (2019). An overview of carotenoids, apocarotenoids and vitamin a in agro-food, nutrition, health and disease. *Molecular Nutrition & Food Research, 1801045*. https://doi.org/10.1002/mnfr.201801045

Moret, S., Scolaro, M., Barp, L., Purcaro, G., Sander, M., & Conte, L. S. (2014). Optimisation of pressurised liquid extraction (PLE) for rapid and efficient extraction of superficial and total mineral oil contamination from dry foods. *Food Chemistry, 157*, 470–475. https://doi.org/10.1016/j.foodchem.2014.02.071

Nikolopoulos, D., Korgiopoulou, C., Mavropoulos, K., Liakopoulos, G., & Karabourniotis, G. (2008). Leaf anatomy affects the extraction of photosynthetic pigments by DMSO. *Talanta, 76*(5), 1265–1268. https://doi.org/10.1016/j.talanta.2008.05.037

Parniakov, O., Apicella, E., Koubaa, M., Barba, F. J., Grimi, N., Lebovka, N., … Vorobiev, E. (2015). Ultrasound-assisted green solvent extraction of high-added value compounds from microalgae Nannochloropsis spp. *Bioresource Technology, 198*, 262–267. https://doi.org/10.1016/j.biortech.2015.09.020

Parniakov, O., Barba, F. J., Grimi, N., Marchal, L., Jubeau, S., Lebovka, N., & Vorobiev, E. (2015). Pulsed electric field assisted extraction of nutritionally valuable compounds from microalgae Nannochloropsis spp. using the binary mixture of organic solvents and water. *Innovative Food Science and Emerging Technologies, 27*, 79–85. https://doi.org/10.1016/j.ifset.2014.11.002

Rebolloso-Fuentes, M. M., Navarro-Pérez, A., Ramos-Miras, J. J., & Guil-Guerrero, J. L. (2000). Biomass Nutrient Profiles of the Microalga *Phaeodactylum Tricornutum*. *Journal of Food Biochemistry, 25*(1), 57–76.

Rodriguez-Concepcion, M., Avalos, J., Bonet, M. L., Boronat, A., Gomez-Gomez, L., Hornero-Mendez, D., … Zhu, C. (2018). A global perspective on carotenoids:

Metabolism, biotechnology, and benefits for nutrition and health. *Progress in Lipid Research, 70*. https://doi.org/10.1016/j.plipres.2018.04.004

Santos Assunção, L., Quênia Muniz Bezerra, P., Stahl Hermes Poletto, V., de Oliveira Rios, A., Graça Ramos, I., Duarte Ferreira Ribeiro, C., Aparecida Souza Machado, B., Izabel Druzian, J., Alberto Vieira Costa, J., & Larroza Nunes, I. (2021). Combination of carotenoids from Spirulina and PLA/PLGA or PHB: New options to obtain bioactive nanoparticles. *Food Chemistry*, *346*. 10.1016/j.foodchem.2020.128742.

Selvakumar, P., Karthik, V., Kumar, P. S., Asaithambi, P., Kavitha, S., & Sivashanmugam, P. (2021). Enhancement of ultrasound assisted aqueous extraction of polyphenols from waste fruit peel using dimethyl sulfoxide as surfactant: Assessment of kinetic models. *Chemosphere, 263*, Article 128071. https://doi.org/10.1016/j.chemosphere.2020.128071

Stinco, C. M., Benítez-González, A. M., Meléndez-Martínez, A. J., Hernanz, D., & Vicario, I. M. (2019). Simultaneous determination of dietary isoprenoids (carotenoids, chlorophylls and tocopherols) in human faeces by Rapid Resolution Liquid Chromatography. *Journal of Chromatography A, 1583*. https://doi.org/10.1016/j.chroma.2018.11.010

Stinco, C. M., Fernández-Vázquez, R., Escudero-Gilete, M. L., Heredia, F. J., Meléndez-Martínez, A. J., & Vicario, I. M. (2012). Effect of orange juices processing on the color, particle size, and bioaccessibility of carotenoids. *Journal of Agricultural and Food Chemistry, 60*(6). https://doi.org/10.1021/jf2043949

Tokuşoglu, Ö., & Ünal, M. K. (2003). Biomass nutrient profiles of three microalgae: *Spirulina platensis*, *Chlorella vulgaris*, and *Isochrisis galbana*. *Journal of Food Science, 68*(4), 1144–1148. https://doi.org/10.1111/j.1365-2621.2003.tb09615.x

Vaz, B. da S., Moreira, J. B., Morais, M. G. de, & Costa, J. A. V. (2016). Microalgae as a new source of bioactive compounds in food supplements. *Current Opinion in Food Science*, *7*, 73–77. 10.1016/j.cofs.2015.12.006.

Zhang, Y., Soldatov, S., Papachristou, I., Nazarova, N., Link, G., Frey, W., & Silve, A. (2022). Pulsed microwave pretreatment of fresh microalgae for enhanced lipid extraction. *Energy, 248*, Article 123555. https://doi.org/10.1016/j.energy.2022.123555

Zhou, J., Wang, M., Carrillo, C., Zhu, Z., Brncic, M., Berrada, H., & Barba, F. J. (2021). Impact of pressurized liquid extraction and pH on protein yield, changes in molecular size distribution and antioxidant compounds recovery from Spirulina. *Foods, 10*(9), 2153. https://doi.org/10.3390/foods10092153

Zhou, J., Wang, M., Berrada, H., Zhu, Z., Grimi, N., & Barba, F. J. (2022). Pulsed electric fields (PEF), pressurized liquid extraction (PLE) and combined PEF + PLE process evaluation: Effects on Spirulina microstructure, biomolecules recovery and Triple TOF-LC-MS-MS polyphenol composition. *Innovative Food Science and Emerging Technologies, 77*. https://doi.org/10.1016/j.ifset.2022.102989

Zhou, J., Wang, M., Saraiva, J. A., Martins, A. P., Pinto, C. A., Prieto, M. A., … Barba, F. J. (2022). Extraction of lipids from microalgae using classical and innovative approaches. *Food Chemistry, 384*, Article 132236. https://doi.org/10.1016/j.foodchem.2022.132236