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Role of infrared-assisted extraction of antioxidant phytochemicals in enhancing antimicrobial activity of *Phaeodactylum tricornutum*

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ABSTRACT

Microalgae are a sustainable source of highly bioactive and nutritious biomolecules such as polyphenols, and carotenoids. The efficiency of a novel infrared (IR) technology in terms of optimization of phytochemical (phenolic compounds, chlorophylls and carotenoids) recovery from *P. tricornutum* was studied using Response Surface Methodology (RSM). Optimized antioxidant activity (870 ± 22 μg TE/mL) and phenolic compound concentration (562 ± 7 mg GAE/100 g DM) were obtained using IR at 33 °C for 12 min at pH 7. In addition, chlorophyll A (26.54 ± 2.78 mg/100 g DM) and carotenoids (9.61 ± 0.40 mg/100 g DM) were recovered at the simultaneous optimal IR conditions: 56 °C, 36 min, pH 9.5. Compared to conventional aqueous extraction, IR enhanced by 10.06% the recovery of phenolic compounds and ameliorated their biological activities by 36.24% for DPPH, 17.24% for CUPRAC, 14.50% for TEAC, and 4.60% for FRAP. Finally, IR bioactive extracts also exhibited antibacterial effects against *S. aureus*. In conclusion, the richness of *P. tricornutum* in phytochemicals makes it a valuable alternative food source. IR technology proved to be an efficient method for the recovery of high-added value compounds from microalgae. Antioxidant and pigment-rich microalgae extracts can be used as dietary supplements.

1. Introduction

In recent decades, research into alternative food sources such as microalgae has attracted growing interest. This is mainly due to the need of highly productive and cost-effective products to solve the nutritional and environmental issues caused by growing populations and industrial development. Furthermore, human-induced climate change has led to problems such as drought that affect the production of traditional crops (Farooq et al., 2012; Rafi et al., 2022). Microalgae are a sustainable substitute with a high nutritional value thanks to their content of proteins, vitamins and phytochemicals such as polyphenols and carotenoids (Gong & Bassi, 2016). In addition, they are distinguished for their concentration in polyunsaturated fatty acids (PUFA), including eicosapentaenoic acid (EPA) with strong antioxidant effects (Gohara-Beirigo et al., 2022). Microalgae are also beneficial for animal health since their addition in feed increases the quality of the final product in an environmentally sustainable way (de Medeiros et al., 2021). They act as effective CO_2 sinks with minimal water requirements for their development and are used to remove heavy metals from marine waters and industrial wastes (Al Khawli et al., 2021a; Hepburn et al., 2019).

Phaeodactylum tricornutum is a marine unicellular diatom that is considered a source of PUFA, EPA, chlorophylls, phenolic compounds, and carotenoids such as fucoxanthin, a pigment of marine origin that shows anti-inflammatory, antioxidant and anticarcinogenic effects (Fajardo et al., 2007; Neumann et al., 2019). However, both growth and nutritional composition depend not only on the selected microalgae species but also on factors such as temperature, salinity, nutrient availability, and pH (Kumar et al., 2020). For this reason, it is of interest

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to study the microalgae *P. tricornutum* as a source of bioactive compounds that can be used in dietary supplements to reduce the increasing prevalence of concomitant diseases such as inflammation and oxidative stress, which in turn contribute to the development of cancer (Fajardo et al., 2007). In fact, the study published by Kang et al. (Kang et al., 2013) showed that the ingestion of *P. tricornutum* extract in mice fed high-fat diet resulted in a reduction in body weight and adipose tissue, as well as a decrease in serum triglycerides, insulin, and leptin levels.

To recover the bioactive compounds from *P. tricornutum*, several extraction methods can be used, including conventional and alternative methods. Conventional extraction has several disadvantages such as long extraction times, high temperature leading to degradation or coagulation of components and the use of large amounts of organic solvents (Roselló-Soto et al., 2016). For this reason, the use of alternative technologies such as pulsed electric fields, supercritical fluids and infrared-assisted extraction is currently gaining importance (Qu et al., 2016).

Infrared-assisted extraction (IAE) is an innovative technique that is increasingly applied to the extraction of components from plant tissue for its numerous advantages: shorter extraction times, higher extraction yields, lower temperatures, cost-effectiveness and ease of use (Chen et al., 2010). This technology is based on the emission of radiation with a wavelength ranging between 0.78 and 1000 μm to excite the atoms and molecules of the target sample. The vibration of the atoms causes a rise in temperature that leads to the evaporation of the liquid cell content and the disruption of the tissues, releasing the compounds into the external medium (Xiang et al., 2022). This technology has recently been applied to extract flavonoids and polyphenols from different plant materials such as orange peels (El Kantar et al., 2020) and *Eryngium creticum* (Hammoud et al., 2022).

Considering the variability in the extract composition caused by the extraction conditions, the main aim of this study was the optimization of an infrared-assisted extraction method of total phenolic compounds, chlorophylls, and carotenoids, from the microalgae *P. tricornutum* using the Response Surface Methodology (RSM). The concentration of the compounds recovered under optimal conditions, their antioxidant and antibacterial activities will also be evaluated as compared to conventional water-based extraction.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals used in the experiments were of analytical grade. Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), were purchased from Sigma-Aldrich (Darmstadt, Germany). The kits used for the different antioxidant capacity assays (FRAP, ABTS, CUPRAC) were purchased from Bioquochem (Asturias, Spain).

2.2. Sample preparation

P. tricornutum cultures were produced in four 800 L GemTube (LGEM, Rotterdam, The Netherlands) photobioreactors at the National Algae Pilot Plant (Mongstad, NAM, Norway) in a greenhouse with natural light and supplemental artificial lighting (EAX 170 W LED, Evolys AS, Oslo, Norway). The photobioreactors operated at a pH of 7.8, CO_2 addition as needed, and culture temperatures of 15–35 °C. Microalgae were grown in a modified medium based on natural seawater (Fensfjorden, Mongstad, salinity of 31 ppt) and enriched with Guillard's (F/2) Marine Water Enrichment Solution (Sigma-Aldrich, Steinheim, Baden-Wuerttemberg, Germany). After harvesting, the biomass was dewatered using a spiral plate centrifuge (Evodos 25, Evodos b. v., Raamsdonksveer, The Netherlands). The result was a cake of 20% dry weight that was vacuum-packed and stored at −20 °C for further analyses.

2.3. Extraction procedure and experimental design

The *Ired-Irrad*® extraction prototype consists of a ceramic infrared emitter connected to a proportional-integral-derivative (PID) control system. For each extraction, a 1:30 ratio of *P. tricornutum* to water-based solvent (*w:v*) was added to a round bottom flask and irradiated at a distance of 1 cm from the ceramic IR emitter. Resulting extracts were filtered using folded qualitative filter paper with 8–12 μm particle retention size, centrifuged (10 min, 4500 rpm), and used for chemical analyses.

Regarding the experimental conditions, Response Surface Methodology (RSM) was adopted as a statistical/mathematical tool to optimize the extraction process. In addition, it allows quantifying of both the linear and quadratic effects of the operating parameters as well as the interactions that may exist between them. In our case, the study was conducted to determine how the variables: time, pH and temperature affect the biological activities of the *P. tricornutum* extracts as obtained by the IR technology. These three independent variables were coded at five levels ($-\alpha$, -1, 0, $+1$, $+\alpha$). Time ranged between 3.2 and 36.8 min, pH values between 4.5 and 9.5 and temperature values varied from 33.2 to 66.8 °C. In this sense, the pH for the different extractions was adjusted with NaOH/HCl aqueous solution (0.1 M) and a rotatable central composite design (8 factorial, 6 central and 6-star points) was performed according to Table 1, with 20 runs, thus allowing 10 degrees of freedom. This design was used for the optimization of total phenolic compounds, antiradical activity, chlorophyll A and carotenoids.

The infrared-assisted extracts at optimal conditions were compared with extracts obtained by water-based (WB) conventional methodology performed using a digital water bath (JSR JSWB -22 T, Gongju-city, Korea). For the study of antibacterial activity, the liquid extracts under optimal conditions were lyophilized and the powder was used for analyses.

2.4. Chemical analyses

2.4.1. Total phenolic compounds

Total phenolic content (TPC) was quantified using Folin-Ciocalteu method (Singleton et al., 1999). 0.2 mL of the extracts and 1 mL of tenfold diluted Folin-Ciocalteu reagent were mixed with 0.8 mL of sodium carbonate (Na_2CO_3) (75 g/L). The final mixture was incubated at 60 °C for 10 min and cooled for an additional 10 min. Subsequently, absorbance was measured using a UV–vis spectrophotometer (Biochrom Ltd, Cambridge, England) at 750 nm. Gallic acid was used as the standard for the calibration curve, and the extraction solvent as a blank

Table 1
Infrared-assisted extraction experimental conditions.

Run	Time (min)	pH	Temperature (°C)
1	30.0	8.5	60.0
2	20.0	7.0	50.0
3	20.0	4.5	50.0
4	20.0	7.0	50.0
5	10.0	5.5	40.0
6	20.0	7.0	50.0
7	30.0	8.5	40.0
8	20.0	7.0	50.0
9	10.0	5.5	60.0
10	20.0	7.0	50.0
11	20.0	9.5	50.0
12	20.0	7.0	33.2
13	20.0	7.0	50.0
14	30.0	5.5	40.0
15	3.2	7.0	50.0
16	10.0	8.5	40.0
17	36.8	7.0	50.0
18	30.0	5.5	60.0
19	20.0	7.0	66.8
20	10.0	8.5	60.0

control. Results were expressed as mg gallic acid equivalents per 100 g of dry matter (mg GAE/100 g DM).

2.4.2. Antiradical and antioxidant activities

The diphenyl-2-picrylhydrazyl radical scavenging (DPPH) assay was used to measure antiradical activity (Blois, 1958). 1.45 mL of DPPH radical (0.06 mM) was added to 50 μL of microalgae extracts or Trolox (positive control). After incubation for 30 min in the dark at room temperature, the absorbance of DPPH was measured using a spectrophotometer at 515 nm and methanol as a blank. The inhibition percentage of DPPH was determined using the following equation [1] and expressed as μg Trolox equivalent per ml (μg TE/mL):

$$\text{Inhibition Percentage} = [(\text{absorbance of negative control} - \text{absorbance of sample})/\text{absorbance of negative control}] \times 100 \quad [1]$$

The assay kit Ferric Reducing Antioxidant Power (FRAP) was used to measure the antioxidant activity of the extracts capable of reducing the ferric complex in an acidic medium in the presence of an appropriate antioxidant solution (Benzie & Strain, 1996). The reaction is proportional to the antioxidant capacity of the sample. Briefly, 10 μL of the diluted extracts or standard were combined with 220 μL of the FRAP working solution. A plate reader was used to measure the absorbance at 593 nm after 4 min of mixing. Antioxidant activity was expressed as μM iron (II) equivalent.

The 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay (ABTS) kit was performed according to the study published by Millet et al. (Miller et al., 1993). 200 μL of the ABTS solution were mixed with 5 μL of the diluted extracts or standard. After 5 min of continuous stirring, the absorbance was measured spectrophotometrically on a plate reader at 734 nm. The ABTS scavenging capacity was expressed in mM ascorbic acid equivalent (mM AA).

Cupric ion Reducing Antioxidant Capacity assay (CUPRAC) was also used to measure the antioxidant activity of the extracts (Apak et al., 2010). Oxidation of copper (II)-neocuproine in the CUPRAC assay allows the evaluation of total antioxidant capacity. 40 μL of the diluted extracts or standard were added to 200 μL of the working solution. The mixture was incubated at room temperature for 30 min, and absorbance was measured at 450 nm using a plate reader. Results were expressed as mM Trolox equivalent (mM TE).

2.4.3. Chlorophyll and carotenoids content

A UV/vis Lambda 2 spectrophotometer (PerkinElmer, Rodgau-Juegesheim, Germany) was used to determine total carotenoids, chlorophyll A content based on their absorption maxima (A) between 400 and 700 nm (Lichtenthaler & Buschmann, 2001). Extracts were diluted with distilled water and the following equations [2,3] were used to calculate their corresponding contents:

$$\text{Chlorophyll A } (\mu g/mL) = (13.36*A_{664.1}) - (5.19*A_{648.6}) \quad [2]$$

$$\text{Total carotenoids } (\mu g/mL) = (1000*A_{470} - 2.13*Ca - 97.64*Cb)/209 \quad [3]$$

The results were expressed in mg/100 g dry matter (mg/100 g DM).

2.5. Antimicrobial activity

2.5.1. Microbial strains

In order to assess the antimicrobial effect of the extracts, three microbial strains isolated from clinical samples were tested: Gram-negative *Escherichia coli*, Gram-positive *Staphylococcus aureus* and the yeast *Candida albicans*.

2.5.2. Determination of minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC)

The macrodilution broth method (Feβler et al., 2018) was performed to determine the minimum inhibitory concentration (MIC), which is the lowest concentration of the *P. tricornutum* extract that inhibits visible microbial growth. *S. aureus* and *E. coli* were tested with respect to the results of the disc diffusion test. Microbial suspensions were adjusted to 0.5 McFarland and then diluted to 10^6 CFU/ml 1 ml of Mueller-Hinton broth (MHB) was added to 7 tubes. For each bacterial strain, 400 mg/ml of the extract were added to the first tube and then serially diluted with MHB. Then, 1 ml of the bacterial suspension was added to each tube. The final concentration of microalgae powder in the broth tubes ranged from 100 to 1.56 mg/ml. The negative control consisted of 2 ml of MHB and the positive control consisted of 1 ml of MHB to which 1 ml of bacterial suspension was added. All tubes were incubated at 37 °C for 24 h. The next day, 50 μl from each macrodilution tube was applied separately to Mueller-Hinton agar (MHA) and incubated for an additional 24 h to determine the minimum bactericidal concentration (MBC), which is the lowest concentration at which 100% bacterial killing is achieved.

2.5.3. Agar well diffusion method

Agar well diffusion method was performed according to Magaldi et al. (Magaldi et al., 2004). An overnight culture of each microbial suspension was adjusted to 0.5 McFarland, diluted to 10^6 CFU/ml, and plated on Mueller-Hinton agar. Wells of 6 mm diameter were made in the agar with a Pasteur pipette (one well per plate). The wells were then filled with 50 μl of a 200 mg/ml extract powder dissolved in dH_2O (giving 10 mg/well for each microbial isolate). The antibiotic to which the bacterial strain was most sensitive served as the positive control (gentamicin 10 μg for *S. aureus* and fosfomycin 50 μg for *E. coli*), and bleach (2–6%) was used as the positive control for *C. albicans*. 50 μl of dH_2O was used as a negative control for each bacterial suspension. The plates were then incubated at 37 °C for 24 h.

2.6. Statistical analysis

Data were examined using analysis of variance (ANOVA) and least significant difference (LSD). A p-value <0.05 was considered statistically significant. All data related to RSM were analyzed using Statgraphics Centurion software XVII (Statpoint Technologies, Inc., Warrenton, VA, USA). Each analysis was performed in triplicate. Standard deviations are represented by error bars in the figures. All data related to the statistical model comparing factors and interactions is shown in Table S1.

3. Results and discussion

3.1. Effect of time, temperature and pH on the extraction of bioactive compounds and pigments

Infrared-assisted extraction of *P. tricornutum* was optimized using Response Surface Methodology (RSM) to maximize values determined by TPC (mg GAE/100 g DM), DPPH (μg TE/mL) and pigment recovery: chlorophyll A and carotenoids (mg/100 g DM).

3.1.1. Total antioxidant activity and total phenolic content

The phenolic content and antioxidant activity for each extraction conditions are shown in Table 2. As can be seen, the values obtained for phenolic compounds and antioxidant activity after the analysis of the 20 samples ranged from 465 ± 19 to 562 ± 7.11 mg GAE/100 g DM and 242 ± 21 to 870 ± 22 μg TE/mL, respectively. According to the assay design, the highest content of phenolic compounds (562 ± 7 mg GAE/100 g DM) was obtained after an application of 20 min, pH 7 and 66 °C, while the highest content of antioxidant compounds (870 ± 22 μg TE/mL) measured by DPPH assay was obtained after applying 20 min, pH 7 and 33 °C.

Fig. 1 shows the simultaneous optimization of antioxidant and phenolic bioactive compounds expressed by the desirability function, which indicates the influence of temperature, pH, and time on infrared-

Table 2
TPC (mg gallic acid equivalents (GAE)/100 g dry matter (DM)) and DPPH (μg Trolox equivalents (TE)/mL) of the extracts obtained after the application of infrared-assisted extraction at different conditions (time, pH and temperature).

Run	TPC (mg GAE/100 g DM)	DPPH (μg TE/mL)
1	511	460
2	468	397
3	514	421
4	489	290
5	547	625
6	470	281
7	478	535
8	480	242
9	527	544
10	504	347
11	525	386
12	552	870
13	495	251
14	522	517
15	465	365
16	530	738
17	487	326
18	512	335
19	562	389
20	523	362

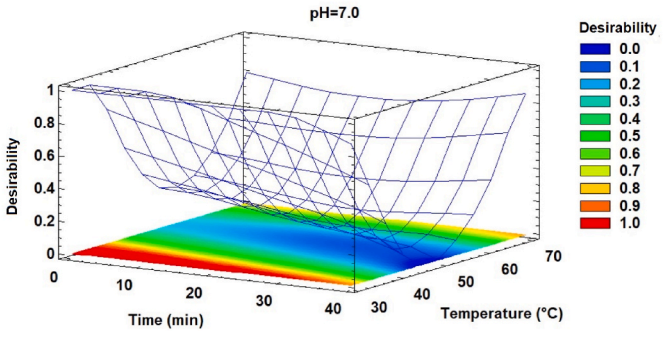

Fig. 1. Effect of different extraction conditions on the recovery yield of phenolic compounds and antioxidant activity. The least relevant factor (highest *p*-value) was set to its optimal value.

assisted extraction. The obtained desirability was 1.0, indicating that both reactions show similar behavior except for the pH factor, which is not significant ($p > 0.05$), allowing maximum simultaneous recovery with high influence of the applied temperature. As can be seen, temperature is the factor with the most significant influence ($p < 0.05$) on the extraction of bioactive compounds, with higher content at lower temperatures. In this regard, the optimal conditions for the extraction of bioactive compounds were 33 °C, 12 min and a pH of 7.

In this sense, the individual pattern of the antioxidant and phenolic compounds shown in Fig. 2 were observed. Similar tendencies were observed for both factors, with temperature being the factor with the greatest influence on yield ($p < 0.05$), followed by time ($p > 0.05$). In both cases, low values led to a higher yield of bioactive compounds and avoid their degradation caused by high temperatures over a longer period. Moreover, pH is the factor with the least influence on extraction ($p > 0.05$), showing similar tendency for phenolic content and antioxidant compounds between 5.5 and 8.5. According to the obtained results, the highest recovery of antioxidant (738 ± 4 μg TE/mL) and phenolic (547 ± 4 mg GAE/100 g DM) compounds by infrared-assisted extraction in *P. tricornutum* considering the analyzed ranges (10–30 min, 40–60 °C and pH between 5.5 and 8.5) was obtained after applying 10 min, 40 °C and pH of 8.5 and 5.5, respectively.

The influence of temperature, pH, and time on the recovery of bioactive compounds by IR-assisted extraction has been reported by other authors. The study published by Xiang et al. (Xiang et al., 2022)

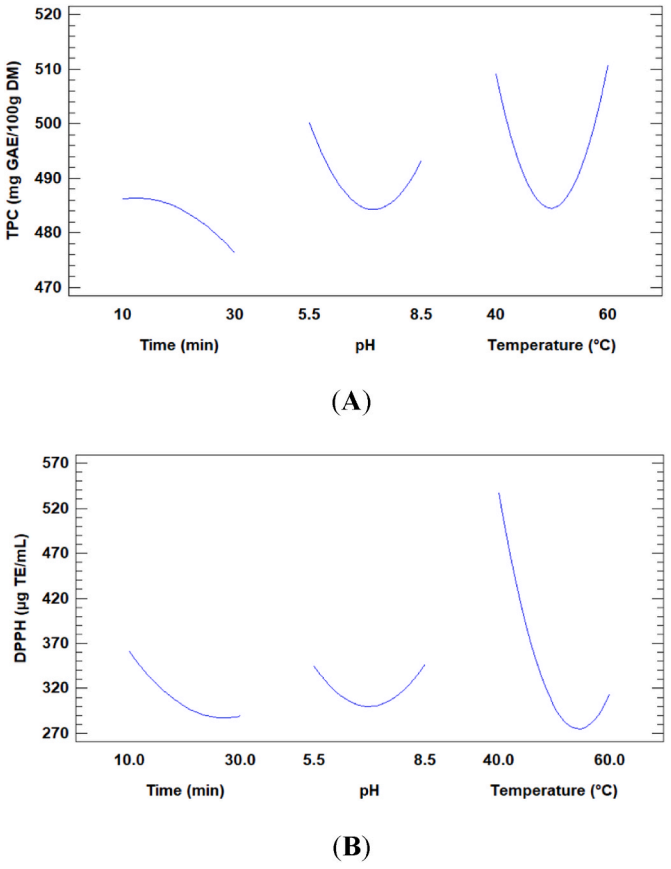

Fig. 2. Diagram of different extraction conditions effect on (**A**) TPC recovery yield and (**B**) antioxidant activity measured by DPPH assay.

showed that prolonged temperature and time in IR-assisted extraction may lead to the degradation of certain thermosensitive compounds. Thus, it is observed that the recovery of antioxidant and phenolic compounds decreases with increasing time. This result is consistent with many studies (Fu et al., 2011; Huang et al., 2014; Li et al., 2012; Mou et al., 2017), in which indicate that the high efficiency of the extraction process allows optimal extraction in short times of up to 10 min. Moreover, it is necessary to consider not only the thermal degradation caused by the temperature increase, but also the increase of impurities in the solution, which affect the mass transfer and the observed results. Temperature-induced destruction of cell membranes reduces the selectivity of infrared-assisted extraction (Xiang et al., 2022), which would explain the increase in recovery at 60 °C and is likely due to impurities acting as interfering factors in the chosen analytical methods.

In terms of pH, similar behavior of *P. tricornutum* is observed with different extraction methods. According to Al Khawli et al. (Al Khawli et al., 2021b), pH had no significant effect ($p < 0.05$) on the ultrasound-assisted extraction of bioactive and phenolic compounds. Nevertheless, it is necessary to continue the research on the behavior of bioactive compounds towards the different factors by studying broader areas with precise methods that allow determining the specific compounds that are affected. However, it is necessary to consider the thermal degradation caused by high temperatures over a long period of time, which affects the antioxidant and phenolic compounds as well as the pigments.

3.1.2. Pigments

Pigment contents of the 20 samples of *P. tricornutum* ranged from 11.18 ± 1.20 to 26.54 ± 2.78 mg/100 g DM and 2.50 ± 0.01 to 9.61 ± 0.40 mg/100 g DM for chlorophyll A and carotenoids, respectively. According to the experimental design, the highest contents of

chlorophyll A (26.54 ± 2.78 mg/100 g DM) were obtained after applying 20 min, 50 °C and pH 9.5 and 20 min, 66 °C, pH 7 for carotenoids (9.61 ± 0.40 mg GAE/100 g DM), as shown in Table 3.

The simultaneous optimization of the pigments for the factors considered (temperature, time and pH) is shown in Fig. 3 with a desirability of 0.99. It can be observed that temperature is the factor with the least influence ($p > 0.05$), while pH and time have a similar influence, with a tendency for extraction to increase as the value of these factors increases. As a result, the optimal extraction conditions for chlorophyll A and carotenoids were 56 °C, 36 min and a pH of 9.5.

The behaviour of the pigments in relation to the factors considered is similar to that obtained in the global optimization, with an increase in pigment recovery with increasing time, pH and temperature, as shown in Fig. 4. The effect of the factors on the recovery of chlorophyll A shows the same tendency with a stronger influence of pH, while in the extraction of carotenoids, temperature has a stronger influence. According to the results obtained, the highest recovery of chlorophyll A (24.06 ± 1.12 mg/100 g DM) and carotenoids (7.68 ± 0.67 mg/100 g DM) was obtained by infrared-assisted extraction in *P. tricornutum* considering the ranges evaluated (10–30 min, 40–60 °C and pH between 5.5 and 8.5) after applying 30 min, 40 °C and pH of 8.5 for chlorophyll A and 5.5 for carotenoids. As with the bioactive compounds, pH is the factor with the greatest variability with respect to the optimal recovery point of each of the compounds studied.

The influence of temperature in the application of various extraction techniques on pigment recovery has been described by several authors. In the study published by Gilbert-López et al. (Gilbert-López et al., 2017), an increase in carotenoid recovery was observed when the temperature was increased to 170 °C, a value considered as the optimal point in the optimization design applied for pressurized-liquid extraction. The results obtained showed the same tendency of an increase in yield with increasing temperature, considering that the temperature range was lower with a maximum at 60 °C. However, the behaviour of carotenoids with respect to temperature depends on the extraction method used. In microwave-assisted solvent extraction, the yield of carotenoids increases with increasing temperature due to the cell matrix disruption with a maximum of 55 °C due to the degradation of the compounds, setting as optimum point the application of low/medium microwave power level (Sarma et al., 2022). In ethanolic *P. tricornutum* extracts, the optimal MAE temperature was 30 °C (Gilbert-López et al., 2017). However, further studies are needed to determine the optimal extraction temperature for the different pigments in more extreme temperature ranges and to observe pigment degradation with increasing infrared-assisted extraction temperature.

On the other hand, the study published by Al Khawli et al. (Al Khawli et al., 2021b) on ultrasound-assisted extraction from *P. tricornutum* also showed the difference in optimal pH when comparing the extraction of chlorophylls and carotenoids, with an optimal extraction pH of 5.5 and 8.5, respectively. In this sense, a similar behavior in terms of pH and extraction time was observed when different methods were used, with an increase in pigment recovery with increasing extraction time until the optimal point of 30 min was reached, a result consistent with what was observed in the present study.

Table 3
Chlorophyll A and carotenoid content of the extracts obtained after the application of infrared-assisted extraction at different conditions (time, pH and temperature).

Run	Chlorophyll A (mg/100 g DM)	Carotenoids (mg/100 g DM)
1	16.01	7.58
2	17.21	4.58
3	19.31	7.39
4	17.43	5.38
5	12.66	6.64
6	24.06	4.73
7	15.51	3.12
8	14.52	4.13
9	14.94	6.26
10	20.34	3.50
11	16.90	9.33
12	21.78	8.56
13	19.28	2.50
14	26.54	7.68
15	9.69	5.34
16	12.36	6.40
17	11.18	7.88
18	11.38	6.76
19	13.73	9.61
20	12.99	7.39

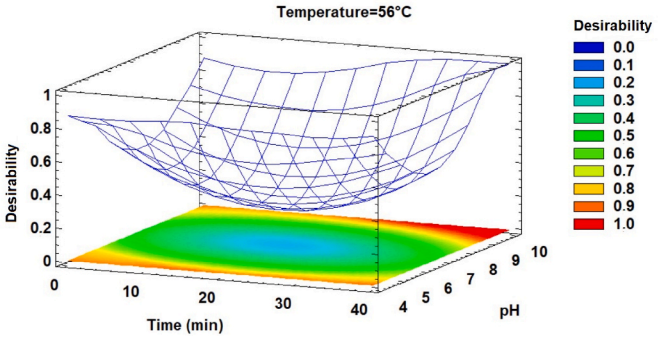

Fig. 3. Effect of different extraction conditions on the recovery yield of *P. tricornutum* pigments (chlorophyll A and carotenoids). The least relevant factor (highest *p*-value) was set to its optimal value.

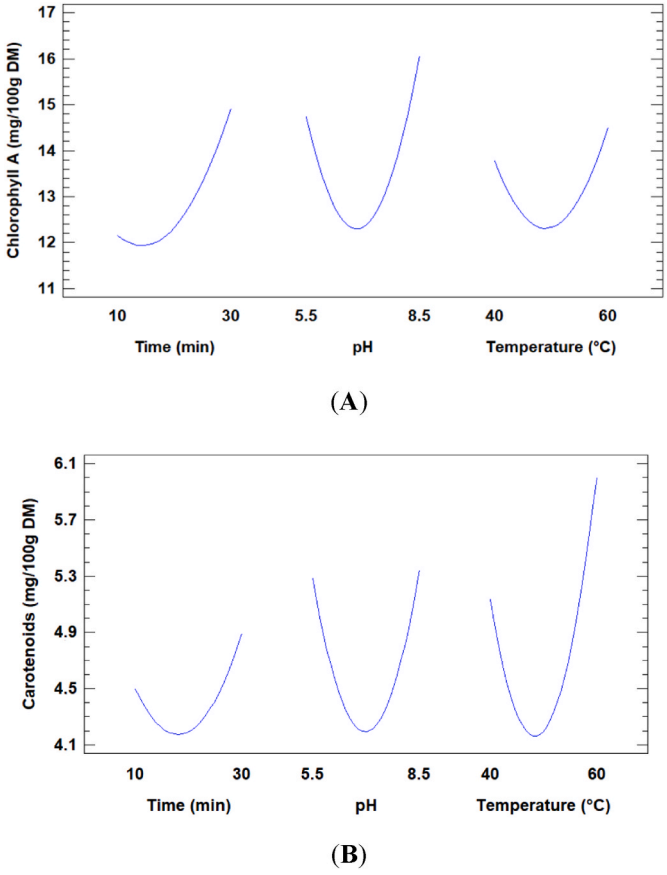

Fig. 4. Diagram of different extraction conditions effect on total (**A**) chlorophyll A and (**B**) carotenoid content recovery yield.

3.2. Recovery of antioxidant and phenolic compounds in infrared-assisted extraction at optimal conditions

3.2.1. Total antioxidant and phenolic content

The content of bioactive compounds obtained under the optimal conditions described in the previous section is shown in Fig. 5. The values obtained ranged from 896.51 ± 4.41 to 1221.39 ± 13.25 μg TE/mL for DPPH, 1450.00 ± 20.09 to 1700.00 ± 194.28 μM TE for CUPRAC, 2306.23 ± 124.48 to 2640.72 ± 49.79 mM AA for ABTS, 308.33 ± 2.35 to 322.50 ± 1.18 μM Iron (II)-E for FRAP, and 467.00 ± 1.19 to 514.00 ± 0.47 mg/100 g DM for TPC, corresponding to conventional aqueous extraction and infrared-assisted extraction, respectively. According to the results, significant differences ($p < 0.05$) were observed in the recovery of bioactive compounds depending on the extraction method, resulting in higher recovery of these compounds after infrared-assisted extraction in all analyses performed.

As can be seen in Fig. 5, IR-assisted extraction improved the extraction of antioxidant compounds compared to conventional aqueous extraction for all analyses performed, with increases of 36.24% for DPPH, 17.24% for CUPRAC, 14.50% for TEAC, and 4.60% for FRAP. Furthermore, the recovery of phenolic compounds was also higher, with an increase of 10.06% for TPC.

The effectiveness of IR-assisted extraction of bioactive compounds has been observed by other authors on plant matrices. Cai et al. (Cai et al., 2011) showed that the recovery of epicatechin, catechin, and procyanidin B2 in grape seeds was increased by 56.10%, 15.81%, and 67.35%, respectively, when IR technology was applied compared to the conventional hydroalcoholic method with heat. In addition, significantly higher yields were also obtained with IR compared to conventional ultrasound (USN) extraction and microwave-assisted extraction (MAE) for all compounds in a significant way ($p < 0.05$). The increase in yield by IR -assisted extraction over these technologies was also observed by Cheaib et al. (Cheaib et al. n. d.) on apricot pomace, obtaining increases in the extraction of antioxidants of 116% over microwave-assisted extraction and 125% over ultrasonic extraction when the same energy is maintained, and an increase of 6% and 23.25%, respectively, when the same time is maintained.

3.3. Antimicrobial activity

The study showed that *P. tricornutum* water extract exhibited antibacterial activity against some of the pathogenic microbial strains tested. This result is in agreement with other studies where it was found that a single phenolic extract could exhibit varying degrees of antimicrobial activity depending on the microbial species tested (Arguelles, 2018).

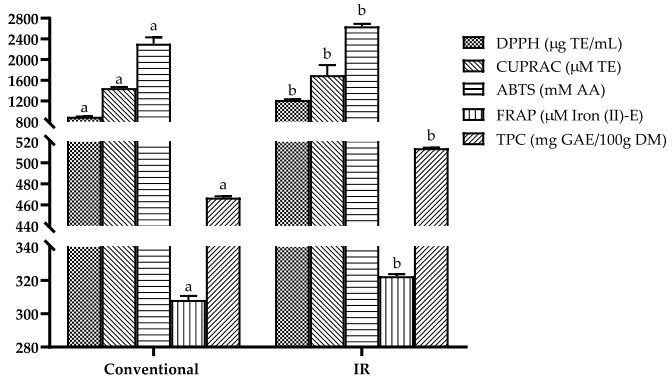

Fig. 5. DPPH, CUPRAC, ABTS, FRAP and TPC values in conventional and infrared-assisted extraction of *P. tricornutum*. Different lowercase letters in the same parameter indicate statistical differences related to the extraction methodology.

In the present study, water-based conventional (WB) and IR extracts were able to inhibit the growth of *S. aureus* with a MIC value of 25 mg/mL. The fact that the antibacterial activity of WB and IR extracts was similar, suggests that the active compounds were not impacted by the IR extraction process. Nevertheless, IR extracts showed a higher inhibition than conventional extracts, as can be seen in Fig. 6.

Similarly, agar well diffusion results revealed that the diameter of the inhibition zone of *S. aureus* was nearly the same (2 mm) for both WB and IR extracts.

As regards to the antimicrobial activity (MBC), results have shown significant reduction of *S. aureus* growth with the increase of the concentration of the extract in the agar plate from 25 mg to 100 mg in both cases, with IR showing higher inhibition than WB.

However, against the *E. coli* and *A. candida* strains, no antimicrobial activity was observed. Most probably due to the differences in the cell wall composition and permeability characteristics of different tested strains as the presence of the outer membrane, porins and the periplasm may account for the resistance of gram-negative bacteria, i.e. *E. coli*, against antimicrobial agents (Denyer & Maillard, 2002). In a similar manner, presence of chitin in the fungal cell wall may confer resistance against antifungal agents (Ruiz-Herrera & San-Blas, 2003).

Based on these findings, we could conclude that *P. tricornutum* extract showed a stronger antimicrobial activity against Gram-positive strains than against Gram-negative and yeast strains. Further studies of susceptibility and resistance of microbial species including more strains from each one will be needed to confirm these results. Nevertheless, MBC results have highlighted the improvement of the antibacterial activity of the IR extract compared to the conventional WB extract. This demonstrates the synergetic effect of phenolic compounds present in each extract, which even at the same polyphenol concentration, resulted in modified biological efficiency for WB and IR extracts. The synergistic effect of phenolics against the growth of *S. aureus* using IR was also observed for olive leaves extracts (Abi-Khattar et al., 2019).

4. Conclusions

The results obtained show that under optimal conditions, infrared-assisted extraction (IR) significantly increases the recovery of antioxidant bioactive compounds compared to the conventional method, with temperature being the most influential factor with a decrease in recovered bioactive compounds with increasing temperature due to thermal degradation. In addition, pH is an important factor to consider in the extraction of chlorophylls, with a basic pH allowing a higher yield. Furthermore, the antimicrobial analysis showed the inhibitory potential of the extracts obtained against *S. aureus*, with a minimum inhibitory concentration (MIC) of 25 mg/ml.

These results suggest that the extracts obtained by infrared-assisted extraction from *P. tricornutum* possess efficient bioactive and antimicrobial compounds. For these reasons, the potential applications of these extracts in the food, cosmetic and nutraceutical industries were highlighted, indicating the relevance for different industrial sectors not only in relation to *P. tricornutum*, but also in relation to the application of sustainable and innovative technologies that could increase the effectiveness and efficiency of industrial processes.

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CRediT authorship contribution statement

Mara Calleja-Gómez: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Anna-Maria Abi-Khattar:** Visualization, Methodology, Investigation, Formal analysis, Data curation. **Espérance Debs:** Writing – review &

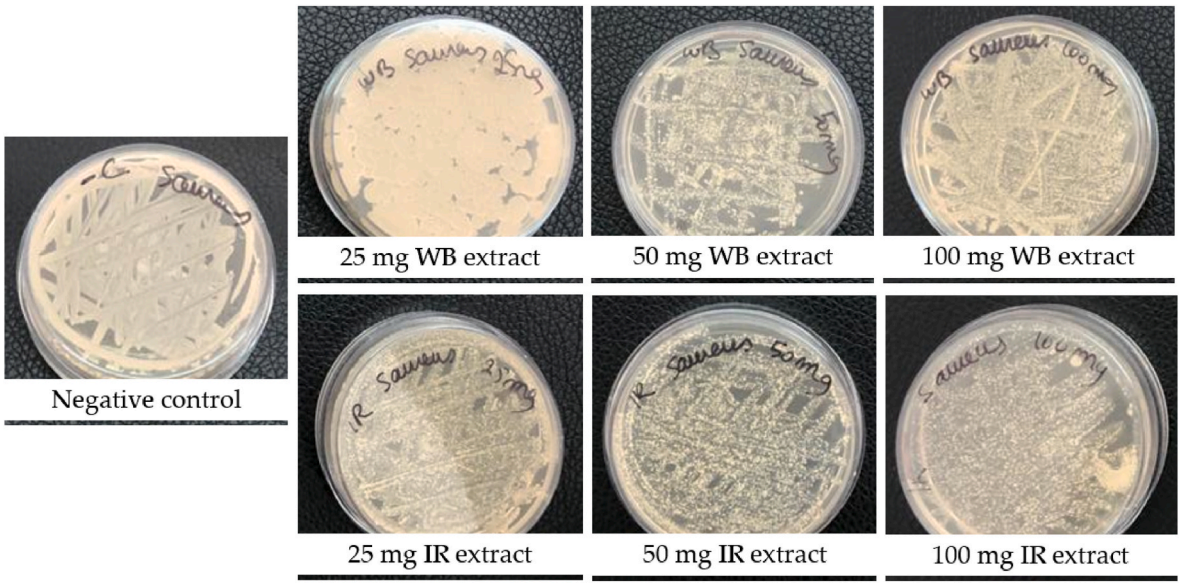

Fig. 6. Reduction of *S. aureus* growth with the increase of the concentration of the conventional water bath (WB) and infrared-assisted extraction (IR) extracts.

editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation. **Hiba N. Rajha:** Writing – review & editing, Formal analysis. **Richard Maroun:** Writing – review & editing, Validation, Resources. **Pedro Vicente Martínez-Culebras:** Supervision, Resources, Conceptualization, Visualization. **Nicolas Louka:** Writing – review & editing, Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Supervision, Validation.

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

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.116072.

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