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Influence of Geographical Location of Spirulina (*Arthrospira platensis*) on the Recovery of Bioactive Compounds Assisted by Pulsed Electric Fields

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Abstract: *Spirulina* (*Arthrospira platensis*) has been consumed by humans since ancient times. It is rich in high added-value compounds such as chlorophylls, carotenoids and polyphenols. Pulsed electric fields (PEF) is an innovative non-thermal technique that improves the extraction of bioactive compounds from diverse sources. PEF pre-treatment (3 kV/cm, 100 kJ/kg) combined with supplementary extraction with binary solvents at different times was evaluated to obtain the optimal conditions for extraction. In addition, the results obtained were compared with conventional treatment (without PEF pre-treatment and constant shaking) and different strains of *Spirulina* from diverse geographical locations. The optimal extraction conditions for recovering the bioactive compounds were obtained after applying PEF treatment combined with the binary mixture EtOH/H_2O for 180 min. The recovery of total phenolic content (TPC) (19.76 ± 0.50 mg/g DM (dry matter) and carotenoids (0.50 ± 0.01 mg/g DM) was more efficient in the *Spirulina* from Spain. On the other hand, there was a higher recovery of chlorophylls in the *Spirulina* from China. The highest extraction of total antioxidant compounds was in *Spirulina* from Costa Rica. These results show that PEF, solvents and the condition of growing affect the extraction of antioxidant bioactive compounds from *Spirulina*. The combination of PEF and EtOH/H_2O is a promising technology due to its environmental sustainability.

Keywords: PEF-assisted extraction; spirulina; bioactive compounds

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1. Introduction

Humans have been consuming microalgae since at least the 16th century. Today, microalgae are found in many food formulations [1–3]. Microalgae are of great interest due to their relevant nutrient content and also because they are a renewable and sustainable source [4]. Many years ago, cyanobacteria started to be grown in large quantities for a variety of industrial applications due to their ability to produce different types of compounds [5]. For instance, as microalgae contain many vitamins (such as A, B, C and E) and minerals (especially calcium and iron), it may be possible to formulate different personalized food products [6].

The most well-known and widely used cyanobacterium is *Spirulina*, which can be divided into *Arthrospira platensis* and *Arthrospira maxima*. It has been estimated that more than 30% of world microalgae biomass production is from *Spirulina* [7]. *Spirulina* is the most widely used in the world due to its high protein content, which has been estimated to reach 50–70% on a dry weight basis under optimal growth conditions [8,9]. Furthermore, numerous spirulina-derived peptides (IRDLDYY, HVLSRAPR, LDAVNR and MMLDF)

with different biological activities have been isolated and structurally characterized and are available in various databases such as BIOPEP-UWM [10–13].

Besides proteins, *Spirulina* is also a rich source of numerous other valuable compounds such as phycobiliproteins, carotenoids, and chlorophylls. All the above-mentioned compounds have shown several potential applications in the food industry [14–16]. Moreover, spirulina-derived pigments possess many health benefits. In this sense, spirulina-derived carotenoids have provitamin A activity and enhance the immune system upon ingestion along with reducing the risk of developing various disorders such as cardiovascular diseases, chronic diseases and cancers [15–17].

Generally, the algal biomass commercialized for food purposes is sold as supplements in the form of tablets or capsules and promoted as a superfood rich in proteins or omega-3 [17]. Currently, *Spirulina* is known to play a vital role in the food industry. In 1967, the International Association of Applied Microbiology recognized *Spirulina* microalgae as a food source for the first time [9]. At present, *Spirulina* is largely used in the food industry to produce several food products that have been launched into the market [17]. However, most of these food products use microalgae as coloring agents. Furthermore, *Spirulina* has been Generally Recognized as Safe (GRAS)—GRN No. 127—by the United States (US) Food and Drug Administration (FDA) and, due to its long history of use as a food, it can also be marketed in the European Union (EU) without the need to comply with Regulation (EU) 2015/2283 on novel food [1].

Different extraction techniques can be used to efficiently recover the various valuable compounds from microalgae. Traditionally, solid–liquid or solid–solid extraction techniques with the help of organic solvents and high temperatures were used to extract bioactive compounds. Recently, there has been growing interest in using green and highly efficient methods for the extraction of bioactive compounds from microalgae. This is due to concern about using toxic organic solvents in extraction procedures [18,19]. Today, various alternative environment-friendly technologies such as supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), ultrasound-assisted extraction (UAE), pulsed electric fields-assisted extraction (PEF) and microwave-assisted extraction (MAE) can be used to extract valuable compounds [20–22]. PEF-assisted extraction offers several advantages compared with traditionally used extraction methods. Käferböck et al., 2020, found that PEF application for cell disruption approximately increase 90% of C-phycocyanin extraction compared to bead milling [23]. Additionally, the phycocyanin fractions and proteins obtained were of higher purity and had an environmental impact that was half that of similar fractions obtained without PEF treatment. Martí-Quijal et al., 2021, also reported that PEF improves the extraction yield of antioxidant bioactive compounds from microalgae [24]. However, Li et al., 2020, reported opposite results in which PEF did not facilitate the release of phycocyanin from dried biomass [25]. In most cases, PEF-assisted extraction is focused on the use of aqueous suspension of microalgae. However, there are very few reports about the use of PEF to extract non-polar pigments such as chlorophylls or carotenoids from microalgae [26,27]. As previously reported, microalgae species have a direct impact on the extraction performance of PEF [19]. Therefore, it is important to consider the specific species involved in the extraction to obtain the necessary information to scale up the PEF process to an industrial level.

The best geographical location for harvesting microalgae is usually based on microalgal productivity, electricity, and land prices [28], and the chlorophyll concentration measured by satellite is used as an indicator of growth [29]. Different geographical locations of harvest could have an impact on the concentration of bioactive compounds. As far as we know, there are no studies that explore the concentration of bioactive compounds from *Spirulina* cultivated in different geographical locations.

Therefore, the present study aimed to investigate the total contents of carotenoids, chlorophyll *a*, chlorophyll *b*, and the antioxidant capacity of *Spirulina* extracts from different geographical locations. In addition, the efficacy of PEF extraction methods in combina-

tion with ethanol or dimethyl sulfoxide (DMSO) to recover valuable compounds such as pigments and total phenolic content from *Spirulina* was evaluated.

2. Materials and Methods

2.1. Chemicals

The DMSO and ethanol, and the salts sodium carbonate (Na_2CO_3), potassium phosphate (KH_2PO_4) and sodium phosphate (Na_2HPO_4) were supplied by VWR (Saint-Prix, France). In addition, the reagents trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid, 2,2′-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS), 2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH), fluorescein, Folin–Ciocalteu reagent and the salt potassium persulfate ($K_2S_2O_8$), were purchased from Sigma-Aldrich (Steinheim, Baden-Württemberg, Germany).

2.2. Samples

Spirulina (*Arthrospira platensis*) strain 15016 from Spain, Costa Rica, and China was used in this study. The *Spirulina* from Serra (Spain) was cultivated in raceway ponds near Valencia in a greenhouse under natural sunlight. Culture pH varied from 9.8 to 10.4. It was regulated by the addition of CO_2 at the time of harvesting. The *Spirulina* from Costa Rica was grown in a greenhouse-controlled environment, and the pH of the culture was maintained in a range from 9.8 to 10.2 by adding sodium bicarbonate. Finally, *Spirulina* from Hainan Island (China) was grown in open raceway ponds. No artificial light was used, and shadow nets partially covered the cultivation ponds, which allowed for the control of the pigments' production. At the time of harvesting, all samples were dried with dry air at 60 °C.

2.3. Extraction Procedure

The PEF treatment of *Spirulina* samples was carried out in a PEF-Cellcrack III (German Institute for Food Technology, DIL) (ELEA, Quakenbrück, Germany) in accordance with a previously reported method [21]. Briefly, 4.0 g of *Spirulina* (dry biomass) was mixed with 200 mL of deionized water to make a 2% solution of biomass. The voltage applied was 3 kV/cm, the specific energy input was 100 kJ/kg, and the number of pulses was 45. A portable conductivity meter ProfiLine Cond 3310 (WTW, Xylem Analytics, Weilheim in Oberbayern, Germany) was used to measure the temperature and conductivity before and after the treatment.

After the PEF treatment, ethanol (EtOH) and dimethyl sulfoxide (DMSO) was added 1:1 (*v*:*v*) to the initial 2% biomass solution, respectively, and mixed by stirring for 180 min at RT [21]. Then, the samples were centrifuged at 4000 rpm for 10 min using a 5810R centrifuge (Eppendorf AG). Finally, the supernatant was collected and stored at −20 °C for further experiments.

The control sample was extracted in the same way described above but without carrying out the PEF treatment. Briefly, 4.0 g of *Spirulina* (dry biomass) was mixed with 200 mL of deionized water and then EtOH or DMSO was added in a 1:1 proportion and mixed by stirring for 180 min.

2.4. Chemical Analysis

2.4.1. Total Phenolic Content (TPC)

The total phenolic content was determined by following the method previously reported by Parniakov et al., 2015, based on oxidation/reduction reaction [26]. Briefly, 3.0 mL of Na_2CO_3 was mixed with 100 µL of samples extract. After that, 100 µL of Folin–Ciocalteu reagent was added to the reaction mixture and incubated at room temperature (RT) for 1 h. After incubation, the absorbance was measured at a wavelength of 750 nm using a spectrophotometer Perkin-Elmer UV/Vis Lambda 2 spectrophotometer (Perkin-Elmer, Rodgau-Jügesheim, Germany).

2.4.2. Trolox Equivalent Antioxidant Capacity (TEAC)

The Trolox equivalent antioxidant capacity assay (TEAC) was used to evaluate the total antioxidant capacity of *Spirulina* extracts according to the previously reported method of Re et al., 1999, based on the decolorization of ABTS radical [30]. Briefly, $ABTS^+$ radical stock solution (7 mM) was prepared with the help of potassium persulfate and kept at room temperature in darkness for further analysis. After that, the $ABTS^+$ radical stock solution was diluted with ethanol until an absorbance of 0.70 ± 0.02 was reached at 734 nm. Finally, the diluted $ABTS^+$ radical solution (2.0 mL) was mixed with 100 µL of sample and incubated for 3 min in darkness. After incubation, the absorbance was measured at 734 nm in a Perkin-Elmer UV/Vis Lambda 2 spectrophotometer (Perkin-Elmer, Rodgau-Jügesheim, Germany). The TEAC analysis was carried out in triplicate. The total antioxidant capacity was calculated using a Trolox standard curve and expressed as µM Trolox equivalents (µM TE).

2.4.3. Oxygen Radical Absorbance Capacity (ORAC)

The ORAC analysis was carried out according to the previously reported method of de la Fuente et al., 2019, with minor modifications [31]. Briefly, *Spirulina* extract (50 µL) was mixed with 50 µL of fluorescein and incubated at 37 °C for 10 min in 96 well cell culture plate. After that, 25 µL of AAPH solution was added to the reaction mixture. The 96-well plate was placed in a VICTOR3 1420 multilabel plate counter reader (PerkinElmer, Turku, Finland), and the fluorescence was recorded every minute for 60 min under an excitation wavelength of 485 nm and an emission wavelength of 528 nm. The PBS (phosphate buffer) was used as a blank, and 100 µM Trolox solution was used as the antioxidant standard. The results were expressed as µM Trolox equivalents (µM TE).

2.4.4. Chlorophyll *a*, Chlorophyll *b* and Carotenoids

The total carotenoids (C_{x+c}), chlorophyll *a* (C_a), and chlorophyll *b* (C_b) were evaluated spectrophotometrically by following the previously reported method of Poojary et al., 2016 [22]. For the determination of total carotenoids (C_{x+c}), chlorophyll *b* (C_b), and chlorophyll *a* (C_a), absorbance was measured at 470, 653 and 665 nm, respectively, for samples dissolved in ethanol and at 480, 649, and 664 nm for those dissolved in DMSO [32]. The total carotenoid, chlorophyll *a*, and chlorophyll *b* contents were calculated according to the following equations:

EtOH equations:

$$C_a\ (\mu g/mL) = 13.36A_{664} - 5.19A_{648} \quad (1)$$

$$C_b\ (\mu g/mL) = 27.43A_{648} - 8.12A_{664} \quad (2)$$

$$C_{x+c}\ (\mu g/mL) = \frac{1000A_{470} - 2.13C_a - 97.64C_b}{209} \quad (3)$$

DMSO equations:

$$C_a\ (\mu g/mL) = 12.47A_{665} - 3.62A_{649} \quad (4)$$

$$C_b\ (\mu g/mL) = 25.06A_{649} - 6.5A_{665} \quad (5)$$

$$C_{x+c}\ (\mu g/mL) = \frac{1000A_{480} - 1.29C_a - 53.78C_b}{220} \quad (6)$$

2.5. Statistical Analysis

To study the differences between the data reported for each factor (PEF pre-treatment, solvents and extraction time) and each variable measured (chlorophyll, carotenoids, TPC, TEAC and ORAC concentrations) analysis of variance (ANOVA) was used. All experiments were carried out in triplicate. Data were expressed as mean ± standard deviation in all cases. A significance level of 95% ($p < 0.05$) was considered. Finally, the LSD (*Least Significant Differences*) test was performed to determine the differences between the means of the obtained values. All statistical analyses were performed with the software STATGRAPHICS Centurion XVI 16.1.03 (Statgraphics Technologies Inc., Princeton, NJ, USA).

3. Results

3.1. Total Phenolic Content (TPC)

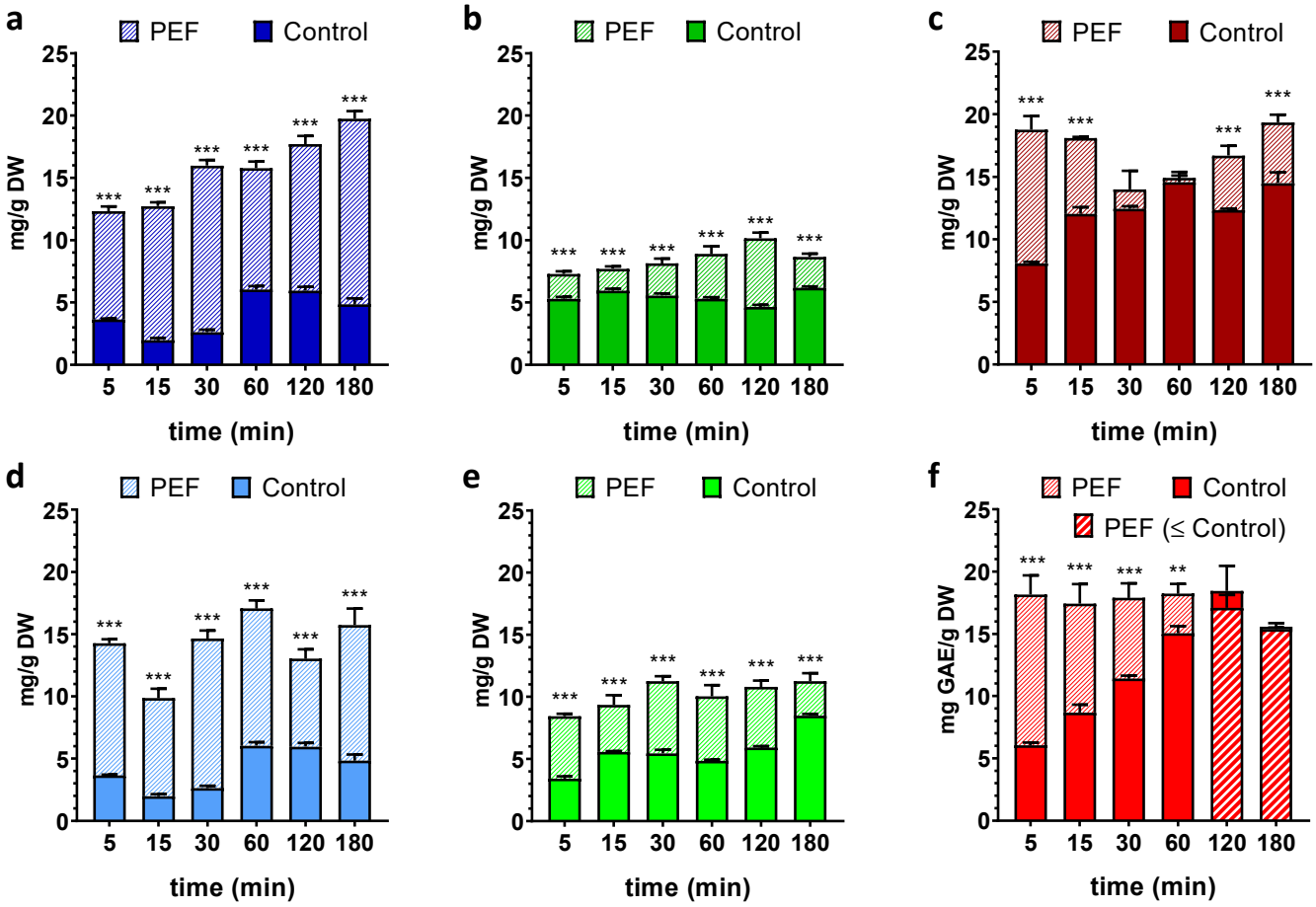
The combined effect of PEF treatment, geographical location, and binary solvents (DMSO and ethanol 50% v/v in water) on antioxidants and pigments extraction from the *Spirulina* cultivated in Spain, China, and Costa Rica regions were evaluated. The results for TPC are represented in Figure 1 for ethanol solvent and DMSO solvent. Comparing the extraction procedure, the samples treated with PEF for almost all the geographical sources and extraction times reached a higher TPC. There were only two exceptions for the *Spirulina* from China extracted with ethanol (30 and 60 min) and with DMSO (120 and 180 min) where there were no differences between the PEF-treated sample and the control. In both cases, this could be related to variations during analysis, but no statistical differences were found in terms of extraction time. Comparing the geographical location for 180 min, the results indicated that after PEF treatment for ethanol solvent, the TPC content for *Spirulina* cultivated in Spain (19.76 $\pm$ 0.50 mg/g DM) and China (19.36 $\pm$ 0.61 mg/g DM) was significantly higher ($p < 0.05$) compared to *Spirulina* cultivated in Costa Rica (8.66 $\pm$ 0.34 mg/g DM). These results are in agreement with those obtained by other authors, who observed a higher extraction of TPC when the PEF pretreatment was used [33]. The higher extraction of phenolics compounds could be related to the disruption of the cell membranes due to the electroporation effect [34,35]. This process is produced when an electrical treatment creates pores in the cell membrane due to an electrical breakdown. Then, the release of intracellular compounds increases, improving the yield of extraction [36]. On the other hand, for DMSO solvent, the TPC differs in time for PEF-pretreated *Spirulina* cultivated in different geographical regions (Figure 1b). The results are in agreement with those reported by Parniakov et al., 2015, for *Nannochloropsis* microalgae [37]. These authors found that the polyphenols extraction yield was at a minimum when DMSO 50% in water (v/v) was used as a solvent. On the other hand, ethanol 50% in water (v/v) achieved the maximum extraction level for polyphenols.

In addition, for both ethanol and DMSO 50%, the recovery remains stable throughout extraction. This could be explained from the point of view of the different polarities and interactions that could take place during the extraction between the microalgae samples and the ethanol (polar and protic solvent) and DMSO (polar and aprotic solvent) [38].

3.2. Chlorophyll a, Chlorophyll b and Carotenoids Contents

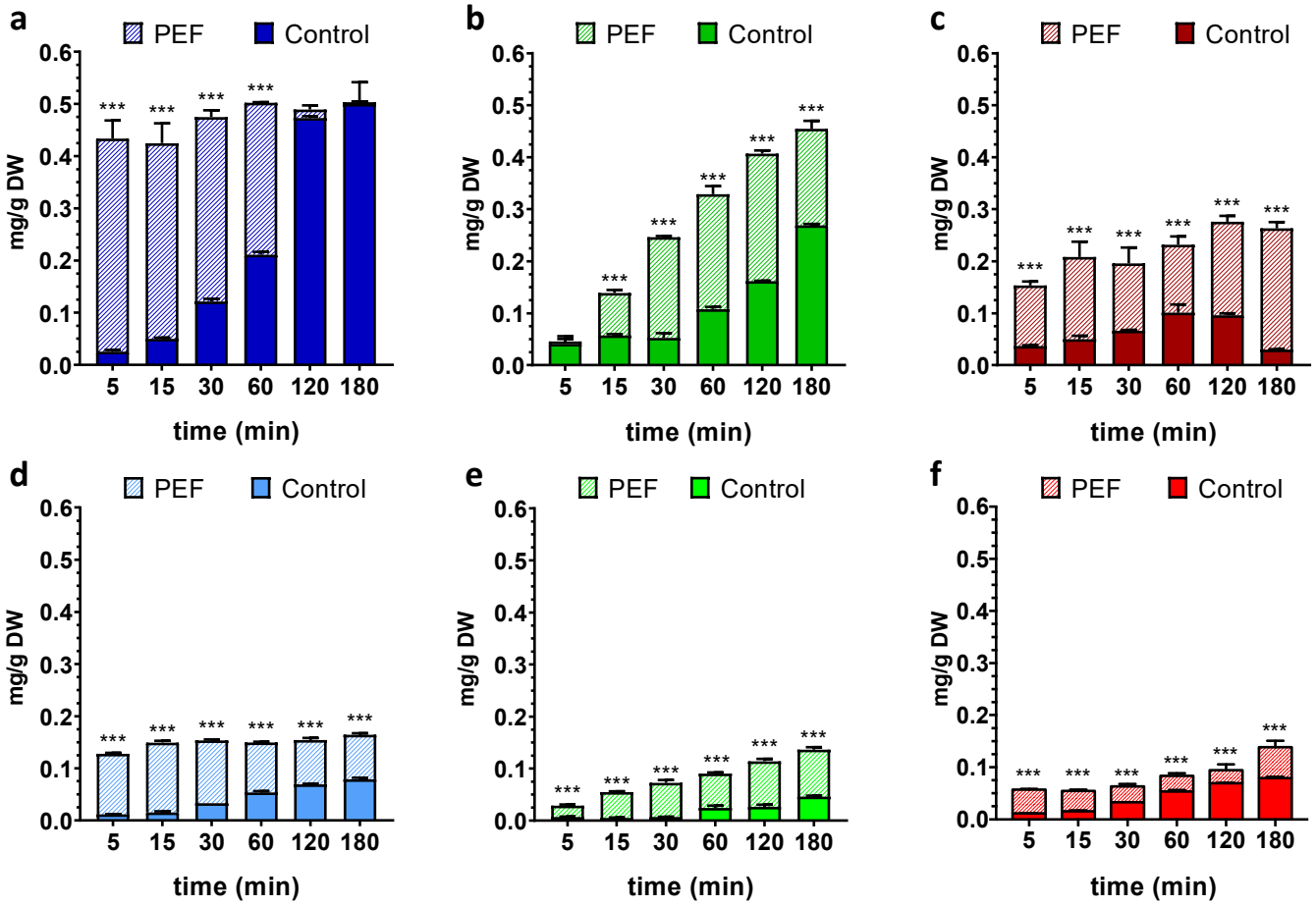
In Figure 2, the total carotenoid content of the extracts of *Spirulina* strain from different regions is presented, using ethanol and DMSO 50% (v/v) in water, respectively, as solvent after treatment with PEF compared with the control. In almost all cases, the PEF treatment significantly increased the content of carotenoids. The extraction time was a very important factor for the control samples, allowing an increase in the carotenoid concentration till the end of the process. A higher carotenoid content (0.50 $\pm$ 0.01 mg/g DM) was detected for PEF treatment of *Spirulina* from Spain compared with those from China and Costa Rica. On the other hand, the lowest carotenoid content (0.26 $\pm$ 0.01 mg/g DM) was detected for *Spirulina* from China without treatment. It was suggested that the accumulation of carotenoids might be affected by growth conditions. In our case, the carotenoid content was similar to or even higher than in previously reported studies [32,37,39]. Again, the recovery of carotenoids was lower in the case of the extracts obtained with DMSO/H_2O when compared to those obtained with EtOH/H_2O.

Finally, the contents of pigments (C_a and C_b) were significantly different between the *Spirulina* strains, especially after EtOH-assisted PEF extraction (Figures 3 and 4). The ANOVA test shows that all parameters analyzed have a significant effect ($p < 0.05$) on C_a and C_b extraction. This may be due to the differences in pigment levels between the *Spirulina* strains and in the extraction efficiency between the employed solvents. The highest C_a contents (2.25 $\pm$ 0.07 at 60 min, and 1.33 $\pm$ 0.02 mg/g DM, at 180 min) were obtained for PEF-treated *Spirulina* from China in the extract of EtOH and DMSO, respectively (Figure 3).



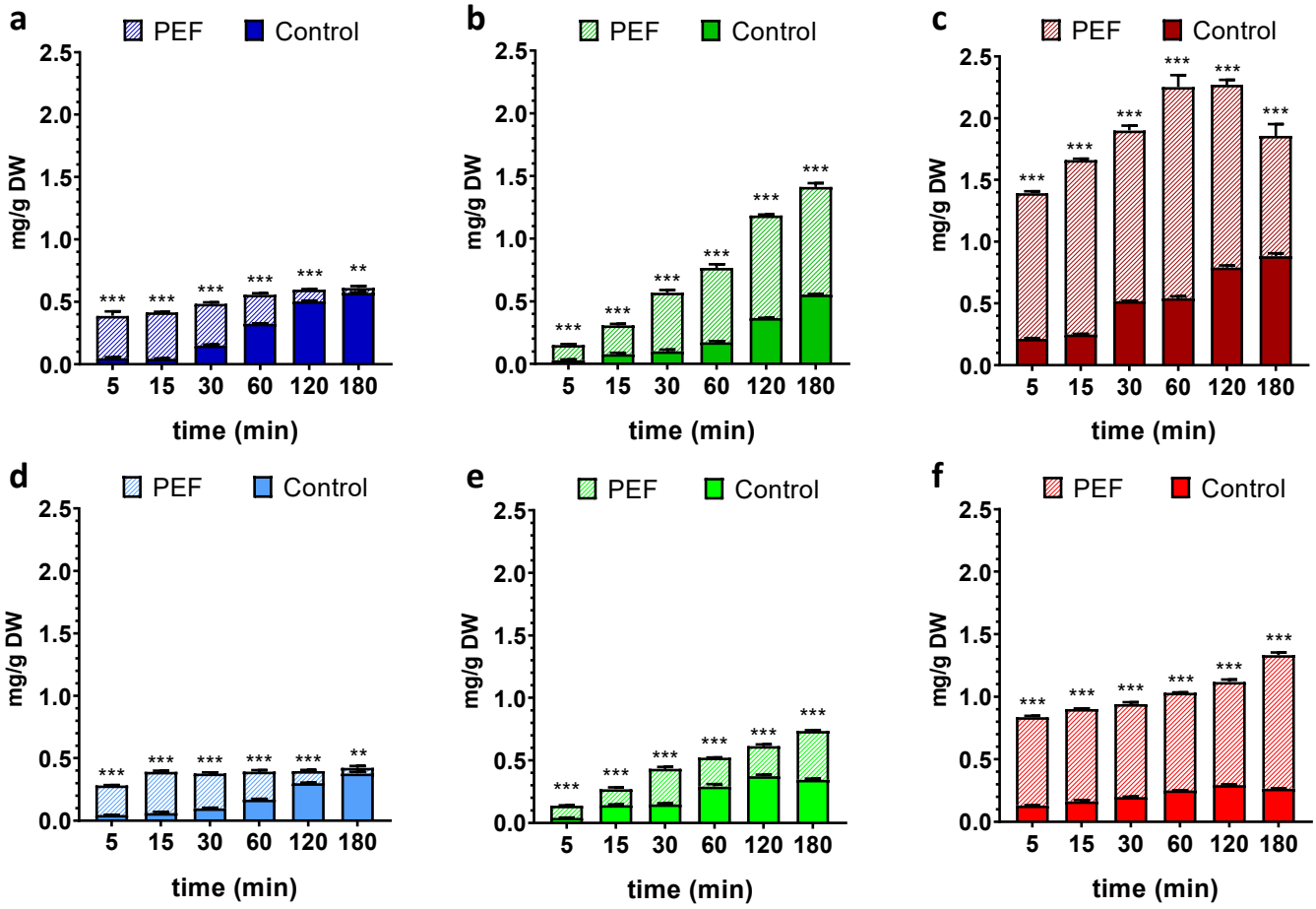
Time	Spain				Costa Rica				China			
	EtOH		DMSO		EtOH		DMSO		EtOH		DMSO	
	Control	PEF	Control	PEF	Control	PEF	Control	PEF	Control	PEF	Control	PEF
5	ab	c	b	a	ab	b	c	b	b	a	c	a
15	b	c	ab	a	ab	b	bc	ab	ab	a	bc	a
30	ab	b	ab	a	ab	ab	bc	a	ab	a	abc	a
60	a	b	ab	a	ab	ab	bc	ab	a	a	ab	a
120	a	ab	a	a	b	a	b	ab	ab	a	a	a
180	ab	a	ab	a	a	ab	a	a	a	a	ab	a

Figure 1. Total phenolic content of *Spirulina* extracts with PEF treatment or without PEF treatment (Control) for ethanol:water (**a**–**c**) and DMSO:water (**d**–**f**) solvent from *Spirulina* cultivated in different geographical regions (blue: Spain, green: Costa Rica, red: China). Statistical differences related to the PEF treatment are represented by asterisks (*). Statistical differences related to the extraction time are represented by different lower-case letters in the table. ** = $p < 0.01$; *** = $p < 0.001$.



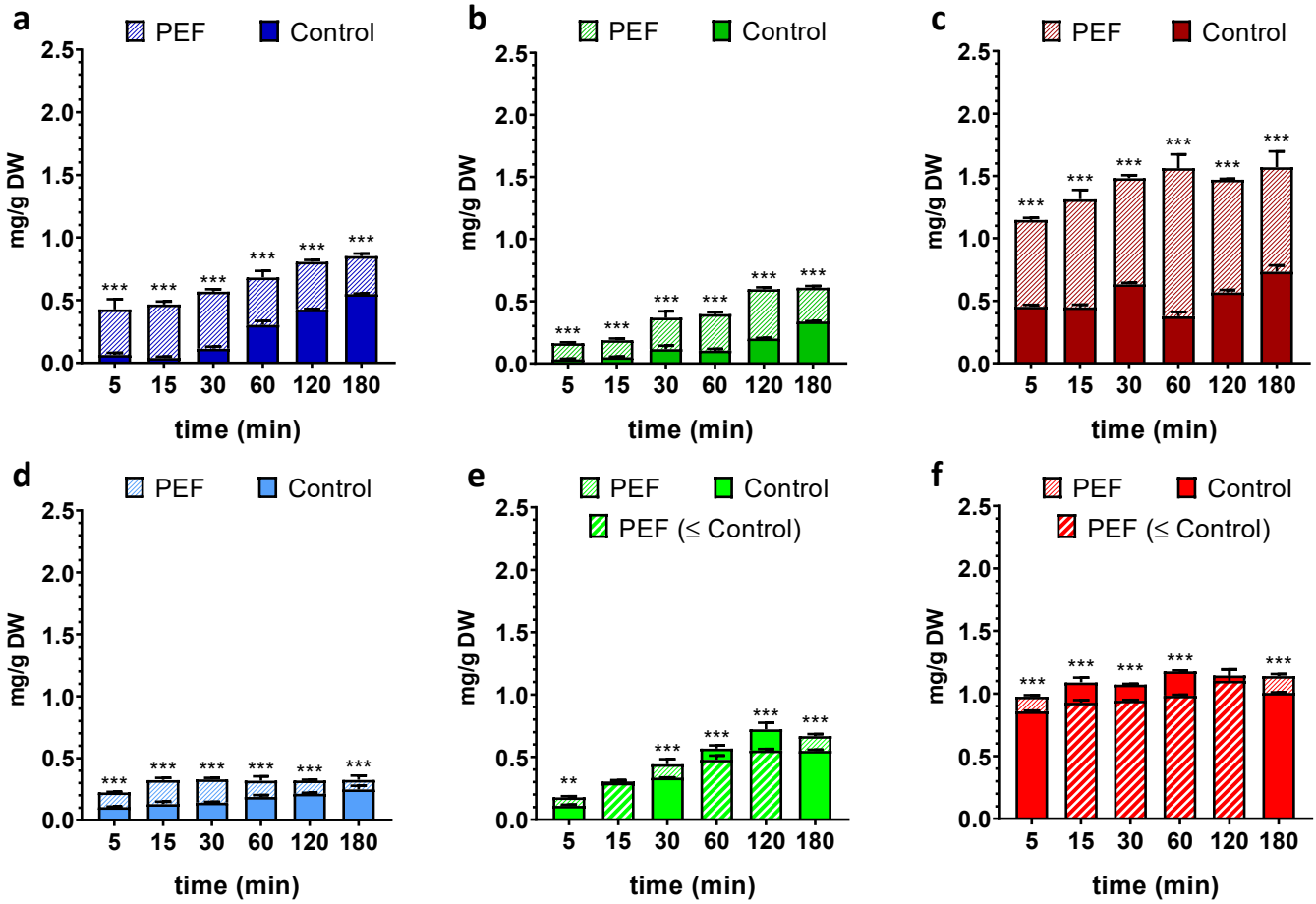
Time	Spain				Costa Rica				China			
	EtOH		DMSO		EtOH		DMSO		EtOH		DMSO	
	Control	PEF	Control	PEF	Control	PEF	Control	PEF	Control	PEF	Control	PEF
5	c	a	d	b	d	d	c	e	a	c	d	d
15	c	a	d	a	d	cd	c	de	a	abc	d	d
30	bc	a	cd	a	d	bc	c	cd	a	bc	cd	cd
60	b	a	bc	a	c	ab	b	bc	a	ab	bc	bc
120	a	a	ab	a	b	ab	b	ab	a	a	ab	b
180	a	a	a	a	a	a	a	a	a	ab	a	a

Figure 2. Total carotenoid content of *Spirulina* extracts with PEF treatment or without PEF treatment (Control) for ethanol:water (**a**–**c**) and DMSO:water (**d**–**f**) solvent from *Spirulina* cultivated in different geographical regions (blue: Spain, green: Costa Rica, red: China). Statistical differences related to the PEF treatment are represented by asterisks (*). Statistical differences related to the extraction time are represented by different lower-case letters in the table. *** = $p < 0.001$.



Time	Spain				Costa Rica				China			
	EtOH		DMSO		EtOH		DMSO		EtOH		DMSO	
	Control	PEF	Control	PEF	Control	PEF	Control	PEF	Control	PEF	Control	PEF
5	c	c	e	b	e	d	c	d	c	b	d	e
15	c	c	de	a	d	cd	bc	cd	c	ab	cd	de
30	c	bc	d	a	d	bc	bc	bc	b	ab	bcd	d
60	b	ab	c	a	c	b	ab	ab	b	a	abc	c
120	a	a	b	a	b	a	a	ab	a	a	a	b
180	a	a	a	a	a	a	a	a	a	ab	ab	a

Figure 3. Chlorophyll *a* content of *Spirulina* extracts with PEF treatment or without PEF treatment (Control) for ethanol:water (**a**–**c**) and DMSO:water (**d**–**f**) solvent from *Spirulina* cultivated in different geographical regions (blue: Spain, green: Costa Rica, red: China). Statistical differences related to the PEF treatment are represented by asterisks (*). Statistical differences related to the extraction time are represented by different lower-case letters in the table. ** = $p < 0.01$; *** = $p < 0.001$.



Time	Spain				Costa Rica				China			
	EtOH		DMSO		EtOH		DMSO		EtOH		DMSO	
	Control	PEF	Control	PEF	Control	PEF	Control	PEF	Control	PEF	Control	PEF
5	c	d	d	b	d	d	c	d	b	b	b	b
15	c	d	d	a	cd	cd	bc	cd	b	ab	ab	b
30	c	cd	cd	a	c	bc	bc	bc	ab	ab	ab	b
60	b	bc	bc	ab	c	b	ab	abc	b	a	a	b
120	ab	ab	ab	ab	b	a	a	ab	ab	ab	ab	a
180	a	a	a	a	a	a	ab	a	a	a	ab	a

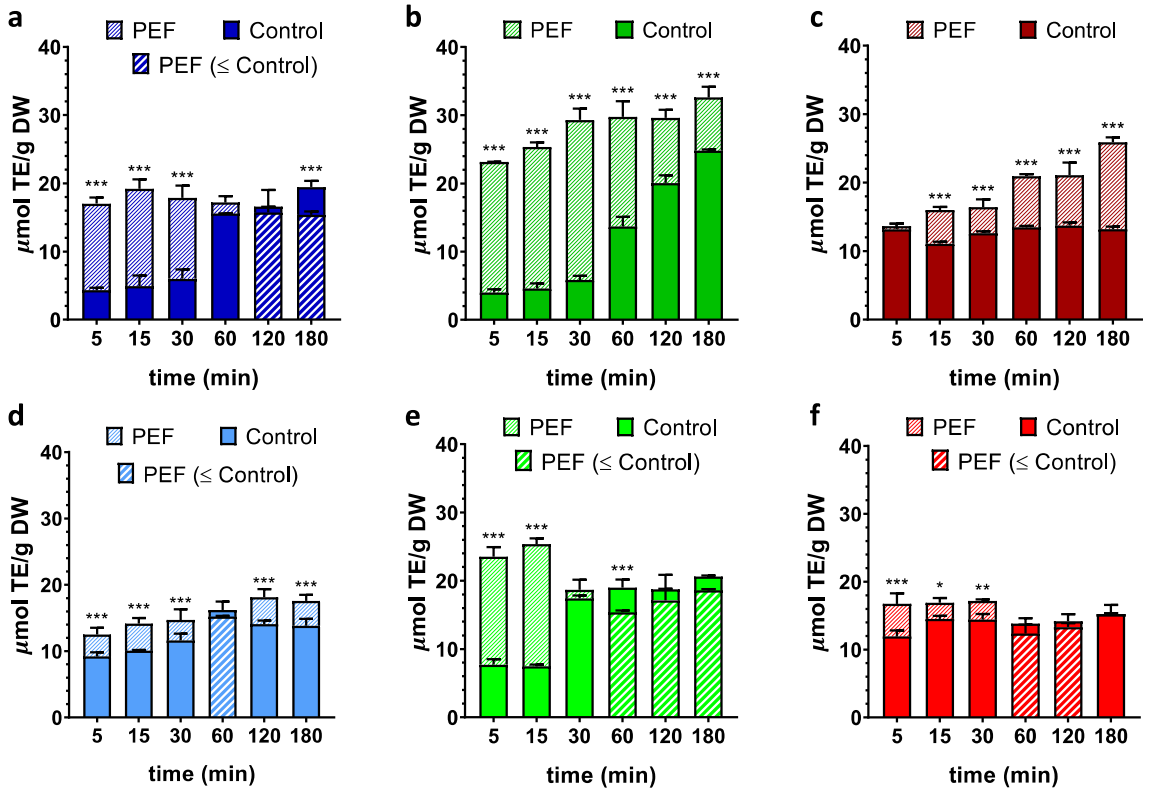
Figure 4. Chlorophyll *b* content of *Spirulina* extracts with PEF treatment or without PEF treatment (Control) for ethanol:water (**a**–**c**) and DMSO:water (**d**–**f**) solvent from *Spirulina* cultivated in different geographical regions (blue: Spain, green: Costa Rica, red: China). Statistical differences related to the PEF treatment are represented by asterisks (*). Statistical differences related to the extraction time are represented by different lower-case letters in the table. ** = $p < 0.01$; *** = $p < 0.001$.

Similarly, the highest C_b contents (1.57 ± 0.09 and 1.14 ± 0.02 mg/g DM) were obtained for PEF-treated *Spirulina* from China in the extract of EtOH and DMSO, respectively. However, in this case, PEF pre-treatment did not produce an interesting improvement in C_b extraction (Figure 4).

Once again, it can be observed how PEF treatment improves the extraction process by increasing the yield of pigments extraction. As mentioned before, this is because of the creation of pores in the cell membrane due to electric potential, which facilitates the release of intracellular compounds to the extracellular media. This helps to extract the compounds of interest faster and in a more sustainable way.

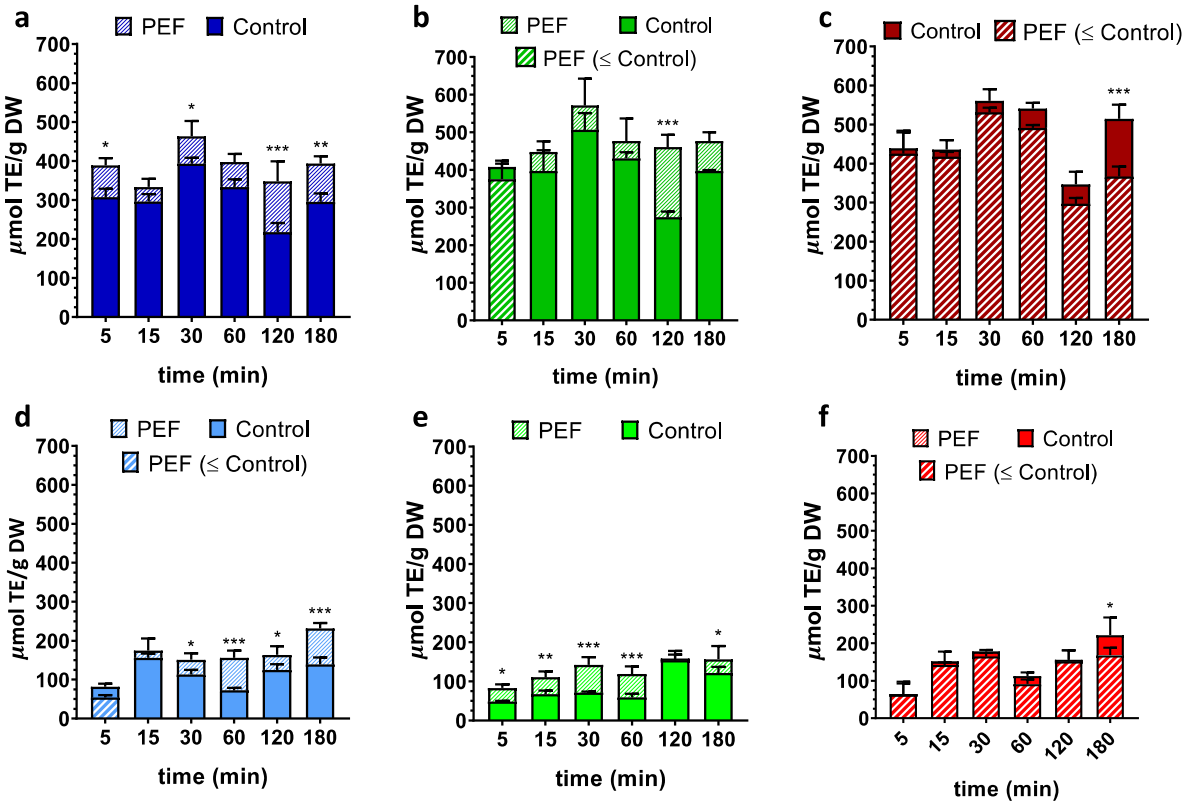
3.3. Antioxidant Potential

To characterize the antiradical potential of *Spirulina* extracts, the antioxidant capacity was determined using different methods, such as TEAC and ORAC (Figures 5 and 6). The statistical analysis showed that TEAC (Figure 5) and ORAC (Figure 6) antioxidant activities present significant differences ($p < 0.05$), affected by PEF treatment, the solvent used and also the geographical location of *Spirulina*. Moreover, TEAC activity was also dependent on treatment time. As pigments and polyphenols are the main responsible compounds of antioxidant activity, these results agree with that reported previously in this work. The ORAC assay showed higher antioxidant activity in ethanolic extracts compared to DMSO extracts. This can be explained by the higher extraction of bioactive compounds, especially carotenoids, as this test is more sensible for lipophilic substances [40]. Therefore, our results confirm the relevance of parameters such as PEF conditions and the *Spirulina* strain employed, which have a direct impact on the extraction procedure and should be considered in order to scale up the PEF-assisted extraction process to an industrial level.



Time	Spain				Costa Rica				China			
	EtOH		DMSO		EtOH		DMSO		EtOH		DMSO	
	Control	PEF	Control	PEF	Control	PEF	Control	PEF	Control	PEF	Control	PEF
5	b	abc	b	c	d	c	bc	ab	ab	c	b	a
15	b	a	b	c	d	bc	c	a	b	c	ab	a
30	b	ab	ab	c	d	ab	ab	ab	ab	c	ab	a
60	a	abc	a	bc	c	ab	a	b	a	b	ab	a
120	a	bc	ab	a	b	ab	a	ab	a	b	ab	a
180	a	c	ab	ab	a	a	a	ab	ab	a	a	a

Figure 5. Trolox equivalent antioxidant capacity of *Spirulina* extracts with PEF treatment or without PEF treatment (Control) for ethanol:water (**a**–**c**) and DMSO:water (**d**–**f**) solvent from *Spirulina* cultivated in different geographical regions (blue: Spain, green: Costa Rica, red: China). Statistical differences related to the PEF treatment are represented by asterisks (*). Statistical differences related to the extraction time are represented by different lower-case letters in the table. * $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.



Time	Spain				Costa Rica				China			
	EtOH		DMSO		EtOH		DMSO		EtOH		DMSO	
	Control	PEF	Control	PEF	Control	PEF	Control	PEF	Control	PEF	Control	PEF
5	ab	a	a	b	ab	b	c	b	a	ab	c	b
15	ab	a	a	a	ab	ab	bc	ab	a	ab	abc	ab
30	a	a	a	ab	a	a	bc	a	a	a	ab	ab
60	ab	a	a	ab	ab	ab	bc	ab	a	a	bc	ab
120	b	a	a	a	b	ab	a	a	a	b	abc	ab
180	ab	a	a	a	ab	ab	ab	a	a	ab	a	a

Figure 6. Oxygen radical antioxidant capacity of *Spirulina* extracts with PEF treatment or without PEF treatment (Control) for ethanol:water (**a**–**c**) and DMSO:water (**d**–**f**) solvent from *Spirulina* cultivated in different geographical regions (blue: Spain, green: Costa Rica, red: China). Statistical differences related to the PEF treatment are represented by asterisks (*). Statistical differences related to the extraction time are represented by different lower-case letters in the table. * $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

4. Conclusions

From the results obtained in this study, it can be concluded that the geographical area, the extraction solvent, and PEF pre-treatment have a significant impact on the recovery of antioxidant compounds. A greater extraction of TPC and carotenoids was obtained in *Spirulina* from Spain after treatment with PEF and EtOH/H_2O solvent. On the other hand, the maximum content of chlorophylls was obtained with the use of ethanol solvent, PEF treatment, and *Spirulina* from China. TEAC values were also increased after PEF treatment and ethanol solvent in *Spirulina* from Costa Rica, mainly due to the greater overall extraction of antioxidant compounds compared to the other *Spirulina* samples. The use of PEF in the extraction of antioxidant bioactive compounds and pigments from *Spirulina* would be interesting, as it could be an environmentally sustainable technology.

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