frontiers in Food Science and Technology

TYPE Original Research
PUBLISHED 19 March 2026
DOI 10.3389/frfst.2026.1800155

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RECEIVED 30 January 2026
REVISED 24 February 2026
ACCEPTED 27 February 2026
PUBLISHED 19 March 2026

CITATION
Dehn L, Psarianos M and Schlüter OK (2026) Extraction and stability of R-phycoerythrin from the macroalga *Palmaria palmata* using conventional and nonthermal processes.
Front. Food Sci. Technol. 6:1800155.
doi: 10.3389/frfst.2026.1800155

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Extraction and stability of R-phycoerythrin from the macroalga *Palmaria palmata* using conventional and nonthermal processes

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Macroalgae are highlighted for their rich nutritional composition and established consumption traditions. The red seaweed species *Palmaria palmata* is recognized as a valuable source of R-phycoerythrin (R-PE). Pulsed electric fields (PEF) is a nonthermal process often applied to food products to increase the extraction yield of intracellular compounds. In the current study, PEF was applied to *Palmaria palmata* as a pre-treatment to improve R-phycoerythrin extraction. Different extraction (solvent, particle size) and PEF processing parameters (voltage, treatment duration) were tested to identify the most efficient extraction conditions and the most critical parameters for obtaining the highest yield. The storability of R-PE was also assessed (light presence and temperature). The most efficient solvent was sodium phosphate buffer 0.02 M with a 1:20 solid-to-liquid ratio, leading to a yield of 2.05±0.07 mg/g on a dry basis. A milling step in liquid nitrogen further reduced the macroalgae's particle size from 1600 to 600 µm, doubling the extraction yield of R-PE. The effect of storage conditions on the stability of R-PE was tested, and it was observed that after 2 and 4 h of storage, light exposure led to 23% and 31% pigment degradation, respectively. The best-performing PEF condition increased R-PE yield from 5.39 mg/g to 7.39 mg/g on a dry-basis. The electric field strength and the treatment time were identified as the most critical parameters affecting the R-PE yield. PEF processing and reducing the particle size were the most efficient aspects that improved the extraction yield.

KEYWORDS
pigment recovery, pre-treatment, pulsed electric fields, seaweed, storage

1 Introduction

Macroalgae, or seaweed, are considered novel food sources. Their inclusion in European diets can reduce the global warming potential of the diets, while meeting people's nutritional needs (Parodi et al., 2018; Mazac et al., 2022). Furthermore, macroalgae are photosynthate-active and can release oxygen alongside biomass production (Ferrara, 2020). Furthermore, they have a rich composition, including proteins, amino acids, unsaturated fatty acids, sugars, and bioactive compounds such as pigments, phenolic compounds, and vitamins (Alghazeer et al., 2022; Ismail et al., 2023). The use of seaweed as food is widespread, with approximately 300 species being harvested in 50 countries, and the majority being red algae (Moreira et al., 2022). They can be consumed as parts of salads, as dried ingredients, as bulking agents (flours), as a

source of dietary supplements, as edible wrapping (e.g., in sushi), as natural colorants and as powders for texture modification (Blikra et al., 2021). Among edible macroalgal species, the red alga Palmaria palmata stands out as a delectable food, with high protein and sugar content depending on the season (Maribu et al., 2025) that is already widely used as food (Lopes et al., 2019).

Among the valuable compounds found in the alga *Palmaria palmata* is the red pigment R-phycoerythrin (R-PE), which is part of the protein-pigment complex of phycobilisomes that are found in cyanobacteria and red algae (Basheva et al., 2018). In the food sector, R-PE is often used as a colorant in products such as sweets and dairy, and as an ingredient in cake decorations and drinks due to its fluorescent properties (García et al., 2021; George and John, 2023). Additionally, R-PE from seaweed is known to exhibit bioactive properties, such as antimicrobial, antioxidant and cytotoxic activity (Sudhakar et al., 2023). However, the extraction of R-PE can be challenging because phycobilisomes are anchored to thylakoid membranes inside chloroplasts. Therefore, the extraction of R-PE would require its dissociation from the phycobilisomes, apart from the diffusion from the cell membrane (Martínez et al., 2019). Efficient cell disruption of the algae would allow diffusion of the phycobilisomes into a solvent, where the R-PE would dissociate from them.

Conventional cell disruption methods for phycoerythrin extraction include freezing-thawing and grinding in liquid nitrogen (Ghosh and Mishra, 2020). Nevertheless, nonthermal processes have been highlighted as efficient alternatives for disrupting cell membranes and facilitating the extraction of intracellular compounds. One of these technologies is pulsed electric fields (PEF), in which the product is placed between two electrodes and processed with high-voltage pulses for a short period, leading to electroporation due to an increase in transmembrane potential (Arshad et al., 2021). The efficiency of PEF treatment is influenced by multiple parameters, such as field strength, pulse shape, and therapy duration, yet studies often do not report all processing parameters, making the standardization of the process challenging (Raso et al., 2016).

PEF has been applied to *P. palmata* for the extraction of phenolics, flavonoids and carbohydrates and successfully reduced the extraction time compared to thermal treatment (Castejón et al., 2021). At the same time, PEF has successfully improved the extraction of B-PE from the algal species *Porphyridium cruentum* (Martínez et al., 2019), as well as other algae-derived pigments, such as C-phycocyanin from *Artrosphira platensis* (Martínez et al., 2017). Nevertheless, it has not been used to improve R-PE extraction from *P. palmata*. Additionally, to the best of the authors' knowledge, there is no publication on the stability of the recovered R-PE after extraction and during storage prior to use as an ingredient. Therefore, the current study aimed to explore PEF as a method for cell disruption of *P. palmata* to extract R-PE and to investigate the stability of R-PE under various storage conditions. Considering the need for the highest R-PE recovery and a consistent understanding of the pre-treatment, extraction, and storage conditions of R-PE, multiple parameters were tested and compared to understand their individual and combined effects.

2 Materials and methods

2.1 Materials

Fresh algae (*P. palmata*) were provided by AlgenProjekt Meeresalgenland UG (Ostenfeld, Deutschland). The algae were delivered in sealed polyvinyl chloride bags in a cooling container. Inside the bag, the algae were stored in a 3.3% NaCl aqueous solution. The solution was previously disinfected using a UV-C filter (Wasserhaus Deutschland GmbH, Potsdam, Germany). Upon delivery, the algae were washed with distilled water and then blotted with absorbent paper to remove excess water. Then the algae were stored at −20 °C for further experiments. When algae were used for experiments, they were thawed at room temperature for 90 min. All chemicals were purchased by Carl Roth GmbH + Co. KG (Karlsruhe, Germany).

2.2 Extraction of phycoerythrin (R-PE)

2.2.1 Overall process and influence of extraction conditions on the R-PE yield

After thawing, the algae were milled for 5 s at 10,000 rpm using a Retsch laboratory mill (Retsch GM 200, Retsch GmbH, Haan, Germany), and a selected amount was mixed with the chosen extraction solvent at the selected ratio. The evaluated extraction conditions were the extraction medium and the solid-to-liquid ratio. The tested solvents were water, 0.02 M sodium phosphate buffer at pH 7, and 0.1 M sodium phosphate buffer at pH 6.8, which have been successfully used for the extraction of R-PE (Xu et al., 2020; Arias et al., 2025; Bastos-Filho et al., 2025). The mixtures were stirred at 350 rpm for 30 min, and then left to stand for 5 min to allow sedimentation. The samples were then centrifuged for 5 min at 10,000 rpm, and 20 °C and the supernatant was collected and transferred to UV-transparent cuvettes. Samples were measured using a spectrometer (Specord® 210 plus, Goebel Instrumentelle Analytik GmbH, Au in der Hallertau, Germany). The respective solvent was used as a blank. The quantification of R-PE is described in Section 2.2.3.

2.2.2 Quantification and purity of R-PE

The R-PE concentration was quantified using Equation 1 as first described by Beer and Eshel (1985) and applied by Pereira et al. (2020).

$$\mathrm{R-PE}\ (\mathrm{mg/g\,d.m.}) = \frac{[(A_{564} - A_{592}) - (A_{455} - A_{592}) \cdot 0.2] \cdot 0.12 \cdot V}{m} \tag{1}$$

where m (g) is the amount of algae in dry matter, V (mL) is the volume of each respective solvent that was used as an extraction medium, 0.2 is the absorbance coefficient of R-PE, and 0.12 is a correction constant. The values A_{564}, A_{592} and A_{455} represent the maximum absorbance of R-PE at a wavelength of 564 nm and the minimum absorbance at wavelengths 592 and 455 nm, respectively (Beer and Eshel, 1985; Pereira et al., 2020).

The purity index (PI) was calculated to estimate the purity of the R-PE using Equation 2.

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$$PI = \frac{A_{564}}{A_{280}} \quad (2)$$

where A_{564} and A_{280} represent the maximum absorbance of R-PE at a wavelength of 564 nm and the absorbance of the total protein content at a wavelength of 280 nm, respectively (Bastos-Filho et al., 2025).

2.3 Evaluation of pre-treatment for the extraction of R-PE

2.3.1 Milling with liquid nitrogen and stability of the recovered R-PE under storage

Upon delivery, 10 g of fresh algae was washed with distilled water and the surface excess moisture was removed by blotting with absorbent paper. Then, samples were placed in a small mill (IKA A11 basic, IKA Werke GmbH and Co. KG, Staufen im Breisgau, Germany) and doused with liquid nitrogen (Nguyen et al., 2017; Ji L. et al., 2022). The sample was milled for 1–2 s and then sieved using a conventional kitchen mesh (Fackelmann GmbH + Co. KG, Hersbruck, Germany) to obtain two powders with different particle sizes. The average particle size of each powder was measured using a particle size analyzer (Partica LA-960, Horiba, Kyoto, Japan). The average particle size of the finer powder was 600 µm, while that of the coarser powder was 1600 µm. Afterwards, the powders were subjected to extraction using 0.02 M sodium phosphate buffer at pH seven at a solid-to-liquid ratio of 1:20 for 90 min, as described in 2.2. Specifically, both samples were weighed in triplicate in 22 mL snap-cap vials to 0.5 g and mixed with 10 mL of the buffer solution (1:20, w/v). All samples were stirred for 90 min at 350 rpm. After stirring, 2 mL of liquid supernatant was pipetted from each sample three times using an Eppendorf pipette into 2 mL Eppendorf tubes. The samples were centrifuged together at 10,000 rpm and 20 °C for 5 min and R-PE, as well as the PI, were quantified as described in Section 2.2.2. The stability of the recovered R-PE was investigated as a function of temperature and light exposure, conditions under which the extracts are expected to be used. The examined temperatures were 4 and 20 °C, representing storage at room temperature and cold storage. The examined light exposure levels were 0% and 100%. The illumination intensity during the light exposure treatments was 1000 µmol $m^{-2}{\cdot}s^{-1}$. The storage experiment was conducted inside a climatic chamber (Vötsch Industrietechnik GmbH, Balingen, Germany) at controlled conditions of temperature (4, 20 °C), light exposure (0, 100%) and time (0–4 h), since these storage parameters are known to possibly influence the R-PE stability (Munier et al., 2014; Nowruzi et al., 2022).

2.3.2 Treatment with pulsed electric fields (PEF)

An amount of 10 g of algae (frozen, thawed and milled according to Sections 2.1, 2.2.1) was mixed with 100 mL of sodium phosphate buffer 0.02 M (pH 7) and placed inside a batch treatment chamber. The treatment chamber was then placed inside an HVP-5 PEF unit (DIL, Quackenbrück, Germany) connected to an oscilloscope (Voltcraft DSO-1062D, Conrad Electronics, Hirschau, Germany) with two channels, corresponding to voltage and current. Initially, treatments were performed using a batch chamber with a 40 mm electrode gap across different pulse widths (15–30 µs), numbers of pulses (100–500), and field strengths (0.38–0.95 kV/cm). The frequency was kept constant at 15 Hz. The pulse shape was nearly rectangular. The temperature was measured after each treatment, and it never exceeded 33.4 °C. The energy consumption of the PEF treatment was estimated using Equation 3 (Raso et al., 2016).

$$W_{spec.}\ (kJ/kg) = n \cdot \frac{1}{m} \cdot \int_0^{\infty} V(t) \cdot I(t) dt \quad (3)$$

where n refers to the number of pulses, m (kg) refers to the sample mass and V (kV) and I (A) refer to the voltage and current, respectively.

During the treatments, an untreated sample used as a control was mixed with 0.02 M sodium phosphate buffer (pH 7) for the maximum duration across all PEF treatments. After the PEF pre-treatments, the mixture was transferred to a beaker, and an additional 0.02 M sodium phosphate buffer (pH 7) was added to achieve a solid-to-liquid ratio of 1:20. The samples were then subjected to R-PE extraction for 90 min, as described in 2.2. Specifically, samples were placed in a glass beaker with a volume of 250 mL and the additional solvent was transferred using a volumetric cylinder. All samples were stirred for 90 min at 350 rpm. After stirring, 2 mL of liquid supernatant was pipetted from each sample three times using an Eppendorf pipette into 2 mL Eppendorf tubes. The samples were centrifuged together at 10,000 rpm and 20 °C for 5 min and R-PE, as well as the PI, were quantified as described in Section 2.2.2. A treatment was further applied using a batch chamber with a 20 mm electrode gap, where 10 g of sample was mixed with 50 mL of the extraction medium. After the treatment, the remaining buffer was added, and the extraction process was carried out under the same conditions.

The PEF processing parameters that were studied were the ones that are known to affect process efficiency (Naliyadhara et al., 2022; Yan et al., 2025). Preliminary treatments were conducted to determine the maximum field strength that can be achieved during the sample treatment and to determine the range of the field strength that would be studied. The tested ranges of the number of pulses, electrode distance and pulse width were based on previous studies of PEF application on other food substrates (Andreou et al., 2020; Andreou et al., 2022; Psarianos et al., 2022).

2.4 Statistical analysis

All experiments were performed in triplicate, and for each replicate, the amounts of R-PE and PI were measured in triplicate. The Shapiro-Wilk test was used to verify that the data follow a normal distribution. In contrast, Levene's test was used to assess the homogeneity of variance at the 0.5 significance level. The data were analyzed with a One-way analysis of variance (ANOVA) with a level of significance of 0.5 and Tukey's-B test being applied post hoc to separate means. Data that did not follow a normal distribution were normalized prior to the analysis. The software used for these analyses was IBM SPSS Statistics 23 (IBM Corp., Armonk, NY, United States).

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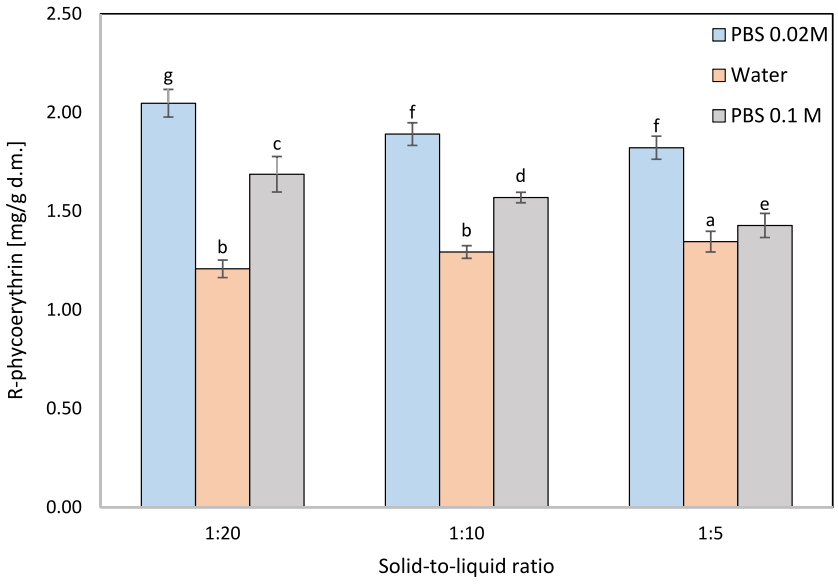


FIGURE 1
Extraction yield of R-PE (mg/g dry matter) with different solvents and solid-to-liquid ratios. The error bars indicate the standard deviation of measurements. Superscript letters (a,b, . . .) indicate the significant differences ($p < 0.05$) between the means of the R-PE yield. PBS: Sodium phosphate buffer.

3 Results and discussion

3.1 Investigation of the extraction parameters of R-PE without pre-treatment

The extraction yield of R-PE using different solvents and solid-to-liquid ratios is presented in Figure 1. Both solvent and the solid-to-liquid ratio exhibited a significant effect ($p < 0.05$) on the R-PE yield. The most efficient extraction configuration was with PBS 0.02 M and a solid-to-liquid ratio of 1:20. The highest yield with water as the solvent was obtained at a solid-to-liquid ratio of 1:5, yielding 1.35 ± 0.05 mg/g d.m. The yield was not significantly different ($p > 0.05$) from that of an aqueous extraction at a solid-to-liquid ratio of 1:10, which was 1.29 ± 0.01 mg/g d.m. Regarding the use of phosphate buffer as an extraction solvent, the solid-to-liquid ratio significantly affected the yield, with 1:20 being the most efficient. This was observed at both buffer concentrations ($p < 0.05$). Extraction with 0.02 M sodium phosphate buffer at a 1:20 solid-to-liquid ratio yielded 2.05 ± 0.07 mg/g d.m., whereas 1:5 and 1:10 ratios yielded 1.82 ± 0.06 mg/g d.m. and 1.89 ± 0.06 mg/g d.m., respectively, without significant differences ($p > 0.05$). Extraction with 0.1 M sodium phosphate buffer at solid-to-liquid ratios of 1:5, 1:10, and 1:20 yielded R-PE yields of 1.43 ± 0.06 mg/g d.m., 1.57 ± 0.03 mg/g d.m., and 1.69 ± 0.09 mg/g d.m., respectively, with all samples showing significant differences ($p < 0.05$).

The choice of solvent also had a significant effect ($p < 0.05$) on the R-PE yield. Sodium phosphate buffer at 0.02 M yielded the highest yield, followed by 0.1 M sodium phosphate buffer and then water, when the same solid-to-liquid ratio was used (Figure 1). Both extraction parameters had a significant effect on the extraction yield ($p < 0.05$). Aqueous extraction yielded lower R-PE than phosphate-buffer extraction across all extraction ratios. Therefore, it was concluded that the most appropriate extraction solvent was 0.02 M sodium phosphate buffer at a solid-to-liquid ratio of 1:20.

The PI ranged from 0.18 to 0.22 across all extraction ratios and solvents, with no significant differences ($p > 0.05$). The only exception was the sample extracted with water at a solid-to-liquid ratio of 1:20, for which the PI was 0.15 ± 0.01. The PI and R-PE yields reported in the present study are consistent with those reported in the literature. Specifically, Pereira et al. (2020) reported a similar PI for R-PE extraction from freeze-dried red alga *Gracilaria gracilis* (PI = 0.19) after freeze-thaw treatment (Pereira et al., 2020). Dumay et al. (2013) determined PIs between 0.12 (freeze-dried and ground algae) and 0.30 (dry cryoground) during R-PE extraction from *P. palmata* by enzymatic hydrolysis using xylanase (Dumay et al., 2013).

It was expected that increasing the solid-to-liquid ratio would increase the extraction yield of R-PE. Applying larger solvent volumes and therefore lower biomass loading increases the contact area of the solvent with the biomass, improving the diffusion of solutes to the liquid. In cases where the biomass loading is high and the solvent is close to the saturation point or to reach equilibrium, the solvent volume needs to be increased substantially to further improve the yield (Patil et al., 2023; Sai-Ut et al., 2023). The latter is likely what was observed for the PBS 0.02 M. The reduction in yield at higher solid-to-liquid ratios with water is likely because the equilibrium has already been reached at 1:5 and a further increase in solvent volume only dilutes the extracted R-PE.

R-PE is highly water soluble in a pH range from 4 to 10 (Galetović et al., 2020), while being most soluble at pH = 7 and becoming unstable in strongly acidic and alkaline environments (Matos, 2019). Glazer (1989) has already established that phycobilisomes can be stabilized during extraction in phosphate

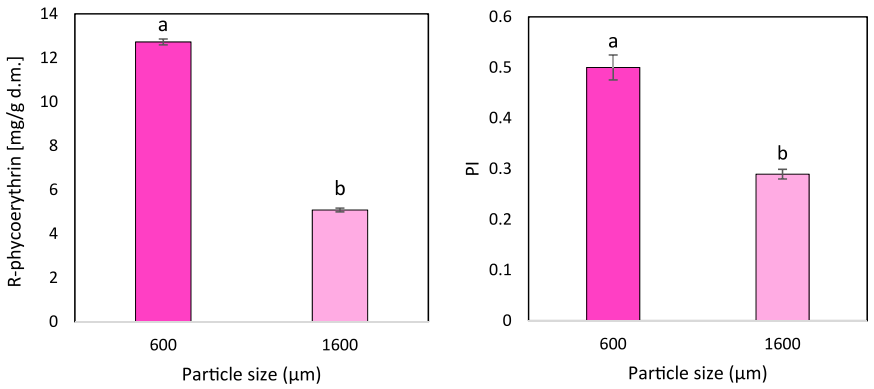


FIGURE 2
Extraction yield of R-PE (left) and PI (right) from milled algae with different particle sizes. The error bars indicate the standard deviation of measurements. Superscript letters (a,b, . . .) indicate the significant differences ($p < 0.05$) between the means of the R-PE yield and the means of the PI.

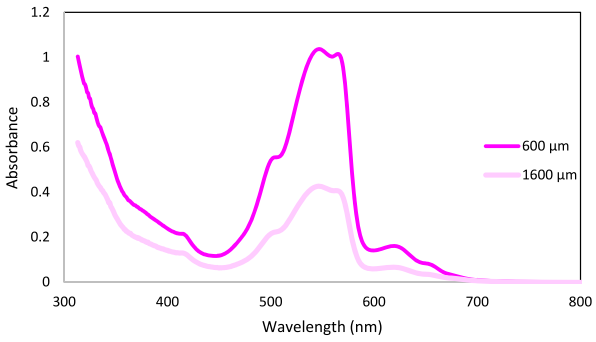


FIGURE 3
Absorbance spectrum of the extracted R-PE from milled algae with different particle sizes. The absorbance data represent the means of the values obtained from the different replications.

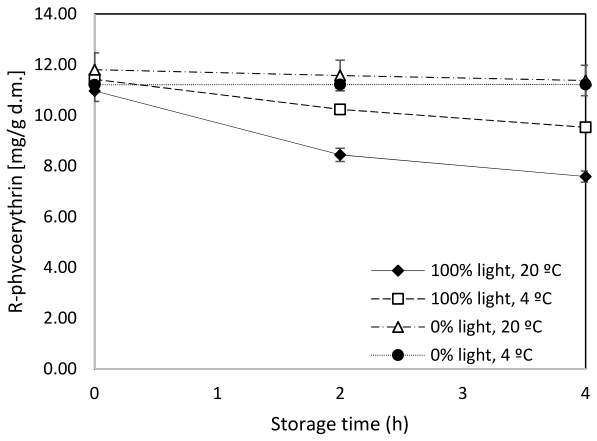


FIGURE 4
Effect of different storage conditions on the stability of R-PE (mg/g d.m.). The error bars indicate the standard deviation of measurements.

buffers at pH = 7–8 (Glazer, 1989). Under extreme acidic or alkaline conditions, phycobilisome proteins denature, leading to color loss, changes in spectral properties, aggregation, and precipitation (Galetović et al., 2020; Carmona et al., 2022). The higher stability of the pH of PBS is likely why it was more efficient than water. During the extraction with the water, the pH may change due to the release of intracellular compounds, affecting the extraction yield. Notably, variations of this R-PE extraction process have been successfully applied to other seaweed species, such as *Gracilaria birdiae* (Linhares de Aguiar et al., 2023), *Gracilaria corticate* (Saraswat et al., 2025) and *Osmundea capsica* (Eshaghzadeh et al., 2023).

3.2 Influence of particle size on the extraction yield of PE

Grinding in liquid nitrogen was used both as a conventional method for extracting R-PE and to investigate the influence of particle size on the R-PE extraction yield. Therefore, after grinding in liquid nitrogen, the algae powder was sieved, yielding two fractions: one fraction with smaller particles and one with larger particles. The sieving separated the particles whose size was about three times smaller. The average particle sizes of the sieved and non-sieved algae powder were approximately 600 µm and 1600 µm, respectively.

According to Figure 2, the R-PE yield from the samples with the small particle size was significantly higher ($p < 0.05$) than that from the larger samples. Specifically, the extraction of R-PE from samples with a particle size of 600 µm yielded 12.72 ± 0.13 mg/g d.m., whereas that from samples with a particle size of 1600 µm yielded 5.08 ± 0.09 mg/g d.m. The PI exhibited a similar trend, with a significantly higher value observed for samples milled to a smaller particle size ($p < 0.05$). Specifically, the PI was 0.50 ± 0.02 and 0.29 ± 0.01 for samples with particle sizes of 600 µm and 1600 µm, respectively. This indicates that reducing particle size not only improved the extractability of R-PE but also facilitated its separation from phycobilisomes.

In addition to buffer concentration, solid-to-liquid ratio, and time, particle size influenced extractability. Reducing particle size geometrically increases the external surface area, thereby increasing

the solid-liquid interfacial contact, shortening diffusion paths and enhancing mass transfer and extraction yield (Dulo et al., 2024). Reducing a solid's surface area exposes more of its surface to the solvent, increasing the mass transfer rate of solid diffusion into the liquid. Furthermore, smaller particles shorten the diffusion distance for solutes within the solid, making it easier for the solvent to penetrate the solid (Rajha et al., 2014; Prasedya et al., 2021; Patil et al., 2023; De Laet et al., 2024). This presumably explains the higher R-PE extraction yield at smaller particle sizes. The particle size and surface area of the product, therefore, have a significant influence on the extraction yield of R-PE.

This finding is further supported by the absorbance spectra of the extracted R-PE, as shown in Figure 3, where the samples with smaller particle size exhibit higher absorbance peaks. The peaks that were considered were the main peaks at 564 nm, 592 nm, and 455 nm, corresponding to its distinct chromophores. The peak at 564 nm corresponds to phycoerythrobilin chromophores, which are responsible for the red color and light-harvesting function. The peak at 592 nm corresponds to bilins related to energy transfer within the protein complex and the peak at 455 nm corresponds to additional accessory pigments or protein-bound chromophores. The spectrum of the extract highlights the complex arrangement of chromophores that enable energy transfer in the macroalgae. These peaks are consistent across recovered algae-based R-PE (Munier et al., 2015; Ulagesan et al., 2021; Sathuvan et al., 2022). The higher peaks and absorbance values likely correspond to a higher concentration of the pigment and the phycoerythrobilin chromophores in the extract.

The application of pre-treatments prior to R-PE extraction from macroalgae is a common practice. For instance, ultrasound and high-pressure pre-treatments have been applied to improve the extraction of R-PE from *Sarcopeltis skottsbergii* (Castro-Varela et al., 2022). Apart from applying liquid nitrogen, an alternative would be to mix the macroalgae with the buffer and subject the mixture to cycles of freezing and thawing to disrupt cell membranes and improve the yield, as performed for *Osmundea capsica* (Eshaghzadeh et al., 2023). Furthermore, the application of pre-treatments to *Palmaria palmata*, such as ultrasound and enzymatic treatments, can enhance the aqueous extraction of other solutes, including proteins and sugars, making the resulting extract nutritionally richer overall (Maribu et al., 2025a).

3.2.1 Investigation of storage conditions on the extracts

In order to determine the light and temperature conditions under which the R-PE extract can be stored without undergoing significant pigment degradation, the stability of the R-PE extract was examined after 2 h and 4 h of storage. Figure 4 shows the stability of R-PE during storage at various conditions. The primary factor leading to pigment degradation was the light exposure, which had a combined effect with the temperature at 20 °C. R-PE showed a significant degradation after 2 h of storage under light exposure, with the degradation being higher and gradually increasing when the temperature was set to 20 °C. On the contrary, samples stored in the dark remained stable regardless of the temperature. Specifically, a significant difference in R-PE concentration was observed after 2 h and 4 h of storage at 100% light and 20 °C. Specifically, a loss of approximately 23% of the R-PE concentration was observed after 2 h of storage, and approximately 31% after 4 h, compared with the initial R-PE concentration. Similarly, storage under 100% light exposure at 4 °C resulted in a loss of approximately 10% of R-PE after 2 h and approximately 17% after 4 h relative to the initial R-PE concentration.

When the extract was not exposed to light and stored at ambient temperature (20 °C), only 2% R-PE loss was observed after 2 h and approximately 3% after 4 h, compared to the initial concentration in the extract. When no light exposure was applied, and the storage temperature was 4 °C, no difference in R-PE content was observed after two or 4 h compared with the initial R-PE content.

To effectively assess the pigment's stability during storage, a multifactorial experimental design was used, employing a 2^2-factor plan. The base two corresponds to the two levels (low level and high level), and the exponent two corresponds to the two factors, the so-called influencing variables, which are combined. The evaluation using a multifactorial experimental design allows identification of which factors have a significant effect and which have the greatest effect (Kleppmann, 2011; Otava and Mylona, 2020).

The percentage loss of R-PE concentration from the start to the specified storage time was used as the measured variable. The degree of freedom f was determined to be 8, and, in accordance with the t-values of the Student distribution, the confidence interval for the respective statistical certainty for 2 h and 4 h of storage time was calculated. For the light factor, a significant difference was observed after 2 h and 4 h of storage. When comparing effects and confidence intervals, the light factor had the most significant effect at both 2 and 4 h. The effects of temperature and the combination of light and temperature were not significant.

Storing algae and extracts prior to use can be challenging due to R-PE's sensitivity to oxidation. In addition to freezing the fresh algal mass at −20 °C for storage until analysis, there are other ways to maintain algal stability. Algae can be freeze-dried and stored in a dry environment until analysis or homogenized in liquid nitrogen (Galland-Irmouli et al., 2000; Dumay et al., 2013; Nguyen et al., 2018; Pereira et al., 2020). Tripathi et al. (2007) examined the storage stability of the R-PE content in the cyanobacterium Lyngbya arboricola in a dry state, in the dark, and in the presence of silica gel as a drying agent at 25 °C. After 2 years of storage, only a 9% loss of PE content was observed in the dried cyanobacteria. Additionally, it was found that over a storage period of 56 days, the stability of the extracted R-PE from the cyanobacterium *Lyngbya arboricola* was greatest in the liquid and freeze-dried forms at an ambient temperature of 4 °C compared to 25 °C and −20 °C (Tripathi et al., 2007).

The instability of the R-PE during processing and storage can significantly hinder its utilization in the food industry. The influence of light and temperature on the pigment's stability has already been investigated by Munier et al. (2014) in relation to the pigment extract of the red alga *Grateloupia turuturu*. However, no significant degradation was observed after 8 h of light exposure, so the R-PE was classified as stable for up to 8 h under light. It is possible that the low irradiance that was applied by Munier et al. (2014) (33.57 μmol $\bullet m^{-2} \bullet s^{-1}$) compared to the irradiance in the present study (1000 $\mu mol \bullet m^{-2} \bullet s^{-1}$) did not significantly affect the pigment. However, after 48 h of light exposure, the solution was colorless, and the R-PE concentration decreased by 70%. Munier

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TABLE 1 Parameters of applied PEF pre-treatments on the red algae and the respective R-PE yield (mg/g d.m.) and PI that were obtained from the samples treated with the respective conditions. The results of the R-PE and PI are expressed as mean ± SD.

Electric field strength (kV/cm)	Voltage (kV)	Number of pulses	Electrode gap (mm)	Pulse width (μs)	$w_{spec.}$ (kJ/kg)	R-PE (mg/g d.m.)	PI
Effect of the applied voltage							
Control					0	2.02 ± 0.46[a]	0.32 ± 0.01
0.38	1.5	500	40	15	2.05	3.68 ± 0.24[b]	0.26 ± 0.01
0.50	2.0	500	40	15	3.65	4.31 ± 0.33[b]	0.28 ± 0.01
Effect of the electrode gap							
Control					0	1.16 ± 0.46[a]	0.26 ± 0.02
0.95	2.0	500	20	15	3.83	4.96 ± 0.33[c]	0.33 ± 0.01
0.50	2.0	500	40	15	3.65	4.00 ± 0.13[b]	0.29 ± 0.02
Effect of treatment time and field strength							
Control					0	5.39 ± 0.27[a]	0.33 ± 0.01
0.38	1.5	100	40	15	0.41	5.28 ± 0.37[a]	0.29 ± 0.01
0.38	1.5	500	40	15	2.05	7.39 ± 0.35[c]	0.35 ± 0.02
0.93	3.7	100	40	30	2.22	6.87 ± 0.09[b]	0.32 ± 0.02
0.93	3.7	500	40	30	11.10	7.24 ± 0.48[c]	0.33 ± 0.02

d.m., dry matter. Superscript letters (a,b, . . .) indicate the significant differences ($p < 0.05$) between the means of the R-PE yield within the same experimental batch.

et al. (2014) also investigated the stability of the R-PE extract at 4 °C and 20 °C for 1 h and found no significant degradation, thus confirming the findings of the present study. Furthermore, the storage stability of the R-PE-rich extract was investigated, and the pigment remained stable at 4 °C for 48 h and at −20 °C for 2 weeks (Munier et al., 2014).

R-PE is known to be sensitive to oxidative reactions due to the presence of oxygen, photooxidation, chromophore activity, presence of reactive oxygen species (ROS) and temperature increase. The primary oxidation mechanism is driven by light absorption by R-PE, which excites its chromophores, generating singlet oxygen and other ROS that then oxidize chromophores and the proteins bound to R-PE (Sugawara et al., 2006; Pan et al., 2013; Sun et al., 2023; Wen et al., 2025).

3.3 Investigation of the parameters of the PEF pre-treatment on the R-PE yield

The parameters of the PEF pre-treatment on the algae to improve the extraction yield of R-PE that were studied were the pulse width (μs), the number of pulses, the applied voltage (kV), and the electrode gap (mm). To understand the effect of each processing parameter on the R-PE yield, three independent experiments were conducted, each with a corresponding untreated control. One experiment assessed the effect of the applied electric field strength, with the other parameters being constant. The second experiment assessed the effect of using treatment chambers with different electrode gaps. It is known that some processing parameters, such as the applied voltage, are affected by the conductive properties of the treated mixture, including its conductivity, pH, bubble presence, and medium dimensions (Alkanan et al., 2024; Abad et al., 2025). Therefore, reducing the distance between the electrodes without changing the processing parameter configuration was expected to increase the field strength and, consequently, the PE yield. The third experiment assessed the effect of treatment time by varying the number of pulses and pulse widths, resulting in longer treatments and longer exposure of the sample to the applied electric fields. At the same time, varying field strengths were applied to explore potential combined effects. The results are presented in Table 1.

It was observed that the applied PEF treatments significantly increased the R-PE yield ($p < 0.05$), with all tested parameters showing a positive effect. The PI was not significantly affected by the PEF treatments under any applied conditions, ranging from 0.26 to 0.35. Notably, most PEF pre-treatments yielded results similar to grinding with liquid nitrogen and obtaining a fraction with a larger particle size. Nevertheless, samples ground to 600 μm without PEF pre-treatment still exhibited the highest R-PE yield among all PEF pre-treatments.

To investigate whether the electrode gap affects the R-PE extraction yield, two gaps were compared: 20 mm and 40 mm. When quantifying the R-PE, a smaller electrode spacing of 20 mm increased the R-PE yield. Thus, with an electrode spacing of 20 mm, a R-PE yield of 4.96 ± 0.33 mg/g d.m. was achieved, 24% higher than that obtained with the chamber at 40 mm electrode spacing under the same processing conditions. This effect was attributed to the higher electric field strength of 0.95 kV/cm that was achieved with the smaller electrode gap compared to the electric field strength (0.50 kV/cm) achieved with the chamber with a larger electrode gap. This finding is further supported by the experimental results, which tested the effect of the applied voltage. Increasing the electric field strength from 0.38 to 0.93 kV/cm increased the R-PE yield, and

both treatments significantly improved the extraction yield compared with the control when 500 pulses were applied. On the contrary, a minor increase in the applied field strength from 0.38 to 0.5 kV/cm did not lead to a further significant increase in the R-PE yield.

Increasing the treatment duration to 7500 µs (15 µs pulse width and 500 pulses) showed a trend toward higher R-PE extractions than in the untreated sample. The PEF treatment with 15 µs and 100 pulses (total treatment duration of 1500 µs) showed no significant effect ($p > 0.05$). On the contrary, applying a double treatment of 3000 µs (30 µs pulse duration and 100 pulses) resulted in approximately 21% higher PE yield than the untreated control. The highest PE yield in this experiment was achieved at a pulse duration of 15 µs and 500 pulses (treatment duration of 7500 µs), and was 7.39 ± 0.35 mg/g d.m. Under these conditions, approximately 27% more R-PE was extracted compared to the control. An even longer treatment duration (15,000 µs) with a 30 µs pulse and 500 pulses did not result in a further increase in R-PE yield ($p > 0.05$).

Many parameters, such as electric field strength, process duration, pulse shape, temperature, and the conductive medium, influence the efficiency of a PEF treatment (Naliyadhara et al., 2022). The intensity of a PEF treatment is better reflected by the specific energy, which combines all these parameters into a single unit. Therefore, adjusting processing parameters for applying higher-energy treatments to a product can improve process efficiency, while increasing the energy transferred to the food sample can enhance the extraction of intracellular compounds (Ranjha et al., 2021). In the present study, when a higher specific energy input was applied in the first experiment, the PE yield did not increase significantly, likely due to small changes in the processing parameters.

In the experiment with different electrode gaps, although the specific energy was nearly equal, a significant increase in R-PE yield was observed at higher field strength. This finding underscores the importance of field strength, even when the process configuration is not significantly altered. A similar finding was observed for the treatment time, where the sample treated with 0.38 kV/cm, 500 pulses, and 2.05 kJ/kg had a significantly higher yield ($p < 0.05$) than the sample treated with 0.93 kV/cm, 100 pulses, and 2.22 kJ/kg. The PEF treatment with the lowest energy input did not increase PE yield, whereas the treatment with the highest energy input yielded the highest PE.

It is known that increasing the applied voltage during PEF treatment of a biological matrix creates a stronger electric field, thereby facilitating electroporation. Cell membranes are naturally electrically charged across the intracellular and extracellular environments, creating a transmembrane potential. Applying a higher external voltage increases the transmembrane potential by accumulating charged particles on either side, thereby forming temporary or permanent pores in the cell membrane (Ranjha et al., 2021). Therefore, increasing the applied field strength (either by applying higher voltage or by reducing the spacing over which the field is applied) facilitates electroporation and enhances mass transfer between the intracellular and extracellular environments, thereby increasing the extraction yields of intracellular compounds (Naliyadhara et al., 2022).

In particular, the applied electric field and the consequent polarization of the cell align interfacial water dipoles, which are attracted through the lipid bilayer. As a result, the lipids rearrange around the water dipoles, forming hydrophilic pores (Tokman et al., 2013; Emon et al., 2025). The hydrogen bonds between lipids and water are stressed, allowing water to penetrate the cell and pores to stabilize (Tokman et al., 2013; Ji M-H. et al., 2022). While some pores (often small ones) initially expand and then contract when the electric field is removed (reversible electroporation), larger or clustered pores, as well as pores that existed for a longer time, do not close (irreversible electroporation) and lead to cell death (Kotnik et al., 2019; Qu et al., 2025).

This phenomenon has been observed for various compounds and products, where increasing the electric field strength has enhanced the extraction of lycopene and carotenoids from tomato peels (Luengo et al., 2014; Pataro et al., 2020), the extraction of phenolics from grape pomace (Carpentieri et al., 2023) and the extraction of proteins from rice bran (Thongkong et al., 2023). Martinez et al. (2017) used PEF to investigate the extraction of phycocyanin from the cyanobacterium *Arthrospira platensis* (also known as spirulina). They found that increasing the electric field strength to 25 kV/cm (from 20 kV/cm) doubled the yield at the lower pulse duration they tested (75 µs). On the contrary, the same experiment with the double pulse duration (150 µs) did not increase phycocyanin yield (Martínez, 2019). Similarly, increasing treatment time by increasing pulse width or the number of pulses has also shown a stronger effect of PEF on the treated product. The pulse shape can also play a role: for rectangular pulses, the applied field intensity remains higher and more stable for longer than with exponential-decay pulses, where the field intensity gradually decreases during pulse application. Longer treatments provide greater exposure of the product to electric fields, allowing more pulses to create pores and leading to a more efficient process (Naliyadhara et al., 2022; Alkanan et al., 2024).

One further parameter affecting the PEF treatment of foods is temperature. The possible thermal effects are due to ohmic heating, which occurs when an electrical current passes through a semiconductive food product. Even though ohmic heating is always present to some extent during PEF treatment, when the temperature increase is insignificant, the thermal effect is usually not considered; when the temperature increases significantly, the synergistic effect of PEF-induced electroporation and volumetric heating is considered (Xue and Farid, 2015; Rocha et al., 2018). In the present study, the temperature after all treatments did not increase by more than 2 °C. The only exception was observed for the sample treated with a 30 µs pulse for 500 pulses, which showed a temperature increase of 6.5 °C after treatment. However, a maximum temperature of 33.4 °C was recorded, which, according to the literature, has not been shown to adversely affect R-PE (Galland-Irmouli et al., 2000; Dufossé et al., 2005).

4 Conclusion

The present study explored which parameters are most appropriate for handling and processing the red macroalgae

Palmaria palmata to improve the extraction yield and stability of R-phycoerythrin. The most efficient extraction conditions were with a 0.02 M sodium phosphate buffer at a solid-to-liquid ratio of 1:20. The solvent-to-liquid ratio did not significantly affect the purity index. Reducing particle size via milling in liquid nitrogen significantly increased the R-PE yield by more than 100%, while also increasing PI. Storing the recovered extracts under light before further use can cause significant degradation of R-PE and may interact with storage temperature, further accelerating this degradation. The application of PEF increased the R-PE yield compared with untreated samples. Intensifying the PEF treatment by increasing the electric field strength or the process duration and consequently the specific energy input led to a significantly higher R-PE yield. Nevertheless, all PEF pre-treatments were less efficient than milling with liquid nitrogen in increasing the R-PE yield. More studies are required to better understand the influence of extraction conditions and parameters on R-PE from red macroalgae, while also considering additional technologies and algal species.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

LD: Investigation, Data curation, Conceptualization, Validation, Writing – review and editing, Visualization, Writing – original draft, Methodology, Formal Analysis. MP: Visualization, Writing – original draft, Data curation, Writing – review and editing. OS: Resources, Conceptualization, Funding acquisition, Writing – review and editing, Supervision, Writing – original draft, Project administration.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This work was supported by the Federal Ministry for Research, Technology and Aeronautics (BMFTR) based on a decision of the Parliament of the Federal Republic of Germany via the Project Management Jülich (PtJ) within the joint research project Agrarsysteme der Zukunft_2: F4F2 - Nahrung der Zukunft (Grant No. 031B1525C).

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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