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Microwave-assisted extraction of phycobiliproteins from *Porphyridium purpureum*

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Abstract :

In the present study, microwave-assisted extraction was first employed to extract the phycobiliproteins of *Porphyridium purpureum* (*Pp*). Freeze-dried *Pp* cells were subjected to microwave-assisted extraction (MAE) to extract phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC). MAE combined reproducibility and high extraction yields and allowed a 180- to 1,080-fold reduction of the extraction time compared to a conventional soaking process. The maximal PE extraction yield was obtained after 10-s MAE at 40 A degrees C, and PE was thermally damaged at temperatures higher than 40 A degrees C. In contrast, a flash irradiation for 10 s at 100 A degrees C was the best process to efficiently extract PC and APC, as it combined a high temperature necessary to extract them from the thylakoid membrane to a short exposure to thermal denaturation. The extraction order of the three phycobiliproteins was coherent with the structure of *Pp* phycobilisomes. Moreover, the absorption and fluorescence properties of MAE extracted phycobiliproteins were stable for several months after the microwave treatment. Scanning electron microscopy indicated that MAE at 100 A degrees C induced major changes in the *Pp* cell morphology, including fusion of the exopolysaccharidic cell walls and cytoplasmic membranes of adjacent cells. As a conclusion, MAE is a fast and high yield process efficient to extract and pre-purify phycobiliproteins, even from microalgae containing a thick exopolysaccharidic cell wall.

Highlights

- ▶ Phycobiliproteins are high value fluorescent microalgae pigments.
- ▶ Most phycobiliprotein extraction processes imply the use of ionic buffers or enzymes.
- ▶ *Porphyridium purpureum* phycobiliproteins can be efficiently extracted using MAE.
- ▶ MAE gives high extraction yields and reduces extraction time.
- ▶ Absorption and fluorescence properties of extracted pigments are not altered by MAE.

Keywords : Allophycocyanin, MAE, Microalgae, Microwave, Phycobiliprotein, Phycocyanin, Phycoerythrin, Porphyridium, Thylakoid

1. Introduction

Phycobiliproteins (PBP) are hydrophilic $\alpha\beta$ heterodimeric proteins constituting the major light-harvesting pigments of cyanobacteria, red algae, glaucocystophytes, and cryptophytes [1]. Both the α and β -subunits consist of an open-chain tetrapyrrole constituting the chromophore, linked to an N-terminal proteic extension involved in the subunit aggregation to $\alpha\beta$ protomers. The $\alpha\beta$ protomers (referred to as “monomer”) can further aggregate to form triangular-shaped trimers (heterohexamers) and hexamers (heterododecamers), that constitute the building blocks of phycobilisomes (PBS), the light harvesting antennae anchored in the thylakoid membranes [1]. In *Porphyridium purpureum* (*Pp*), PBS mostly contain PE, PC and APC (the three PBP) linked to colorless proteins associated to PE or attaching the PBS core to the thylakoid membranes [2]. PE, PC and APC are spatially organized to transfer energy from higher energy chromophores located in the distal part of the PBS (PE) to the lower energetic ones (PC and APC forming the PBS core) [1]. Because of their unique structural characteristics [1], PBP have intrinsic fluorescence properties which make them highly sensitive fluorophores for various fluorescence based techniques such as immunofluorescence, FACS and fluorimetric microplate assays [3, 4]. PBP are also used in cosmetics [5], nutraceuticals [6], and in oceanography studies as chemotaxonomic markers [1, 7, 8]. Furthermore, recent studies demonstrated that they exert a direct cytotoxicity in cancer cells and potentiate the effect of chemotherapeutic drugs [9–13]. Various processes were thus developed to extract and purify PBP from microalgae and seaweeds. Because phycobilisomes are strongly anchored in the thylakoid membrane, and protected from extraction solvents by a thick exopolysaccharidic cell wall, the first extraction processes used drastic techniques such as mechanical grinding using a French press, in high-ionic strength buffers to prevent phycobilisomes dissociation [1]. Alternative processes were proposed, such as the use of enzymes to hydrolyze the algal cell walls [14] and the use of sucrose density centrifugation to purify phycobilisomes combined to ammonium sulfate fractionation and precipitation, centrifugation, dialysis, isoelectric focusing, ion exchange chromatography and/or gel permeation to purify the chromoproteins [14–20]. Although they allow the purification of PBP with high purity, according to the absorbance ratio A_{PBP}/A_{280} , these processes are time, money and solvent consuming and involve tedious steps that can eventually decrease extraction yields. Microwave-assisted extraction (MAE) is an alternative innovative technology that is used to extract pigments [21, 22], lipids and bioactive molecules from plants, spices, seaweeds and microalgae [23, 24]. The main advantages of MAE are a significant reduction in extraction time, reduced solvent consumption, and high extraction yields that can be increased from 50 to 500% compared to conventional extraction processes [25]. MAE is not restricted to thermoresistant molecules, as the shortened extraction time allows the non-damaging extraction of thermolabile constituents such as human proteins [26], plant polyphenols [27–29], essential oils [30, 31], or microalgae pigments [21]. MAE can also be developed with eco-friendly solvents [32] and be performed at low temperature under vacuum (VMAE) [28] or without the addition of external solvent (the molecules are then solvated in the water sample and collected in a cold trap) . We thus hypothesized that MAE was a high performance process for the extraction of PBP from marine microalgae, and selected *Porphyridium purpureum* as a model species to perform the MAE of phycocyanin (PC), phycoerythrin (PE) and allophycocyanin (APC). The MAE performance was compared to the conventional soaking process on the basis of extraction yields, purity of the extracted PBP, and extraction duration, after optimization of MAE parameters.

2. Material and methods

2.1. Microalgae

Porphyridium purpureum CCAP 1380.3 (bangiophyceae, rhodophyte) was selected as it contains three PBP (PE, PC and APC) in high amounts [2, 33]. *Pp* is one of the most used species to purify PBP, and its exopolysaccharidic cell wall constitutes a mechanical barrier that limits pigments extraction using conventional soaking processes [34, 35].

2.2. Microalgae culture, harvest and freeze-drying

Pp was grown at $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance to achieve a good compromise between growth, biomass and production of PBP [33]. Cells were grown in four units of 50 L column photobioreactors with 35 ‰ salinity seawater enriched by Walne medium [36]. Batch cultures were maintained at 20°C under continuous light provided by fluorescent lamps (Philips TLD 58W 865) and bubbled with 0.22- μm filtered air containing 3% CO₂ (v/v). Microalgae were harvested after 12-16 days of growth and separated from the culture medium by a two-step process. First step used a clarifier separator (Clara 20, Alfa Laval Corporate AB, Sweden) at 100 L·h⁻¹, 9000 g, room temperature. Step two used a soft centrifugation at 4000 g, 20mn, 4°C to separate the slurry. Algal paste was freeze-dried at -55°C and P < 1 hPa, on a freeze-dryer equipped with a HetoLyoPro 3000 condenser and a Heto cooling trap (Thermo, France) to express extraction yields in micrograms of pigments per mg of dry microalgae.

2.3. PBP extraction

MAE was performed using a Biotage initiator microwave reactor (Figure 1.), at atmospheric pressure and in deionized water as PBP are hydrophilic pigments. Because PBP are thermosensitive proteins [37], the extraction temperature and exposure duration to high temperatures are two critical parameters. Optimization of the MAE parameters aimed to efficiently destroy pigments extraction barriers (*Pp* exopolysaccharidic cell wall and PBP anchoring in the thylakoid membrane) and obtain high extraction yields in a fast process, limiting the risk of thermal damage of the extracted PBP. MAE was thus performed from 40°C (lower temperature available in the Biotage reactor) to 120°C, for durations ranging from 10 s (flash irradiation) to 5 min. The solid-to-solvent ratio was constant and fixed to 20 mg freeze-dried *Pp* cells suspended in 7 ml deionized water. MAE was performed in sealed microwave vials, in triplicate independent assays, under constant magnetic stirring (600 rpm).

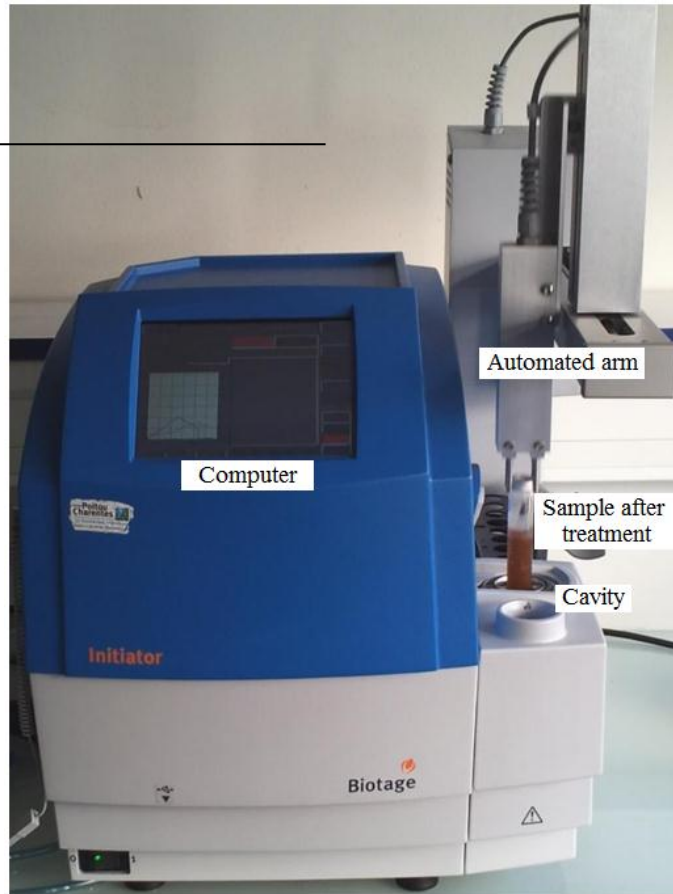


Figure 1. Photograph of the Biotage initiator microwave reactor after PBP extraction using the MAE process. Microwaves irradiation evokes an instantaneous and homogeneous heat transfer in the sample, inducing microalgal cell wall disruption and solid-liquid extraction of thylakoid pigments.

After MAE, extracts were centrifuged (4500 g, 4°C, 5 min) to pellet cell debris and the supernatants were collected in brown vials for PBP dosage. For the absorbance and fluorescence stability study, PBP were stored in brown vials at 4°C before dosage. In order to quantify the reduction of extraction time offered by MAE, soaking extracts were also prepared using the same time, temperature and solvent-to-solid ratios as MAE.

2.4. PBP absorbance

Absorbance of the pigments solutions was recorded at 280, 545, 565, 620 and 650 nm on a Perkin-Elmer lambda 650 UV-visible spectrophotometer. The PE, PC and APC massic concentrations (g.L⁻¹) were calculated using the following equations, according to [38–40].

$$[PC] = \frac{OD_{620} - \left(\frac{APC_{620}}{APC_{650}} \right) * OD_{650}}{PC_{620} - \left(APC_{620} * PC_{650} \right) / APC_{650}} \quad (1)$$

$$[APC] = \frac{OD_{650} - \left(\frac{PC_{650}}{PC_{620}} \right) * OD_{620}}{APC_{650} - \left(APC_{620} * PC_{650} \right) / PC_{620}} \quad (2)$$

$$[\text{PE}] = \frac{\text{OD}_{545} - [\text{PC}] * \text{PC}_{545} - [\text{APC}] * \text{APC}_{545}}{\text{PE}_{545}} \quad (3)$$

The massic extinction coefficients of the 3 PBP were measured with standards solutions (Table 1) and used to simplify the above three equations to (4), (5) and (6) respectively.

(L.g ⁻¹ .cm ⁻¹)	OD 545 nm	OD 620 nm	OD 650 nm
PC	1.26	3.69	0.75
PE	5.73	-	-
APC	0.62	3.99	6.39

Table 1. Massic extinction coefficients of standard phycocyanin, phycoerythrin and allophycocyanin.

$$[\text{PC}] = \frac{(\text{OD}_{620} - 0.62 * \text{OD}_{650})}{3.22} \quad (4)$$

$$[\text{APC}] = \frac{(\text{OD}_{650} - 0.20 * \text{OD}_{620})}{5.73} \quad (5)$$

$$[\text{PE}] = \frac{(\text{OD}_{545} - 1.26 * [\text{PC}] - 0.615 * [\text{APC}])}{5.53} \quad (6)$$

2.5. PBP fluorescence

The fluorescence spectrum of extracted PBP was recorded using a Hitachi F-2500 spectrofluorimeter. Fluorescence was measured from 200 to 800 nm (1500 nm.min⁻¹, slit 2.5 nm) after excitation at 488 nm for PE, 320 nm for PC and 330 nm for APC.

2.6. SDS-PAGE of MAE extracted PBP

Ten microliters of MAE extracts or PBP standards were mixed with 5 µL loading buffer consisting in Tris HCl 50 mM pH 6.8, Glycerol 10 %, SDS 2.5 %, Bromophenol blue 0.002 %, without β-mercaptoethanol and at ambient temperature. PBP were then separated at 50 V for 15 min and 150 V for 75 min on a Mini-protean TGX 12 % gel using a Bio-Rad Power Pack apparatus (Bio-Rad, France). Standard PBP were obtained from Sigma-Aldrich France and consisted, according to the manufacturer, in phycocyanin from *Spirulina platensis* (MW of the native protein = 112 KDa), synthesized allophycocyanin (MW of the native protein = 104 KDa) and phycoerythrin from *Pp* (MW of the native protein = 240 KDa). The prestained PageRuler Plus protein ladder (10 - 250 KDa, Thermo, France) was used as molecular weight marker.

2.7. Scanning electron microscopy (SEM) observation of *Pp* cells

Pp cells were freeze-dried before or after MAE, placed on a conductive double layer carbon support and examined by SEM using a Philips-FEI Quanta 200 ESEM/FEG microscope (environmental mode) equipped with a FEG canon delivering 1 to 30 KV beam current. The backscattered electron mode was used to get a contrast between inorganic salts and biological membranes.

2.8. Statistical analysis

The unpaired Student *t* test ($n = 3$, triplicate independent extractions for each condition) was used to determine if MAE in optimized conditions significantly increased the extraction yield compared to soaking, and if the MAE temperature had a significant positive or negative effect on PE, PC and APC extraction yields for a selected extraction time.

3. Results

3.1. Impact of MAE on *Pp* cell integrity

SEM observation of *Pp* cells before or after MAE (Figure 2.) revealed that the freeze-dry process did not fully disrupt the cytoplasmic membrane, but only evoked a partial cell shrinkage and clustering. These data were coherent with our previous observations with *Dunaliella tertiolecta* (chlorophyte) and *Cylindrotheca closterium* (bacillariophyte) [21]. The presence of salts precipitates surrounding *Pp* freeze-dried cells was evident using the backscattered electron mode and revealed by the contrast between the dark organic material and the very bright salts cristals (Figure 2 B).

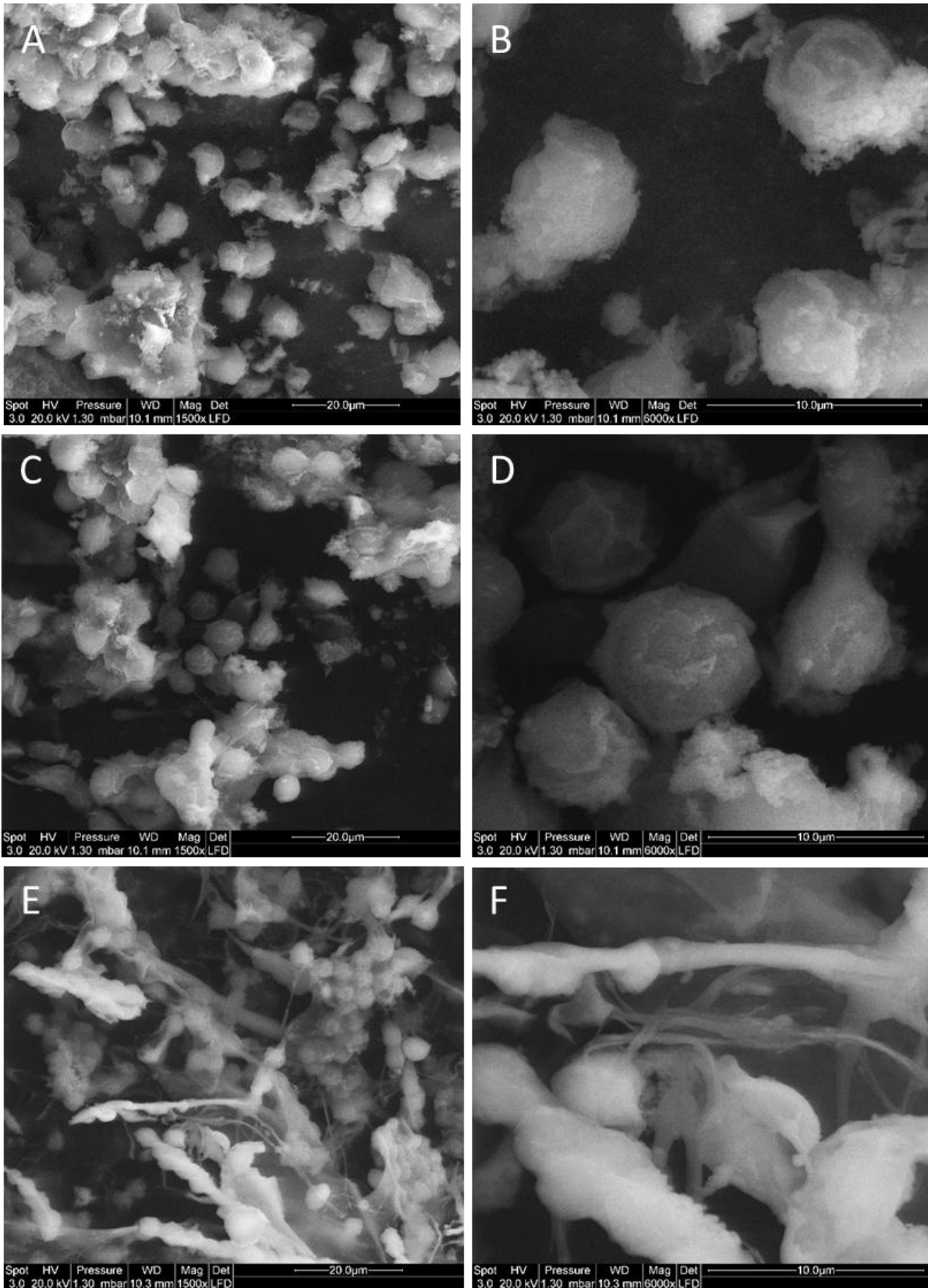


Figure 2. SEM observation of freeze-dried *Pp* cells before MAE extraction (A and B), after 10 s MAE at 40°C (C and D), or after 30s MAE at 100°C (E and F). Magnification: x 1500 (A, C and E); x 6000 (B, D and F).

The presence of a thick exopolysaccharidic (EPS) cell wall surrounding *Pp* cells [34] probably participates to the conservation of the cell morphology during the freeze-dry process by limiting the risk of tearing the cytoplasmic membrane during ice crystals formation. MAE at 40°C had low influence on the cell morphology, although the PE extraction yields at 40°C (see below) evidenced that the inner content of *Pp* cells was accessible for the extraction solvent and that the PBS constituents were partially dissociated from the thylakoid membrane. Occasional fusion of the cell walls surrounding adjacent cells could be observed (Figure 2 D). MAE at 100°C evoked major changes of the *Pp* cells morphology, including systematic fusion of the cell walls in adjacent cells and appearance of long thin filaments that were identified as a mix of cytoplasmic membranes fused with the EPS cell walls (Figure 2 F). PC and APC extraction yields at 100°C (see below) confirmed that the thylakoids membranes were deeply disrupted and that the pigments extractability was not limited by the solvent diffusion across the cell wall and cytoplasmic membrane.

3.2. MAE of phycoerythrin

The maximal PE extraction yield ($73.7 \pm 2.3 \mu\text{g} \cdot \text{mg}^{-1}$) was obtained after 10 s irradiation at 40°C (Figure 3.). Increasing the irradiation time up to 5 min at 40°C had no impact on PE extraction yield, indicating that all extractable PE was recovered during the very first seconds of microwave irradiation, and confirming the previously reported thermostability of PE at 40°C [37].

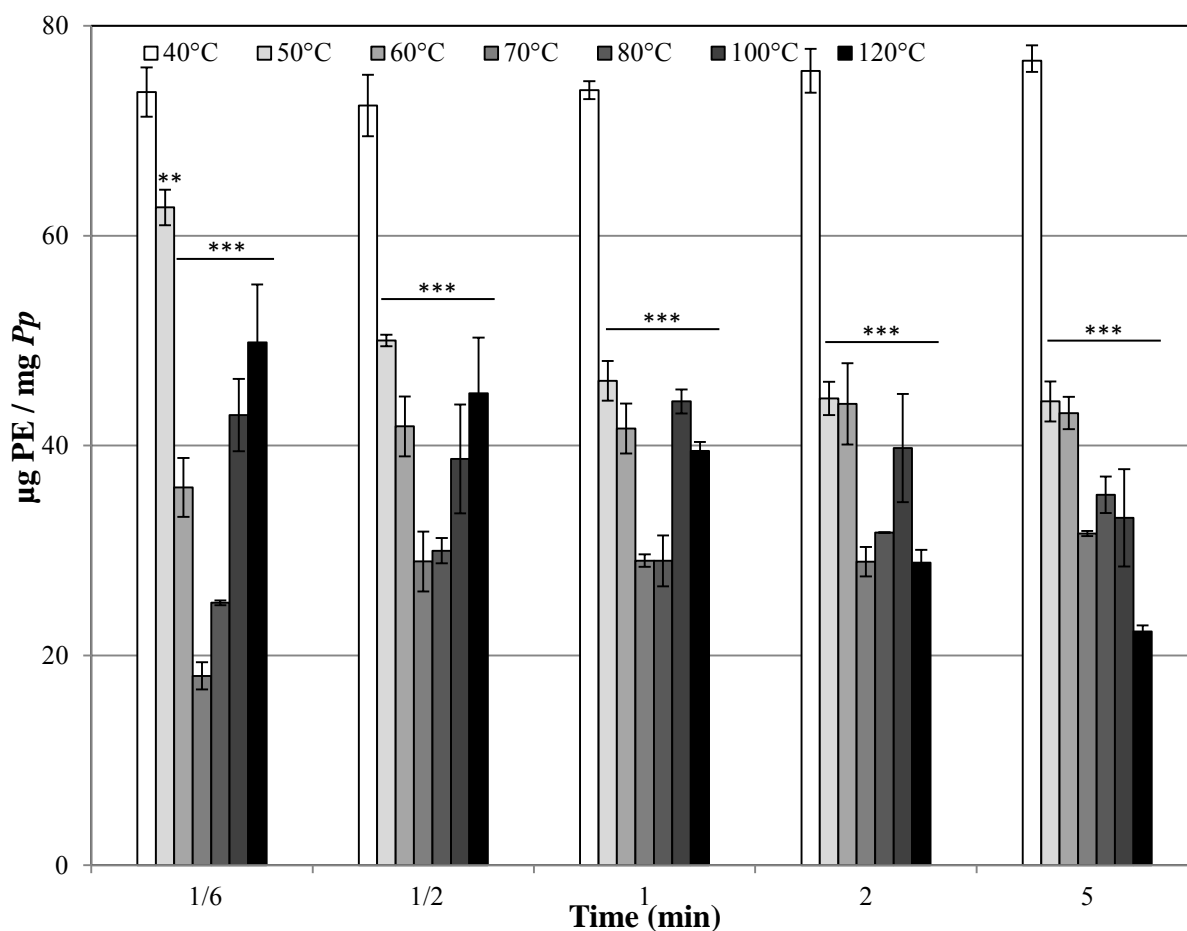


Figure 3. Extraction yields of PE after MAE in a large range of temperatures (40-120°C). The maximal PE extraction yield was obtained after 10 s irradiation at 40°C.

The PE maximal extraction yield was 54.6 fold superior to the value obtained by the aqueous two-phase extraction of *Pp* PE [41]. Although this high extraction yield indicates that MAE is a powerful extraction process compared to aqueous two-phase extraction, additional criteria including purity indexes, industrial scalability, and biomass cost have to be considered to define the global efficiency of the process. MAE gave a PE purity index of 2.2, while soaking and aqueous two-phase extraction respectively lead to purity indexes of 1.3 and 2.8 [42] to 4.3 [41] (Table 2). Considering that the current industrial microwaves reactors can contain up to 70 liters of sample [43], a theoretical mass of 14.74 g of PE could be extracted in a single 10 s MAE from 200 g dry algae, according to our extraction yield and using our solid-liquid ratio. Observation of a maximal PE extraction yield at a relatively low temperature was coherent with the structural model proposed for the *Pp* PBS, in which PE, located at the distal part, is the most accessible of the three PBP. Using conventional soaking in water at 40°C, 60 min were necessary to get an equivalent extraction yield (data not shown), indicating that MAE allowed a 360 fold reduction of the extraction time. For an equivalent extraction time, the difference in PE extraction yields between MAE and soaking was extremely statistically significant ($p < 0.0001$ using the Student unpaired t test). PE corresponded to 83.6 to 84.1% of the total PBP massic content in the MAE extract, compared to 68 to 71% with the soaking process. Thus, MAE not only increased extraction yields but also increased the PE extraction selectivity, and this may be explained by the very short extraction time. Increasing the MAE temperature from 50°C to 100°C significantly decreased the extraction yield, confirming the thermal damage of PE at temperatures superior to 40°C (Figure 3.).

3.3. MAE of phycocyanin and allophycocyanin

The maximal extraction yields of PC and APC was twice to three times lower than that of PE, in accordance with the PC/APC/PE ratio described in the *Pp* PBS model. Irradiation at temperatures ranging from 40 to 80°C gave low extraction yields (5 to 10 $\mu\text{g}\cdot\text{mg}^{-1}$; Figures 4. and 5.), that were explained by the weak extractability of the two pigments tightly bound to the thylakoid membrane compared to PE. The extraction yields significantly increased when the sample was treated at temperatures over 100°C, and the morphological modifications of *Pp* cells at these temperatures confirmed that the pigments extraction barriers were strongly disrupted. However, increasing the irradiation time damaged the extracted PC and APC, as shown by the decrease of extraction yields (Figures 4. and 5.). As a conclusion, a flash irradiation at 100°C was the best MAE process to extract PC or APC, as it combined a high energy, necessary to extract them from the thylakoid membranes and break the exopolysaccharidic cell wall, to a rapid treatment to limit the thermal damage of extracted pigments.

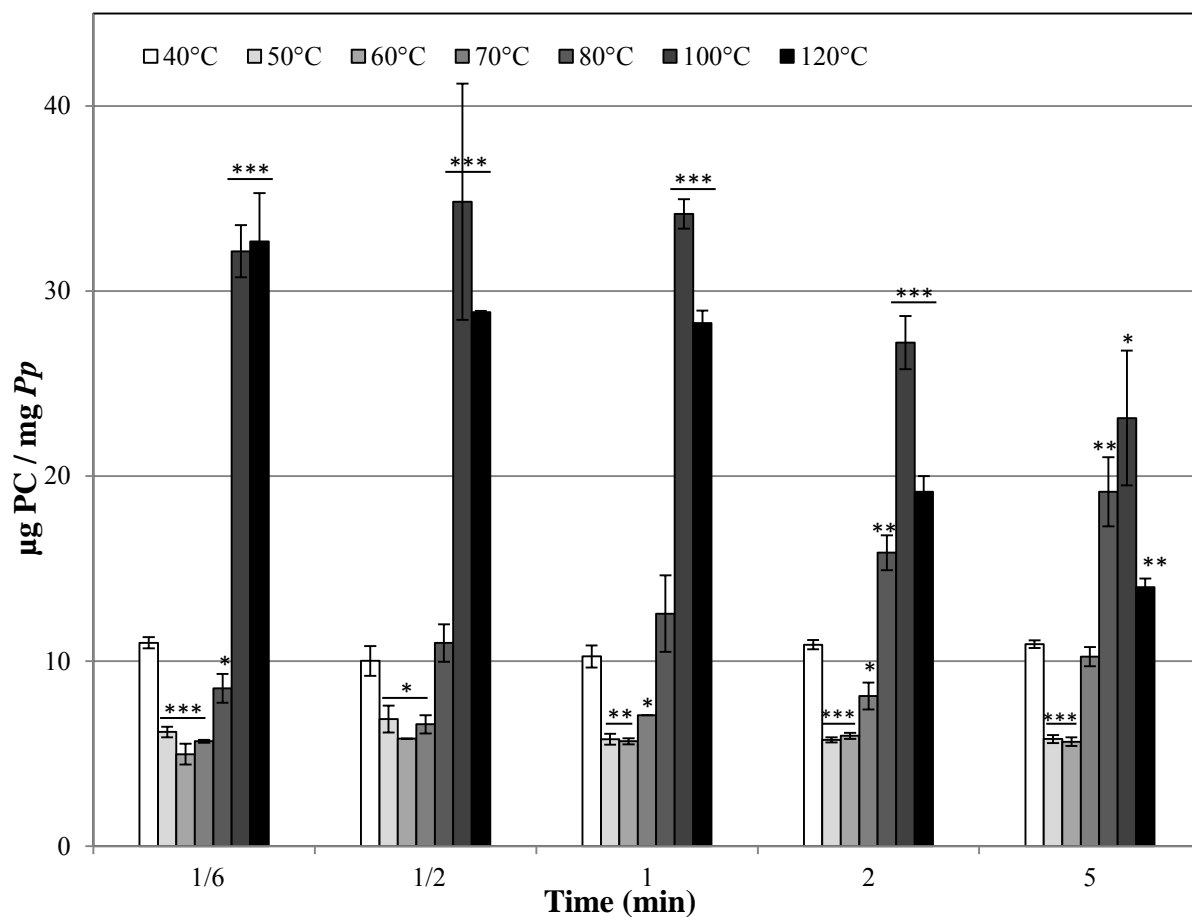


Figure 4. . Extraction yields of PC after MAE in a large range of temperatures (40-120°C). The maximal extraction yield was obtained after a 10 s MAE irradiation at 100°C.

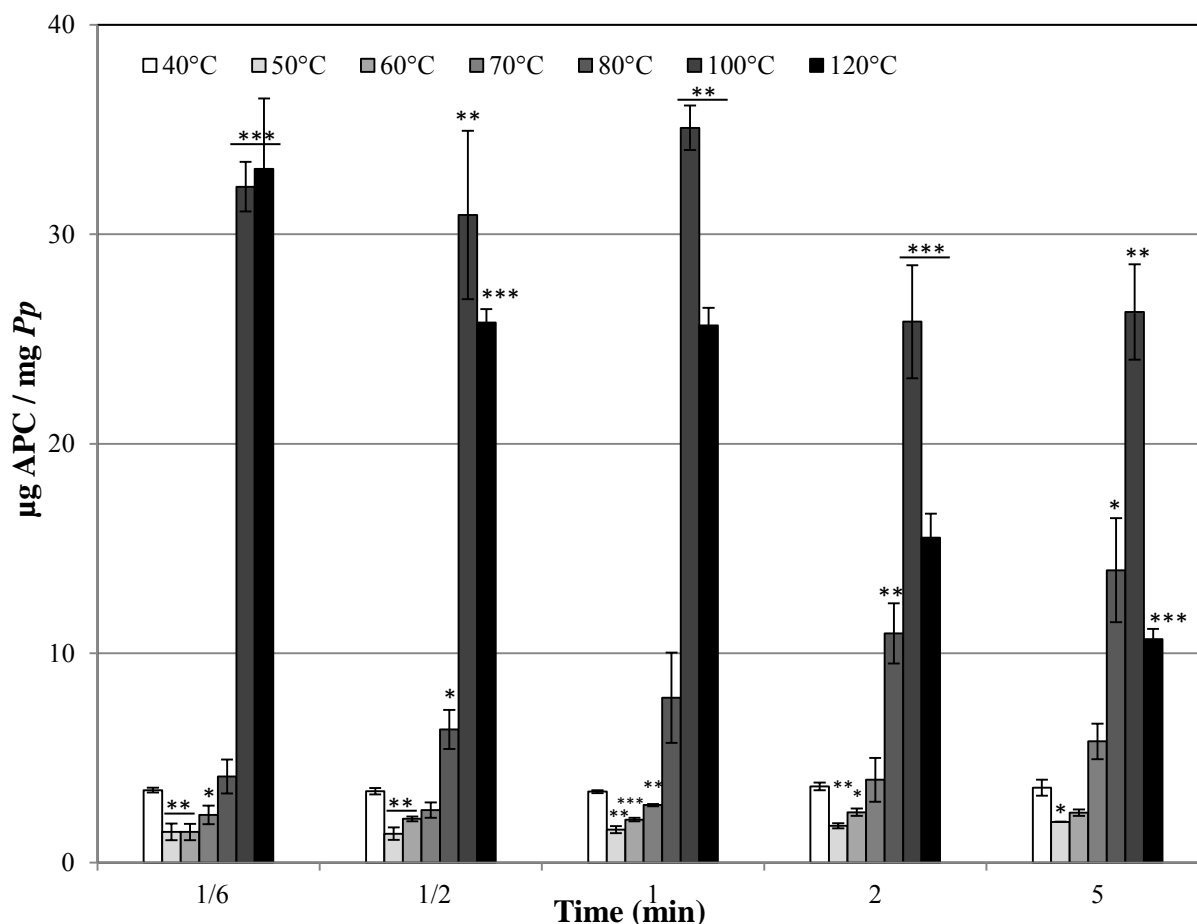


Figure 5. Extraction yields of APC after MAE in a large range of temperatures (40-120°C). The highest extraction yield was obtained in 1 min at 100°C.

The maximal PC extraction yield ($34.8 \pm 6.4 \mu\text{g.mg}^{-1}$) was obtained after 10 s MAE at 100°C, and was equivalent to that measured after 60 to 180 min soaking (data not shown), indicating a 360 to 1080 fold reduction of the extraction time. For APC, the flash irradiation 10s at 100°C gave a high yield ($32.3 \pm 1.2 \mu\text{g.mg}^{-1}$) and 1 min MAE at 100°C (180 fold reduction of the extraction time compared to soaking) was necessary to obtain the highest extraction yield ($35.1 \pm 1.1 \mu\text{g.mg}^{-1}$) (Figure 5.), demonstrating that APC is more tightly bound to the thylakoid membrane than PC, in accordance with the *Pp* PBS model. In these optimized conditions, PC and APC respectively represented up to 33.3% and 30.9 % of the total PBP present in the extract, and PE thermal damage at 100°C probably improved the selectivity of PC and APC extraction (Table 2).

Table 2. Performance of optimized MAE processes to extract and purify PBP from *Pp*. The maximal extraction yields and purity indexes obtained by MAE are compared to the values obtained using soaking or previously reported processes.

PBP	Process	Extraction Yield ($\mu\text{g.mg}^{-1}$)	Purity
PE	MAE 10s 40°C	73.7	2.2
	MAE 5 min 40°C	76.7	2.1
	Soaking 10s 40°C	12.0	0.4

	Soaking 300s 40°C	19.4	0.5
	Soaking 240 min 40°C	92.8	1.3
	Bead mill + Isoelectric precipitation + Aqueous Two-Phase extraction < 10°C	1.35 after cell disruption	2.8 [42] to 4.3 [41] (highly purified)
PC	MAE 10s 100°C	34.8	0.4
	Soaking 10s 100°C	1.9	0.1
	Soaking 60 min 100°C	41.9	0.4
	No available data for PC extraction from <i>Pp</i> (data from <i>Spirulina</i> expressed in % [44])		
APC	MAE 10s 100°C	32.3	0.3
	MAE 1 min 100°C	35.1	0.3
	Soaking 10s 100°C	1.2	0.3
	Soaking 60 min 100°C	44.5	0.4
	No available data for APC extraction from <i>Pp</i> (data from <i>Spirulina</i> expressed in % [44])		

3.4. Optimization of a two-steps MAE process to successively extract PE, PC and APC with high yields from the same *Pp* sample

The best extraction sequence was defined as a first MAE 10 s at 40°C allowing the PE extraction with a selective extraction yield of 84.1%, followed by a second MAE 30 s at 100°C on the remaining pellet, allowing the PC extraction with a selective extraction yield of 29.5 % and APC extraction with a selective extraction yield of 31.1%. One centrifugation step was necessary to collect the concentrated PBP solutions after each microwave irradiation. Soaking allowed to obtain high selective extraction yields but the whole performance of the soaking process was very limited by the total extraction time (270 min against 40 s in MAE).

3.5. Absorbance and fluorescence properties of MAE extracted PBP

MAE extracted PBP using the optimized protocols were stored at 4°C in water solutions and their absorbance and fluorescence were recorded daily to check that their properties and stability were not altered by the MAE treatment. No major absorbance variation was recorded for the 3 PBP for up to 15 days for PE and 6 months for PC and APC (Figure 6.), demonstrating the stability of PBP absorption properties after MAE and their possible storage at 4°C without important absorbance intensity fade out. In a same way, no fluorescence fade out was observed during a cold storage of PC for more than 2 months while PE and APC fluorescence progressively lost 30% intensity in 75 days (Figure 6.). These data are coherent with a previous study indicating a good stability of absorbance and fluorescence properties of PE, in a broad range of pH (4.0-10.0) for 25 days at room temperature, while PC and APC are more sensitive to thermal denaturation [45].

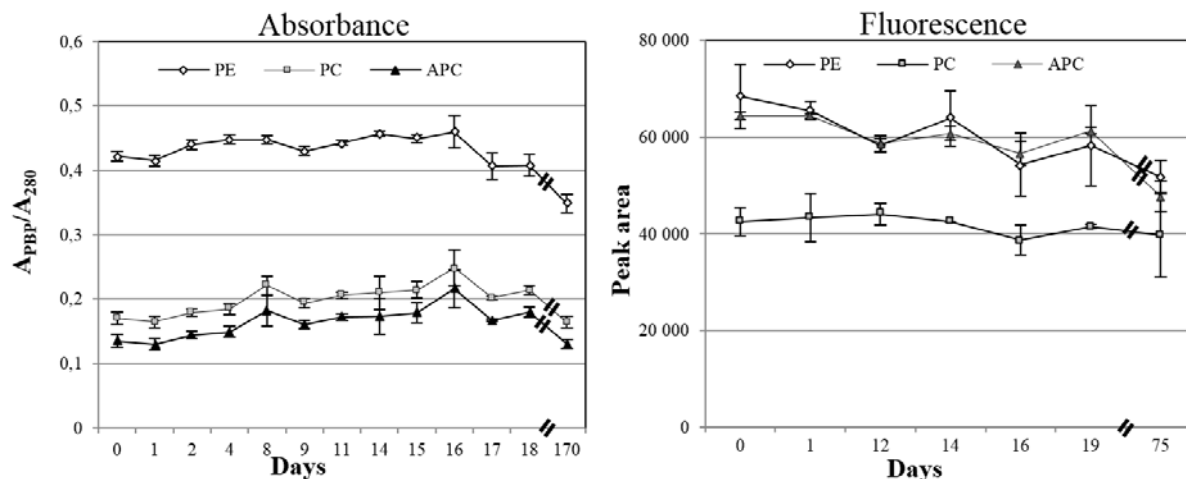


Figure 6. Absorbance and fluorescence of MAE extracted PBP. MAE extracted PBP were kept at 4°C in obscurity and their absorption and fluorescence properties were measured over several weeks after the microwave treatment.

Molecular weight of MAE extracted PBP

Standard PE gave a major red band at 16 KDa that was identified as a mix of α and β subunits [1] (Figure 7.). No band corresponding to an $\alpha\beta$ subcomplex was observed, suggesting that free $\alpha\beta$ protomers disassembled during PE purification. As the SDS-PAGE was performed without β -mercaptoethanol, it also demonstrated that no disulfide bridge linked the α and β subunits to form $\alpha\beta$ protomers. The bands observed at 64, 132 and 155 Kda may respectively correspond to $(\alpha\beta)_2$, $(\alpha\beta)_3\gamma$ and $(\alpha\beta)_4\gamma$ subcomplexes [1]. The *Pp* MAE extract at 40°C contained a major red band at 136 KDa that could correspond to an $(\alpha\beta)_3\gamma$ subcomplex of PE subunits, and two minor bands at 15 KDa (α and β subunits) and 34 KDa ($\alpha\beta$ subcomplex or less probably γ subunit) [1]. Standard PC, APC and MAE extracts at 100°C for 30 s and 60 s gave bands at 12-15 KDa corresponding to the PC and APC α and β subunits [2] (Figure 7.).

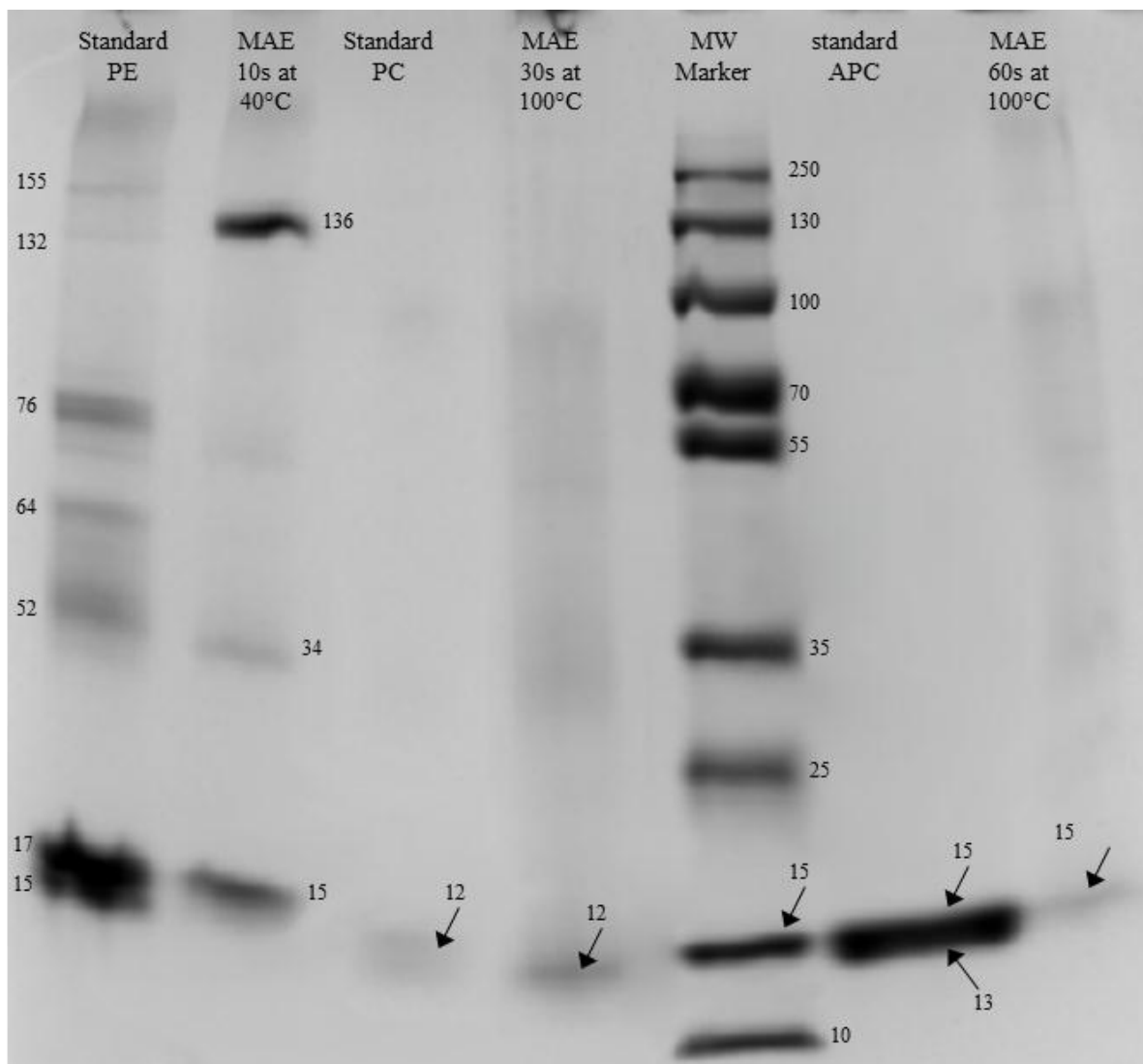


Figure 7. SDS-PAGE electrophoretic pattern of MAE extracted PBP and PBP standards. Molecular weights are expressed in KDa.

4. Discussion

We describe here the successful MAE extraction and pre-purification of phycobiliproteins from *Pp*. MAE combined the qualities of a powerful pigment extraction process: rapidity, easiness, low cost, eco-efficiency, reproducibility, high extraction yields, possibility to selectively extract one PBP, possibility to couple the extraction process with a purification process such as gel permeation, and possibility to work in controlled conditions to limit the thermal degradation of PBP and conserve the spectroscopic qualities of the extracted pigments for several months. We had previously demonstrated that MAE was very efficient to extract pigments from frustulated microalgae, and we show here that it also presents a high interest to get access to pigments deeply buried in thylakoid membranes of species synthesizing a thick exopolysaccharidic cell wall. Compared to soaking, the use of microwaves accelerated pigments extraction kinetics up to 1080 fold, and improved pigment purity up to 3.8 fold before coupling to the purification step. Microwave heating offered the advantage of delivering an instantaneous and homogeneous temperature in the medium, as no heat transfer was required to heat the cells located in the center of the tube. Moreover, MAE allowed a fast and efficient PBP extraction without the need of high pressures, enzymes

(lysozyme, xylanase), salts, toxic solvents, mechanical grinding or sonication. Consequently, limiting the steps number during the extraction process improves the final extraction yields and avoids time-consuming and fastidious protocols. According to the wide range of applications of PBP, and to their economic interest, the development of microwaves pilot units and industrial microwaves reactors should be considered to extract them at industrial scale. Indeed, PBP represent 28 to 68% of the total *Pp* soluble proteins (2.7 to 9.1 pg PBP / *Pp* cell), and their expression can be increased by decreasing irradiance during the microalgae growth [33]. PE is the major PBP in *Pp*, representing about 75% of the total PBP massic content, while PC and APC account together for 25%. Beyond its utility for PBP extraction, this study opens the way for further MAE developments to extract thermosensitive molecules from various marine microalgae species, including those containing a thick exopolysaccharidic cell wall.

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