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Research Article

Variation in the Biochemical Composition of the Edible Seaweed *Grateloupia turuturu* Yamada Harvested from Two Sampling Sites on the Brittany Coast (France): The Influence of Storage Method on the Extraction of the Seaweed Pigment R-Phycoerythrin

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Numerous studies have demonstrated that the biochemical content of seaweeds varies according to seasonality in a restricted area. In this study, the influence of sampling site on the biochemical composition of the edible red seaweed *Grateloupia turuturu* Yamada was investigated, but not its variation over time. Some differences in water-soluble protein ($7.19 \pm 0.74\,mg \cdot g^{-1}$ dw and $19.15 \pm 0.47\,mg \cdot g^{-1}$ dw), water-soluble carbohydrate ($16.05 \pm 0.82\,mg \cdot g^{-1}$ dw and $41.57 \pm 0.66\,mg \cdot g^{-1}$ dw), and lipid contents ($28.07 \pm 5.12\,mg \cdot g^{-1}$ dw and $54.35 \pm 2.05\,mg \cdot g^{-1}$ dw) were recorded between the two sites chosen on the Brittany coast (France). The yield of R-phycoerythrin (R-PE) contained in the seaweed also varied according to the sampling site ($1.16 \pm 0.33\,mg \cdot g^{-1}$ dw versus $4.39 \pm 0.15\,mg \cdot g^{-1}$ dw). In addition, the effect of storage conditions on the preservation of R-PE was studied. The results demonstrated that freezing is the best preservation method in terms of R-PE extraction yield and purity index. In conclusion, this study shows that the sampling site influences the biochemical content of the red seaweed *Grateloupia turuturu*. Moreover, the extraction yield of R-phycoerythrin and its purity index depend on both the sampling site and the sample storage method.

1. Introduction

For many years, marine ecosystems have been subjected to species introductions. Particularly on the Atlantic West Coast of Europe, many seaweed species have been introduced from Asian countries such as China, Korea, and Japan [1]. In these countries, seaweeds are used as human food and provide valuable nutrition [2]. In addition, the R-phycoerythrin pigment extracted from red seaweeds is used in Japan as a food colorant. In European countries, there is no tradition of the use of algae as sea vegetables. Thus, they are mainly harvested for the production of hydrocolloids such as alginates, agar, or carrageenans [3]. However, due to the recent interest in nutrition, the food value of seaweeds has been reassessed by considering the significant amounts of proteins, vitamins, minerals, and lipids which they contain. In France, seaweed consumption is subject to specific regulations [4]. Information about their chemical composition and its variation must be provided for them to be authorized as sea vegetables. To assess the nutritional value of seaweed used as a food product, the determination of its biochemical composition is the first step. The species used as sea vegetables are mainly red and brown algae [5]. Currently, in France, only four red seaweed species are authorized for human consumption

[4], whereas 300 red seaweeds have been identified on the Brittany coast. *Grateloupia turuturu* Yamada is an alien red seaweed species, introduced with the importation of the Japanese oyster Crassostrea gigas in the Mediterranean Thau Lagoon [6]. It was subsequently identified in Brittany at Fort Bloqué (Ploemeur, Morbihan), and its distribution in the Atlantic now extends from the Netherlands to the Canary Islands.

The exploitation of this resource has thus become an ecological and industrial challenge. This seaweed is already used as a sea vegetable in Japan and it contains, like all red algae, R-phycoerythrin ($3.0 \pm 0.3\,\text{mg}\cdot\text{g}^{-1}$ dw) [7], a pigment which plays a key role in photosynthesis [8]. Phycoerythrins are commonly used for various fields of applications, such as immunology, cell biology, flow cytometry, food, and cosmetics [9, 10] and have a high added value (currently up to 300 €/mg). *Grateloupia turuturu* is therefore a biomass of industrial interest. However, studies performed on other algae clearly show that their chemical composition varies according to the species, the geographic area, and the season or environmental conditions [11]. Many studies [12] have investigated not only the long-term adaptation strategies (at the cellular level) of red algae by changes in the anatomy of the thallus, in the cell wall, and differences in chloroplast morphology and thylakoid organization [13], but also the short-term adaptation at the molecular level, including alterations in pigmentation and in photosynthetic membrane composition and functionality [14]. Denis et al. [7] showed seasonal variations in the chemical composition of *G. turuturu* and its potential nutritional value.

In this context, the influence of two sampling sites on the biochemical content of *G. turuturu* was studied with the objective of adding to the knowledge of the criteria which play a role in the biochemical composition of this seaweed. In addition, the effects of the sampling site and two storage methods (freezing and freeze-drying) on the extraction of R-PE were investigated.

2. Materials and Methods

2.1. Sampling. The thalli of *G. turuturu* Yamada (Rhodophyta, Halymeniaceae) were collected on May 18, 2010 during the same tide at two field sites in the intertidal zone of the Atlantic Coast, France: Le Croisic (47°17′53.5″N-2°32′38.6″O) (A) and Batz-sur-mer (47°16′33.2″N-2°29′39.8″O) (B) (Figure 1). Epiphytes were removed and samples were rinsed successively with tap water then distilled water. Subsequently, the algae were immediately frozen.

2.2. Storage. For both sites (A and B), one part of the samples (1) was freeze-dried and studied. Another part of the frozen algae (2) was immediately freeze-dried and then stored for 6 months at room temperature in darkness before extraction and analysis. The last part (3) was frozen and stored for 6 months at −20°C in darkness then freeze-dried just before analysis (Figure 2).

2.3. Extraction of Water-Soluble Compounds. Freeze-dried samples were ground in liquid nitrogen, and the resulting powder was homogenized with sodium phosphate buffer (20 mM; pH 7.1). The extraction was performed with a 1/20 ratio (*w/v*) for 20 min. This procedure and most subsequent manipulations were carried out at low temperature (4°C). Then, the suspension was centrifuged (25,000 ×g, 20 min, 4°C). The resulting supernatant [the aqueous crude extract (CE)] contained the water-soluble compounds. The extractions were performed in triplicate.

2.4. R-Phycoerythrin Determination. R-PE concentration and purity were determined spectrometrically using the classic Beer and Eshel equation (1) [15] and the A_{565}/A_{280} ratio

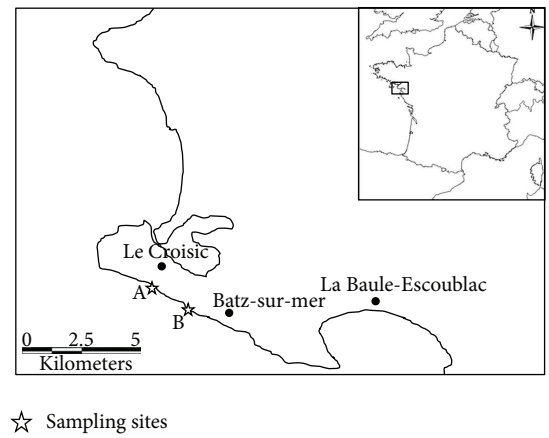

Figure 1: Location of the two field sampling sites of *Grateloupia turuturu* along the Atlantic Coast (France).

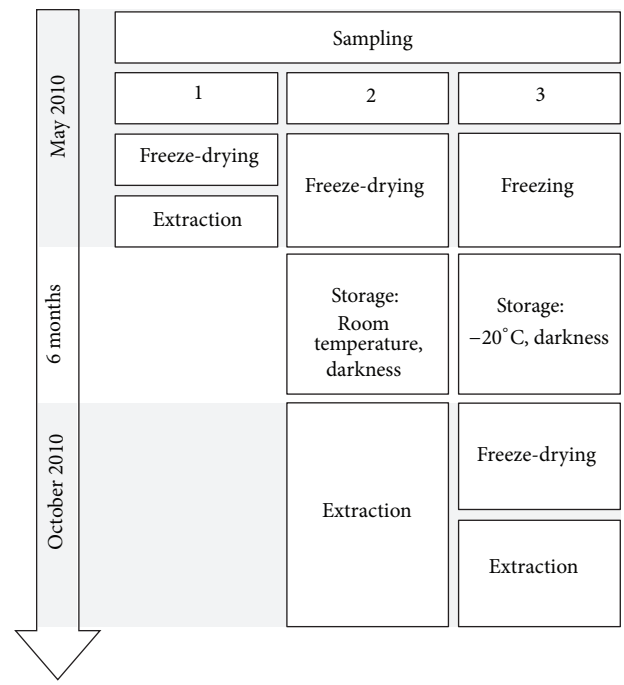

Figure 2: *Grateloupia turuturu*: protocol for the sampling strategy and storage mode.

(=Purity Index or PI) [16–20], respectively. R-PE yield was expressed as mg·g^{-1} dw.

$$[\mathrm{R-PE}] = \left[\left(A_{565} - A_{592}\right) - \left(A_{455} - A_{592}\right) \times 0.20\right] \times 0.12. \quad (1)$$

The R-PE absorption spectra displayed three peaks: two at 495 and 545 nm and one main peak at 565 nm. The spectral profile is commonly used to indicate the nondegradation of R-PE.

2.5. Water-Soluble Carbohydrates. Total water-soluble carbohydrates were analyzed in the CE using the modified colorimetric phenol-sulfuric acid method [21]. Phenol at 5% (200 μL) was added to 200 μL of sample solution or glucose solution followed by 1 mL of concentrated sulfuric acid. The tubes were allowed to stand for 10 min at room temperature before vortexing (10 sec at 3000 rpm), then for 15 min at room temperature and 30 min at 35°C before absorbance was measured at 490 nm. Glucose was used as a standard (range from 0 to 100 mg/L).

2.6. Water-Soluble Proteins. Total water-soluble proteins in the CE were analyzed by the method adapted from Bradford [22]. Bradford reagent (Sigma) (200 μL) was added to 800 μL of sample solution. The absorbance measurement at 595 nm (read immediately after the reaction) and the use of BSA (Sigma) as a standard (from 0 to 50 mg/L) enabled the protein content to be determined.

2.7. Total Nitrogen. The organic nitrogen content was quantified by the Kjeldahl method [23], and an estimate of the total protein content was calculated by multiplying the nitrogen content by a factor of 6.25 [24].

2.8. Ammonium Sulfate Precipitation. The CE was salted out with $(NH_4)_2SO_4$ at 80% saturation at 4°C for 2 h and collected by centrifugation at 25,000 ×g for 20 min at 4°C. The precipitated proteins were resuspended in sodium phosphate buffer (20 mM; pH 7.1). The protein suspension, named protein extract (PE), was collected and stored at 4°C in the dark. R-PE was also analyzed and the purification process was assessed according to the storage method.

2.9. Lipids. Lipids were extracted from fresh algae with a mixture of dichloromethane: methanol (2 : 1, v/v) [25]. NaCl (5%) was added to the aqueous phase to improve phase separation. The lipid content was determined by the gravimetric method.

2.10. Ash Content. Total ash was determined by incineration of 1 g of dry algal material in an oven at 450°C for 4 h. The ash content was expressed as a percentage of dry weight.

2.11. Thalli Transversal Cross-Section. In order to estimate the mean thickness of thalli, transversal cross-sections were carried out on A and B samples. Three slides of a homogenous biomass representative of each site were prepared, eight sections were chosen randomly, and five transects were made to measure the mean thickness. Photomicrographs were made using a light microscope (Olympus AX70/provis) without staining, obtained with a digital camera (Dmx 1200F, Nikon, Japan) and analyzed using Lucia G imaging software (Laboratory Imaging, CZ).

2.12. Statistics and Expression of Results. All analyses were performed in triplicate. Means and standard deviations are given for each experiment. Statistical analyses were conducted using the SigmaStat software version 3.0 for Windows. One-way analyses of variance were used (ANOVA). Significant differences at $P < 0.05$ between the samples were determined by the Student-Newman-Keuls post hoc multicomparison test (SNK).

3. Results and Discussion

3.1. Sampling Site Comparison. Figure 3 shows the differences between the thalli from the two sampling sites in terms of thickness, viscosity, color, and appearance. Clearly, thalli samples from A (Figure 3(a)) are thicker, less viscous, and more greenish than those from B (Figure 3(b)). These observations are summarized in Table 1. Differences were visible on the biological material, since the thalli growing in A were thick and wide, of various shapes, brown, and of low viscosity, while those growing in B were long and thin, red, and very viscous. The difference in thickness was confirmed by transversal cross-section, illustrated in Figures 3(c) and 3(d). Using Lucia G imaging software, the mean thickness was determined as 239 ± 41 μm for A samples against 136 ± 17 μm for B samples. These results were thus significantly different ($P < 0.001$) and confirmed the morphological differences observed between the samples from the two sites. Consequently, there seems to be a relation between the morphology of thalli and perhaps the environmental characteristics of the sampling sites. These ecological characteristics were not determined in our case so, a further study of the criteria such as salinity, hydrodynamism, and water pH will be necessary to validate this hypothesis. The differences observed could also be due to the presence of distinct genetic populations on the sites studied and, in this case, a genetic determination from each sampling will need to be performed in the future.

These differences between samples were observed during the washing step. Effectively, the samples from B gave a pink and fluffy water wash indicating a high R-PE solubility. This was not the case with the samples from A, which were also characterized by highly viscous extracts. This is the first time that this phenomenon has been reported in this species.

Regarding the biochemical composition, the total protein content (217.75 ± 19.72 mg·g^{-1} dw) for B samples (Table 1) was close to that recorded for *G. turuturu* sampled in May 2006 (203.1 ± 19.7 mg·g^{-1} dw) by Denis et al. [7] and in 1984 by Fujiwara-Arasaki et al. [26] with a 200 mg·g^{-1} dw protein content. However, the protein content of A samples (161.58 ± 5.66 mg·g^{-1} dw) was lower than those previously reported values and also than that (220 mg·g^{-1} dw) described for the edible seaweed *Palmaria palmata* [27]. Denis et al. [7] found that proteins in *G. turuturu* were subject to large variations during the year with a maximal concentration

FIGURE 3: *Grateloupia turuturu* thalli collected from two different sites: A and B. (a) Morphological observation of thallus from A. (b) Morphological observation of thallus from B. (c) Transversal section of A thallus. (d) Transversal section of B thallus.

of 300 mg·g^{-1} dw recorded in winter and a minimum of 150 mg·g^{-1} dw during July and August. In this current study, variations in protein content according to the sampling site are reported for the first time. The water-soluble protein content of B samples (19.15 ± 0.47 mg·g^{-1} dw) was also significantly higher than in A samples (7.19 ± 0.74 mg·g^{-1} dw). Using the same extraction protocol presented here, a previous study showed that the water-soluble protein content in *G. turuturu* sampled from another site (located in Piriac sur Mer (47°22′45.6″N- 2°33′15.9″O), near to both sites A and B) was 9.4 ± 2.0 mg·g^{-1} dw [28], further confirming that water-soluble protein content varies according to the sampling site and may differ according to the genetic population concerned. This last hypothesis will need to be verified by a further study about the genetic determination of *G. turuturu* populations on the Brittany coast.

Regarding the total water-soluble carbohydrates, B samples appeared to be the richest (41.57 ± 0.66 mg·g^{-1} dw) compared to A samples (16.05 ± 0.82 mg·g^{-1} dw) (Table 1). It has been previously demonstrated that the amount of water-soluble sugars in this algae is 24.0 mg·g^{-1} dw [28], in agreement with that obtained in this study. These observations suggest that water-soluble carbohydrates (highest in B) do not prevent the extraction of soluble proteins (8.8% of total proteins extracted instead of 4.4% for samples from A) (Table 1).

The lipid content was also significantly different in these samples; 28.07 ± 5.12 mg·g^{-1} dw for A and 54.35 ± 2.05 mg·g^{-1} dw for B. While Denis et al. showed that there was no significant difference in total lipid content throughout the year, the results presented here demonstrate that there were variations between the samples from two different sampling sites. These values were higher than those recorded in Palmaria palmata (15.7 mg·g^{-1} dw) or in Chondrus crispus (6.1 mg·g^{-1} dw). The values obtained for the G. turuturu sample from A were close to those reported for another red edible seaweed, *Porphyra umbilicalis* (33.7 mg·g^{-1} dw) [29].

As seen previously, the composition in proteins, water-soluble carbohydrates, and lipids varies according to the site studied.

Concerning the R-PE, the higher content was recorded for the samples collected in B (Table 1). This is consistent with the previous results about the total protein content and the best extraction of water-soluble proteins already noted for the

Table 1: *Grateloupia turuturu*: morphological aspects and biochemical composition of site A and B samples.

Site	A	B
Appearance of thalli	Thick, low viscosity, brown-greenish	Thin, high viscosity, brown-red
Thickness of thalli (μm)	239 ± 41^a	136 ± 17^b
% cortex	46 ± 8^a	48 ± 9^a
% medulla	54 ± 8^a	52 ± 9^a
Total protein (mg·g^{-1} dw)	161.58 ± 5.66^a	217.75 ± 19.72^b
Water-soluble protein (mg·g^{-1} dw)	7.19 ± 0.74^a	19.15 ± 0.47^b
R-PE (mg·g^{-1} dw)	1.16 ± 0.33^a	4.39 ± 0.15^b
Lipid (mg·g^{-1} dw)	28.07 ± 5.12^a	54.35 ± 2.05^b
Carbohydrates (mg·g^{-1} dw)	16.05 ± 0.82^a	41.57 ± 0.66^b
Dry weight (mg·g^{-1} dw)	127.68 ± 7.31^a	64.61 ± 11.06^b
Ash (mg·g^{-1} dw)	144.42 ± 1.75^a	155.86 ± 2.58^b

Data are expressed as the mean ± SD ($n = 3$). Significant differences at $P < 0.05$ are indicated by different letters (a-b).

B samples. The differences in R-PE yield between samples A and B could also be explained by the difficulty of extracting R-PE from A samples.

The R-PE yield variation was comparable to that of total proteins, with a higher yield in sample B (4.39 ± 0.15 mg·g^{-1} dw) than in sample A (Table 1). This value was close to that reported in a previous study on R-PE from *Grateloupia turuturu* (3.0 ± 0.3 mg·g^{-1} dw) [7], but for A, the R-PE yield (1.16 ± 0.33 mg·g^{-1} dw) was lower than in this study, in agreement with the total protein content results.

The R-PE yield and PI were significantly lower ($115.21 \pm 0.75 \times 10^{-2}$ mg·g^{-1} dw and 0.22 ± 0.00, resp.) in A samples than in B samples ($408.34 \pm 4.08 \times 10^{-2}$ mg·g^{-1} dw and 0.76 ± 0.01, resp.) (Tables 1 and 2). Moreover, for the protein extract from B samples, precipitation in 80% ammonium sulfate saturation led to a transparent supernatant and a reddish-pink pellet for which the PI increased from 0.76 ± 0.01 for B1 CE to 0.93 ± 0.03 for B1 PE (results significantly different). This fractionation step enabled the concentration of R-PE to be increased by a factor of about 1.22 compared to the previous case.

For all samples from A and B, the precipitation in 80% ammonium sulfate saturation led to a PI increase and a yield decrease, but this step was more difficult to achieve with B samples. The different yields and PI values between samples A and B (Table 2) and the spectral differences (Figure 4) showed that the samples studied here reacted differently to the extraction protocol, and that other parameters interfered with this protocol. This step corresponded to a prepurification step but should be adapted according to the biomass.

As noted previously, strong differences in R-PE content were observed between the sampling sites. Some hypotheses could explain these results. Effectively, several authors have already shown an increase in algal population density induced by one environmental factor: hydrodynamism [30, 31]. Plouguerné has reported that other factors, like seawater temperature and changes in salinity, are involved in the recruitment of *G. turuturu* and therefore could have an impact at a biochemical level [32]. Moreover, Denis et al. [7] carried out a study on the seasonal variations of *G. turuturu* and suggested that the best period to harvest this species for R-PE extraction was from February to June. Seasonal variations had an impact at the biochemical level, so light and seawater temperature were two major factors influencing seaweed biochemical content. In this study, only 21% of the dry weight was evaluated for A samples, while around 40% was assessed for B samples. This means that unevaluated matter was composed essentially of fibers (insoluble sugars), which mostly entered the cell wall structure. This difference could perhaps explain the difficulty of extracting R-PE from A samples. The CE spectra (Figure 4(c)) confirmed these differences between samples from A and B. Indeed, using the same extraction protocol, the 565 nm absorbance was five times higher in B than in A.

Table 2: Purity index (PI) and yields of crude extracts and protein extracts from samples from sites A and B and following the three storage modes.

	Fraction	
	PI ($A_{565\,nm}/A_{280\,nm}$)	R-PE yield ($\times 10^{-2}$ mg·g^{-1} dw)
A1 CE	0.22 ± 0.00	115.21 ± 0.75
A1 PE	0.49 ± 0.01	74.42 ± 0.43
A2 CE	0.12 ± 0.03	50.03 ± 2.13
A2 PE	0.17 ± 0.01	31.61 ± 0.75
A3 CE	0.12 ± 0.00	71.50 ± 1.14
A3 PE	0.27 ± 0.01	40.01 ± 0.78
B1 CE	0.76 ± 0.01	408.34 ± 4.08
B1 PE	0.93 ± 0.03	264.56 ± 2.08
B2 CE	0.48 ± 0.01	215.38 ± 15.05
B2 PE	0.57 ± 0.01	182.19 ± 6.47
B3 CE	0.70 ± 0.01	364.32 ± 20.02
B3 PE	0.78 ± 0.01	254.78 ± 7.67

Data are expressed as the mean ± SD ($n = 3$). Note: samples PI values are all significantly different between them. The same is observed for R-PE yield values.

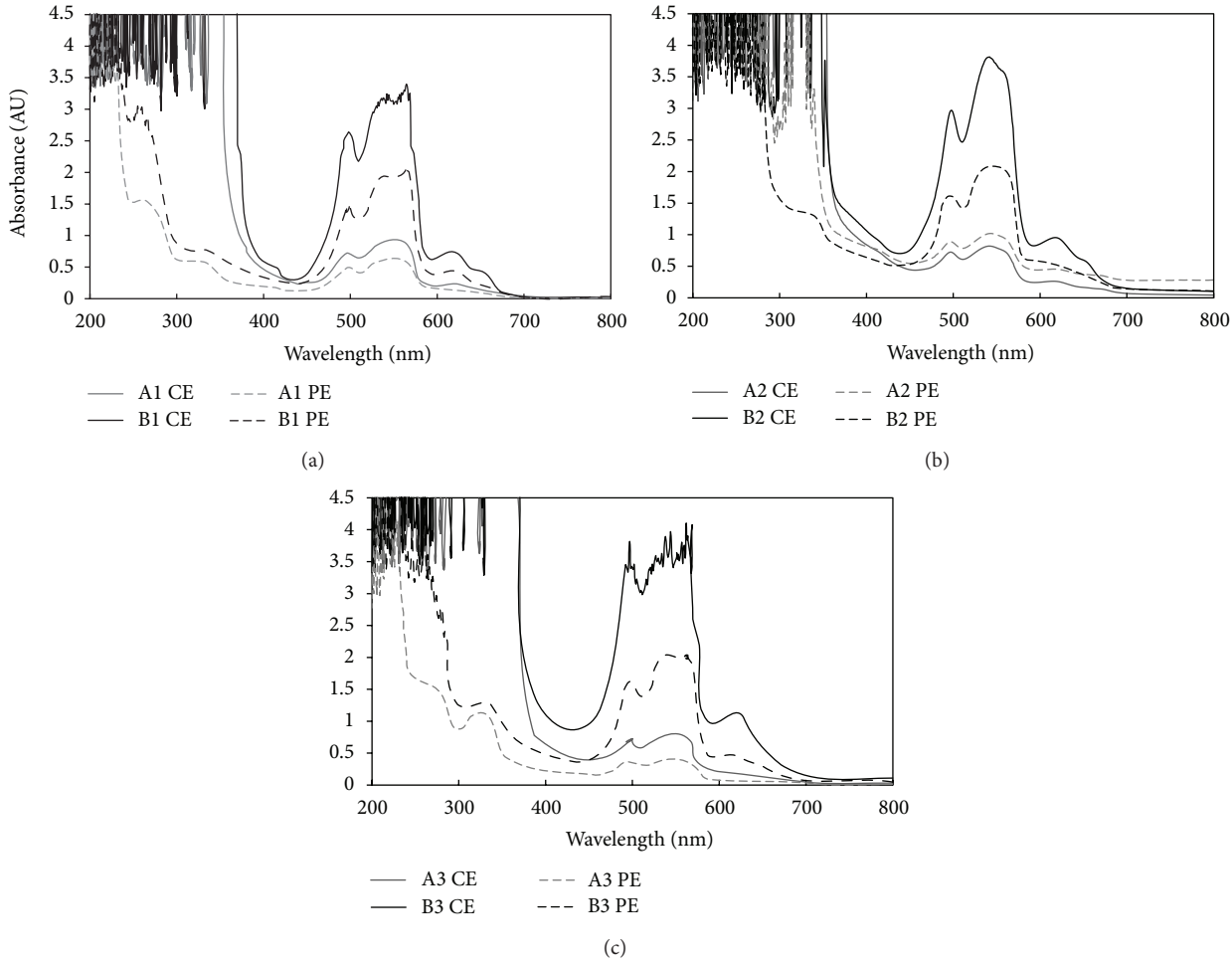

Figure 4: *Grateloupia turuturu*: absorbance spectra of the R-phycoerythrin crude extract (CE) and protein extract (PE). Comparison of storage methods for the three samples; (a): no storage (1), (b): storage at room temperature, darkness, 6 months (2), and (c): storage at −20°C, darkness, 6 months (3), of the two field collection sites A (the gray solid line and the gray dashed line) and B (the black solid line and the black dashed line).

3.2. Storage Method Comparison. To study the impact of the storage method on R-PE extraction yield and purity index, the three following methods were investigated: no storage (1), freeze-dried algae stored for 6 months at room temperature (2), and frozen algae stored for 6 months at −20°C (3). Having demonstrated that the two sampling sites induce different R-PE extraction yields, this part of the work was carried out for both sites (Table 2).

For the two sampling sites, no storage led to the best results in terms of R-PE yield and purity. Storage method 2 had the lowest and significantly different R-PE yields (50.03 ± 2.13× 10^{-2} mg·g^{-1} dw and 215.38 ± 15.05 × 10^{-2} mg·g^{-1} dw, resp.) and the lowest PI (0.12±0.03 and 0.48±0.01, resp.). This was confirmed by the spectra (Figure 4(b)), where the peak feature of the R-PE at 565 nm was not as marked as for other samples, reflecting a deterioration in the R-PE over time. The freezing storage method gave better results. In fact, the B3 R-PE extraction yield (364.32 ± 20.02 × 10^{-2} mg·g^{-1} dw) was greater than and significantly different from that of B2, likewise for the B3 PI (0.70 ± 0.01). Thus, freezing provided better sample preservation than freeze-dried storage at room temperature in darkness (Table 2). The preservation method and the duration seemed to have an impact on samples. For long-term preservation, based on R-PE yield and purity, the best preservation method is freezing of fresh seaweed [3]. Therefore, the sampling site and storage conditions of samples are two major factors that will affect the extraction step and thus the R-PE yield. So, in order to optimize the extraction of this pigment with high added value, it is necessary to use a standardized biomass for which the environmental parameters are known and to optimize the storage conditions to enhance the R-PE extraction.

4. Conclusion

Sampling site and storage method were analyzed for their effects on R-PE extraction from the edible red seaweed *G. turuturu*, harvested on the south Brittany coast (France). The originality of this study is its focus on steps that are upstream of R-PE extraction, that is, the influence of sampling site and

storage method. The aim was to show how these steps have an impact on the extraction of R-PE. By analyzing the R-PE, the study showed that both the yield and the purity of the pigment were significantly different between sampling sites. This result is interesting in an upgrading context, especially in the case of the use of this pigment as a food colorant. Moreover, it was demonstrated that the biochemical content in protein, lipid, carbohydrate, and dry matter varied between the two sites. It was the same regarding the storage method (significant differences in yield and purity of the R-PE between the two storage methods). A previous study [7] showed the effect of seasonal conditions on the variation in biochemical composition of *G. turuturu* collected in Brittany. These results add new information to that already published and highlight the importance of the sampling site environmental factors and the conditions of storage, especially to recover R-PE. The development of a cultivation strategy to produce a standardized biomass for food use is still a perspective which could be investigated.

However, further studies (e.g., temperature, light, hydrodynamism, and salinity impact on biochemical content) will be necessary to optimize the R-PE yield from *G. turuturu*. To improve our knowledge of this species, used in Asia as a food and cosmetic additive, a study is underway in our laboratory to assess R-PE stability toward light, temperature, pH, and time.

Acknowledgments

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