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(RESEARCH ARTICLE)

Study of the influence of the region, the depth and the drying process on the chemical composition of *Gelidium corneum* from Moroccan coast

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Abstract

The *Gelidium corneum* is an algae exploited industrially for its richness in agar. In addition, it is known for its anti-inflammatory, antioxidant and anti-microbial activities. In this study, we established a general overview of the macronutrient composition of *G. corneum* and compared this composition according to harvest site, harvest depth and drying process. Proteins, lipids and carbohydrates of samples are measured by different methods. The analysis of results show that the protein concentrations of the El Jadida and Mohammadia samples are significantly higher than Casablanca samples (0.026 ± 0.0007 µg/µg DM, 0.024 ± 0.001 µg/µg DM, 0.006 ± 0.0007 µg/µg DM, $p < 0.05$ respectively). However, Casablanca samples are significantly richer in total carbohydrates (0.023 ± 0.002 µg/µg DM, $p < 0.05$) and less rich in reducing carbohydrates (0.0001 ± 0.00001 µg /µg DM, $p < 0.05$) compared to other samples. The lipid concentrations of the samples from all harvest sites do not show any significant difference. According to depth, total protein and sugar concentrations were significantly higher in the coast versus depth samples (0.035 ± 0.004 µg/µg DM vs 0.025 ± 0.0007 µg/µg DM, 0.035 ± 0.006 µg/µg DM vs 0.012 ± 0.005 µg/µg DM, $p < 0.05$ respectively). For the drying process, protein, total sugars and lipid concentrations were significantly higher in open air samples compared to oven samples (0.025 ± 0.0007 µg/µg DM vs 0.013 ± 0.0003 µg/µg DM, 0.012 ± 0.002 µg/µg DM vs 0.006 ± 0.002 µg/µg DM, 6% vs 3%, $p < 0.05$ respectively). Our results demonstrate that the chemical composition of *G. corneum* varies according to the harvest region. In addition, samples harvested on the coast and dried in the open air are the most rich in macronutrients.

Keywords: Chemical composition; Depth; Drying; *Gelidium corneum*; Region.

1. Introduction

With 3500 km of coastline, Morocco has a very rich and diverse marine flora such as red algae. These algae are an important source of products used in various fields including medical, pharmacological, cosmetic and food processing. Algae are known to be low in calories, rich in polysaccharides, minerals, vitamins, proteins, steroids and dietary fibres, making them increasingly sought after for commercial purposes [1,2]. They are of potential interest in combating obesity, high blood pressure and free radicals and promoting good alimentary digestion [3].

Morocco is the world's first largest producer of *Gelidium corneum* [4]. Which is a red algae known mainly for its high content of agar [5,6]. Agar is a polysaccharide, characterized by its high gelation capacity. It is considered a good neutral gelling agent, which does not precipitate with proteins and can produce products with high resistance to lactic acid bacteria. It is widely used as a treatment against constipation [7,8] and as a food additive (soups, smoothies, jams, ice

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cream, etc.) [9,10]. The *G. corneum* is not limited to its richness on agar, but also contains other compounds (macro and micronutrients) that have therapeutic and nutritional value. Thus, the objective of this study is to enhance the value of *G. corneum* by determining its composition in macronutrients (proteins, carbohydrates and lipids) and to study the influence of three different factors on this composition, namely: the region, the depth and the drying process.

2. Material and methods

2.1. Harvest site presentation

Three regions were choised for this study. The Tamaris Dar Bouazza beach located 17 km from the center of Casablanca (33°32"0" North, 7°48"30" West), the Sidi Bouzid beach, located 5 km from the El Jadida city (33°13'52" North, 8°32'51" West) and the Sablettes beach located 7 km of Mohammadia city (33°43'0" North, 7°22'0" West). The three regions are located on the Atlantic coast in western Morocco (Figure 1).

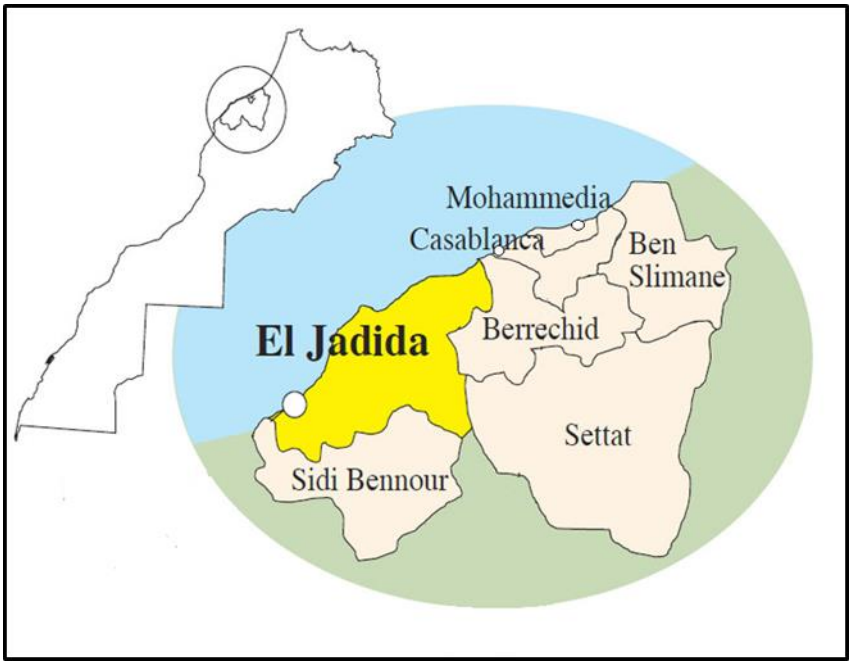

Figure 1 Collection sites of *G. Corneum*

G. Corneum's thalles were harvested in September 2017. At the Casablanca and Mohammadia sites, algal samples were collected from 2 to 12 m depth. In the third study site, El jadida, two depths were considered, the first between 0.5 and 1.5 m (coast) and the second between 2 and 12 m (depth).

2.2. Preparation of the algal sample

2.2.1. Harvesting algae

The seaweed specimens were collected by a professional diver and kept in plastic tubs filled with seawater until they were treated in the laboratory.

2.2.2. Cleaning and identification of algae

After removal of debris, small crustaceans and other algae, *G. corneum* thalle are rinsed with tap water and thoroughly cleaned of epiphytes. The confirmation of the genus and species is made by a biologist of the SETEXAM (Society for the study and exploitation of algae and sea products) in El Jadida, Morocco.

2.2.3. Dryingand conservation of algae

The algal samples from Casablanca and Mohammadia are air-dried for 5 days at room temperature. The El Jadida algae are divided into two batches: the algae of batch 1 are dried in the air shelter for 5 days at room temperature [11] and those in batch 2 are dried at the same temperature but in the oven during the same period of 5 days.

All the samples of dried algae were cut into small pieces, crushed into powder, and then preserved by freezing to –20 °C [12]. The dried seaweeds were cut into small pieces, powdered by a grinder and stored in freezing to –20 °C until the chemical analysis.

2.3. Biochemical composition

2.3.1. Total protein content

Protein extraction

1 g of the algal sample is put in a mortar with ground glass and 7 ml of phosphate bufferand the mixture is ground. Then the mortar is rinsed with 10 ml of buffer to prevent loss of the sample and the contents are placed in a Falcon tube. The upper phase, which contains the proteins to be assayed, is aliquoted into eppendorff tubes.

Protein determination

The protein determination of aliquot of each samples is used by the Bradford protein assay which involves performing a standard range using a reference protein Beef Serum Albumin (BSA). Spectrophotometric reading is performed at 595 nm [13].

2.3.2. Total carbohydrate content

Carbohydrate extraction

Extraction of carbohydrates: 10–15 mg of algae powder are extracted in 1 ml of 70% ethanol (v/v) for about 3 h in a water bath at 70 °C. The samples are then centrifuged for 5 min at 2800 x g [14].

After this extraction we carried out a deproteinization of the sample by trichloroacetic acid (TCA): 500 µl of the extract diluted of 1/10 is added to 1 ml of TCA and 1 ml of distilled water, then the mixture is vortexed.

Carbohydrate determination

The total carbohydrate was measured by the phenol sulfuric acid method of Dubois [15] with and without TCA, using glucose as standard. Spectrophotometric reading at 490 nm.

2.3.3. Total reducing carbohydrate content

Reducing sugars were quantified by the 2, 5-dinotrosalicylic acid (DNS) reagent using fructose (1 mg/ml) as standard. In a glass tube, 1.5 ml of the DNS reagent is mixed with 500 µl of sample. After stirring, the mixture is incubated in boiling water for 5 min. Then the tubes are cooled in tap water and the absorbance is read at 540 nm [16].

2.3.4. Total lipid content

Total lipids are extracted according to the modified method of Folch et al. [17]. 1 g of algal sample is placed in a 50 ml conic tube to which was added 30 ml of Folch reagent (chloroforme–methanol, 2:1 v/v) and vortexed, with the addition of 5 ml of a 0.73% NaCl solution. Finally, the solution is filtered using the cotton inserted in a medical syringe. The extract is then centrifuged for 10 min at 4 °C, and the lower layer is transferred to test tubes. The evaporation of the solution is performed using rotavapor. The lipid content is determined as follows:

$$\left(W_f - W_i\right) = W_s \text{ x } 100\ \%$$

Where Wf is the final weight of the test tube (g), Wi is the initial weight of the test tube (g), and Ws is the dry weight of the seaweed sample (g). Results are expressed as % of dry weight [18].

2.4. Statistical analysis

The results were expressed as mean ± standard deviation. Statistical analysis is done using the t-Student test. Statistical analysis was performed with a Statistical Package for the Social Science SPSS (version 11.0). The results are considered statistically significant when $p < 0.05$.

3. Results

3.1. Total protein content

Results are presented in Figure 2. Indeed, depending on the harvest site, the protein concentrations of El Jadida and Mohammadia samples are significantly (0.026 ± 0.007 and 0.024 ± 0.001 µg/µg DM respectively, $p < 0.05$) higher than that of Casablanca (0.006 ± 0.007 µg/µg DM).

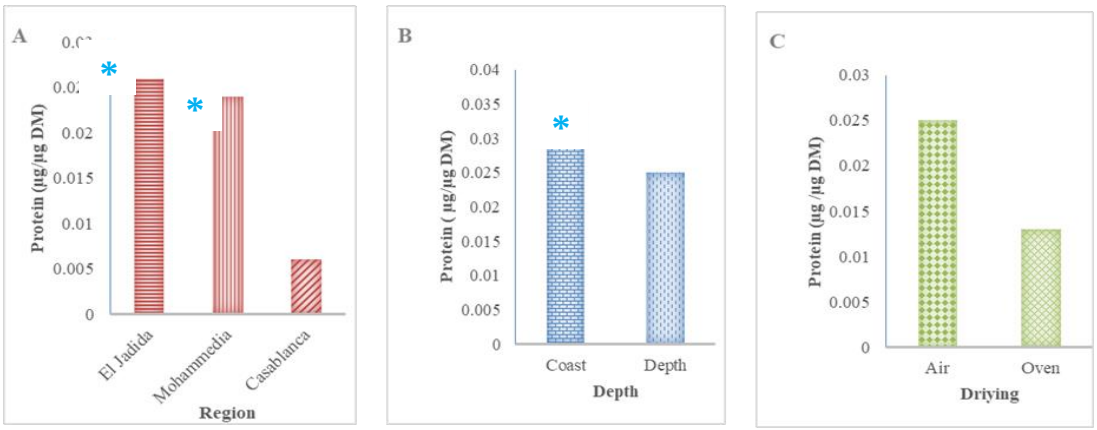

Figure 2 Variation of protein concentrations (µg/µg DM) according to the tree factors studied: (A) region of harvest, (B) depth, (C) drying process. (* $p < 0.05$)

Depending on the depth of the harvest, the protein concentrations are significantly higher in the coastal samples as compared with the deeper samples (0.035 ± 0.004 vs 0.025 ± 0.0007 µg/µg DM, $p < 0.05$). However, there was no significant difference in protein concentrations between air-dried and oven-dried samples (0.025 ± 0.0007 vs 0.013 ± 0.0003 µg/µg DM, $p > 0.05$).

3.2. Total carbohydrate content

Figure 3 shows the results of total carbohydrate concentrations with TCA. The samples from the three study regions have approximately the same total sugar concentrations. On the other hand, these concentrations are significantly higher in the samples of the coast compared to the samples collected from the depth (0.006 ± 0.002 vs 0.004 ± 0.001 µg /µg DM, $p < 0.05$). Depending on the drying process, the results show a significant variation between the samples dried in the open air and those dried in the oven (0.004 ± 0.0001 vs 0.001 ± 0.002 µg/µg DM, $p < 0.05$).

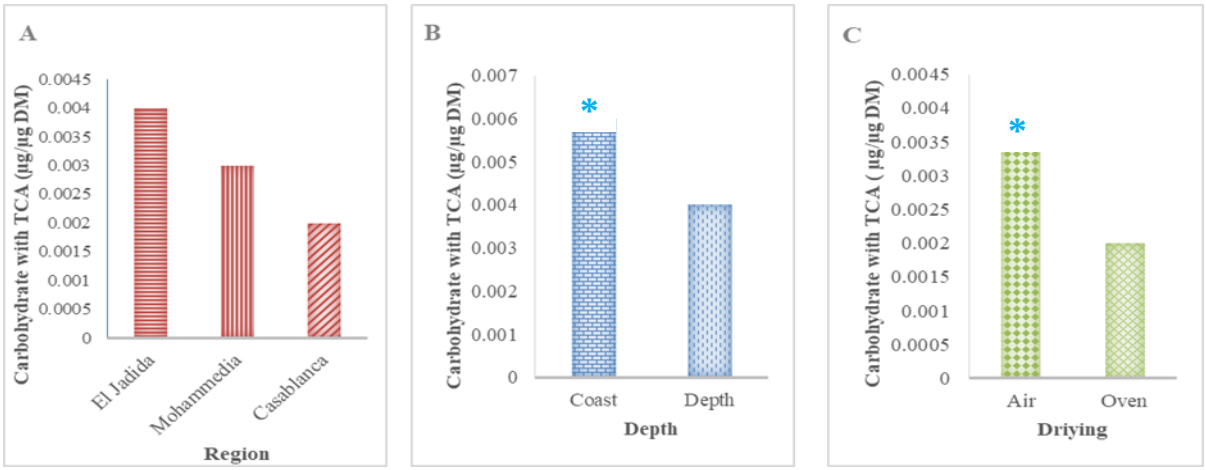

Figure 3 Variation of carbohydrates with TCA concentrations (µg/µg DM) according to the tree factors studied: (A) region of harvest, (B) depth, (C) drying process, (* $p < 0.05$).

Similar results in total sugar concentrations without TCA were obtained as a function of region, depth and drying process (Figure 4). Indeed, depending on the region of harvest, Casablanca samples were significantly higher (0.023 ± 0.002 µg/µg DM, $p < 0.05$) compared to El Jadida and Mohammadia (0.012 ± 0.005 and 0.006 ± 0.0008 µg/µg DM). However, the concentrations are significantly higher in the samples of the coast compared to the samples collected from the depth (0.035 ± 0.006 vs 0.012 ± 0.005µg/µg DM, $p < 0.05$) and are significantly higher in the samples dried in the open air compared to the samples dried in the oven (0.012 ± 0.002 vs 0.006 ± 0.002 µg/µg DM, $p < 0.05$).

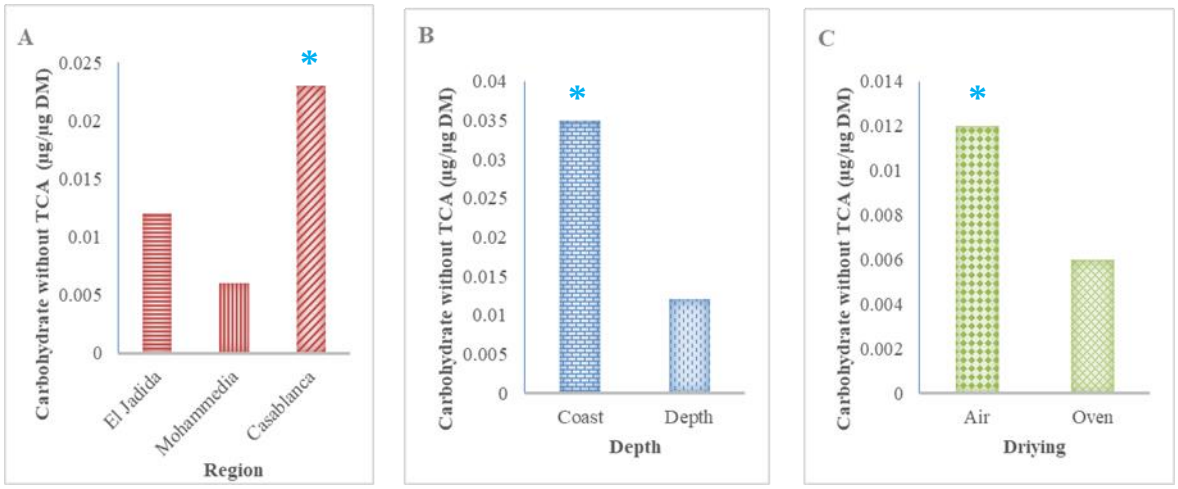

Figure 4 Variation of carbohydrates without TCA concentrations (µg/µg DM) according to the tree factors studied: (A) region of harvest, (B) depth, (C) drying process, (* $p < 0.05$).

3.3. Total reducing carbohydrate content

Our results presented in Figure 5 show that El Jadida samples are significantly richer in reducing sugars (0.0014 ± 0.0006 µg/µg DM, $p < 0.05$) compared to Mohammadia (0.0004 ± 0.0009 µg/µg DM) and Casablanca (0.0001 ± 0.000015 µg/µg DM). In addition, these results show that samples collected from the depth are significantly richer in reducing sugars compared to samples of the coast (0.0014 ± 0.0006 µg/µg DM vs 0.0007 ± 0.00003 µg/µg DM, $p < 0.05$). Concerning the drying process, our results show a significant increase in this concentration in the samples dried in the open air compared to the samples dried in the oven (0.0014 ± 0.00015 vs 0.0007 ± 0.00006 µg/µg DM, $p < 0.05$).

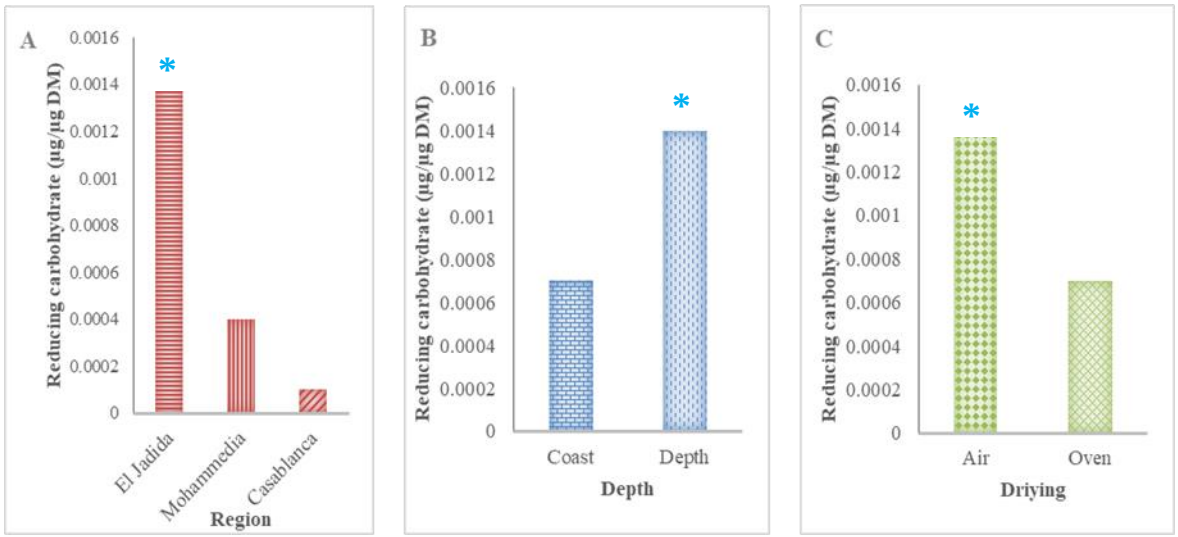

Figure 5 Variation of reducing carbohydrates concentrations (µg/µg DM) according to the tree factors studied: (A) region of harvest, (B) depth, (C) drying process. (* $p < 0.05$)

3.4. Lipid content

The lipid concentrations of the samples showed no significant difference according to the three regions or to depth (Figure 6). However, these lipid concentrations are higher in the samples dried in the open air compared to the samples dried in the oven (6% vs 3%, $p < 0.05$).

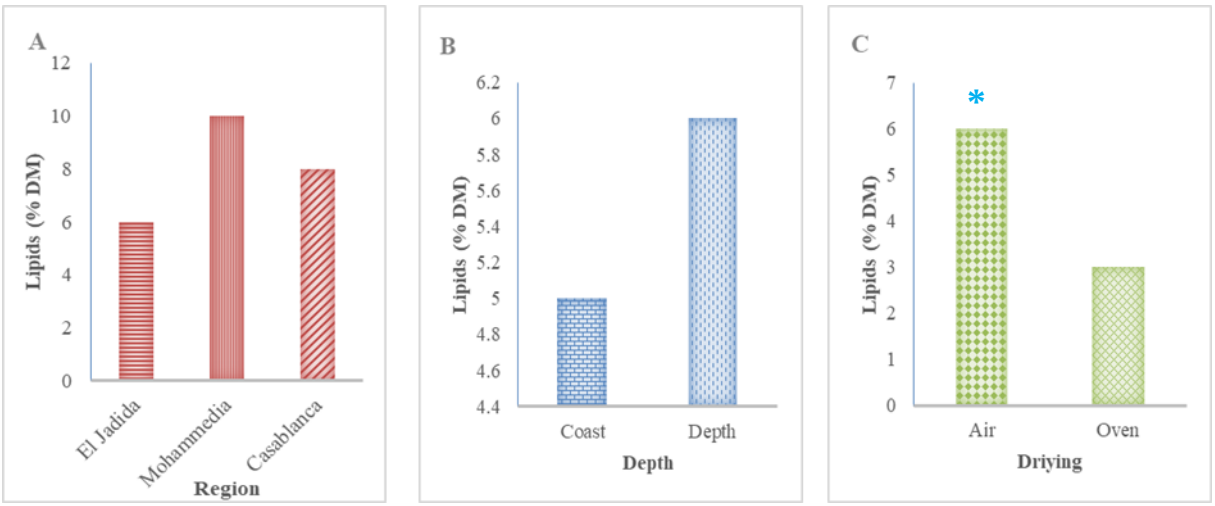

Figure 6 Variation of lipids concentrations (µg/µg DM) according to the tree factors studied: (A) region of harvest, (B) depth, (C) drying process. (* $p < 0.05$).

4. Discussion

In this study, our results show that the chemical composition varies according to the three factors tested, harvest site, depth and drying process.

Depending on the harvest site, our samples from different regions show a variation in chemical composition (proteins, carbohydrates and lipids) with a significant increase in proteins and carbohydrates concentrations in El Jadida samples compared to those from Casablanca and Mohammadia.

Several authors have confirmed that the analysis of secondary metabolites on other species algae harvested from different locations, show a different chemical composition quantitatively and qualitatively [19–22]. This variation in chemical composition can be explained by the variation in geographical, climatic (temperature and precipitation) and environmental (wave frequency) conditions between the three harvest sites, particularly, in chemical composition and salinity of sea water and culture medium. It may also be explained by the genetic diversity present in the samples from each region. These hypotheses can be verified later by genetic studies and by checking the conditions of each region.

The pollution factor can also influence the chemical composition of the algal samples knowing that Casablanca and Mohammadia are two industrial cities and more polluted than El Jadida. A previous study on *Fucus spiralis* algae samples collected from four different sites in the El Jadida city, three of which are characterized as sites of wastewater discharges and increasing industrial activities. The results obtained showed that the accumulation capacity of metals (Cd, Cu, Fe, Mn and Zn) varies according to the sampling sites [21].

For the influence of the depth on the chemical composition of the samples, our results show that the algae harvested on the coast are found to be rich in macronutrients compared to those harvested at depth. Indeed, algae need light to grow and reproduce [23, 24]. They absorb light thanks to pigments and thus produce dioxygen, and transform light energy to make their materials. However, with the depth, the light decreases and the light quality changes, which generates a lack of energy necessary for the production of macronutrients by photosynthesis. This approach is confirmed by the study of Gordillo et al. [25] who found that there is a relationship between light and cyclic AMP.

Depending on the drying process, the drying of the algae in the oven causes the loss of a quantity of the macronutrients compared to drying in the open air. This can be explained by the differences in environmental conditions between the two drying media such as physical properties (temperature, humidity, air speed). Indeed, Ait Mohamed et al. [8] have shown that temperature is a determining factor in the drying of *G. corneum*. The high temperature during the drying

procedure is an imperative reason for loss of value, while the reduction of this temperature has extraordinary potential to improve the nature of the dried samples [26, 27]. In the oven, our samples are dried at a fixed temperature (40 °C), while in the shelter of air, the temperature decreases considerably with the alternation day and night.

The drying time could also lead to a significant loss of nutritional quality at the end of the operation by desirable or undesirable chemical or biochemical reactions which may occur [28].

5. Conclusion

Our results show that the content of proteins, sugars and lipids in *G. corneum* varied according to the region and depth of harvesting and the way the algae are dried. Samples from El Jadida are richer in protein, reducing carbohydrates and sugar with TCA compared to Casablanca and Mohammadia. On the other hand, the lipid concentrations of the samples from the three harvesting sites do not show any significant difference. Thus, the coast of El Jadida presents, more favorable conditions to ensure the *G. corneum* richness in macronutrients and consequently, its valorization on a national and even international scale for nutritional and medical purposes. Our study also affirms that the samples from the coasts with open-air drying are the most advisable given their considerable richness in macronutrients.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors have declared that no competing interest exists.

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