The Invasive Seaweed Agarophyton vermiculophyllum from Oualidia Lagoon (Northwestern Moroccan Atlantic Coast) as a Source of Agar: Yield, Chemical Characteristics, and Rheological Properties

Amal Nadri, Zahira Belattmania, Abdellatif Chaouti, Fouad Bentiss, Charafeddine Jama, Fouzia Hmimid, Abdeltif Reani, Brahim Sabour

Journal of *Marine Science and Engineering*

MDPI

Article

The Invasive Seaweed *Agarophyton vermiculophyllum* from Oualidia Lagoon (Northwestern Moroccan Atlantic Coast) as a Source of Agar: Yield, Chemical Characteristics, and Rheological Properties

Amal Nadri [1], Zahira Belattmania [1], Abdellatif Chaouti [1], Fouad Bentiss [2,3], Charafeddine Jama [3], Fouzia Hmimid [1], Abdeltif Reani [1] and Brahim Sabour [1,*]

[1] Phycology, Blue Biodiversity and Biotechnology RU, Laboratory of Plant Biotechnology, Ecology and Ecosystem Valorization, URL—CNRST n° 10, Faculty of Sciences, Chouaib Doukkali University, P.O. Box 20, El Jadida M-24000, Morocco; nadri.a@ucd.ac.ma (A.N.); belattmania.z@ucd.ac.ma (Z.B.); chaouti@ucd.ac.ma (A.C.); fouzia.hmimid@gmail.com (F.H.); abreani@yahoo.fr (A.R.)

[2] Laboratory of Catalysis and Corrosion of Materials, Faculty of Sciences, Chouaïb Doukkali University, P.O. Box 20, El Jadida M-24000, Morocco; fbentiss@gmail.com

[3] Materials and Transformations Unit, University of Lille, CNRS, INRAE, Centrale Lille, UMR 8207-UMET, F-59000 Lille, France; charafeddine.jama@ensc-lille.fr

* Correspondence: sabour.b@ucd.ac.ma

Abstract: Agar is a hydrophilic biopolymer extracted from red seaweed. This phycocolloid consists of two components: agarose and agaropectin. In the present work, agar extracted from the invasive red seaweed *Agarophyton vermiculophyllum* was characterized using physical, chemical, and spectroscopic analyses to investigate the effect of alkaline pretreatment on agar properties. Two extraction conditions, native and alkali-pretreated agars, were comparatively studied. The native yield (28.4 ± 0.9%) was higher than that of the alkaline-pretreated agar (20.4 ± 0.8%). The alkali-pretreated agar showed higher gel strength (763.8 ± 57.0g cm^{-2}), gelling (36.5 ± 0.9 °C), and melting (83.2 ± 0.6 °C) temperatures and increased 3,6-anhydrogalactose (26.2 ± 1.9%) and decreased sulfate contents (6.2 ± 0.8%) compared with native agar (gel strength: 204.8 ± 17.10g cm^{-2}, gelling temperature: 29.5 ± 0.9 °C, melting temperature: 73.4 ± 0.7 °C, 3,6-AG content: 13.8 ± 0.7%, sulfate content: 10.5 ± 0.5%). The alkaline pretreatment improved the agar's gelling properties and significantly influenced its chemical properties. In view of the obtained results, *A. vermiculophyllum* might potentially be thought of as a viable source for the agar industry in Morocco, serving as a local source of agar.

Keywords: agar properties; alkaline pretreatment; ^{13}C NMR; extraction; FTIR; invasive seaweed; North African lagoon

Citation: Nadri, A.; Belattmania, Z.; Chaouti, A.; Bentiss, F.; Jama, C.; Hmimid, F.; Reani, A.; Sabour, B. The Invasive Seaweed *Agarophyton vermiculophyllum* from Oualidia Lagoon (Northwestern Moroccan Atlantic Coast) as a Source of Agar: Yield, Chemical Characteristics, and Rheological Properties. *J. Mar. Sci. Eng.* **2023**, *11*, 1696. https://doi.org/10.3390/jmse11091696

Academic Editor: Jianheng Zhang

Received: 12 August 2023
Revised: 23 August 2023
Accepted: 25 August 2023
Published: 28 August 2023

1. Introduction

Agar–agar is a gel-forming substance derived from red macroalgae (Rhodophyceae). This phycocolloid is communally used as a gelling, thickening, and stabilizing food additive [1]. A complex mixture of polysaccharides such as agar and carrageenan can be extracted from red seaweed [2]. Agar consists of two main components, agarose and agaropectin. Agarose contributes high-strength gelling properties to agar, while agaropectin is related to weak gelling properties. Agar is a linear polysaccharide consisting of repetitive units of D-galactose and 3-6,anhydro-L-galactose, linked by alternating α-(1 → 3) and β-(1 → 4) glycosidic bonds [3].

Agar presents various characteristics such as gelling, stabilizing, absorbing, and lubrication with different applications in the food, medicine, and biotechnological industries. Agar is the first phycocolloid used in the formulation of human food, accounting for 80%

of its consumption [3]. The utilization of agar in food applications is based on its characteristics, such as a hard texture, the heat tolerance of gels, the easy process of gelation, and stability in acidic environments [2]. Furthermore, agar is being researched as an alternative raw material for medicinal applications because of the strong gels it forms. It has been used in the development of injectable and phase-changeable composite hydrogels for treating cancers with chemotherapy and photothermal therapy [4].

Between 2000 and 2018, the amount of seaweed produced worldwide increased by more than two times, from 10.6 to 32.4 million tons [5]. The agar industry has shown a remarkable 7% yearly growth when referring to carrageenan and alginate. Worldwide industrial agar sales reached 14,500 t with a value of USD 246 million in 2015 [6]. Over the past ten years, the market demand has rapidly increased. Now, the agar business processes nearly 91% of all processed biomass using just *Gracilaria* as an agar source [7]. The value of *Gracilaria* is increasing along with its demand because of the high cost of production deficiency in the genus *Gelidium* and its wild populations. Thus, most of the agarophytes produced in the world contain *Gracilaria* [8]. There has been rising interest in the development of sustainable seaweed aquaculture practices that have the potential to increase the production capacity of seaweed polysaccharides. The overall aim is to improve the production capacity of these biomaterials, making them more available for wider use and enhancing their sustainability. Commercially available agar is primarily extracted from *Gelidium* and/or *Gracilaria*/*Gracilariaopsis*. High-quality agar is traditionally made from *Gelidium* species, while *Gracilaria*/*Gracilariopsis* species result in agar with weaker gel proprieties. *Gracilaria*/*Gracilariopsis* are usually grown in Asian countries, producing about 98% of the world's supply [9]. *Gracilaria* is a genus with a large number of species, many of which have been used for a variety of purposes. The red seaweed *Agarophyton vermiculophyllum* (Ohmi) Gurgel, J.N.Norris et Fredericq, 2018 (previously named *Gracilaria vermiculophylla* (Ohmi) Papenfuss, 1967), originally from the northwestern Pacific, however, has virtually spread to almost all temperate estuaries in the Northern Hemisphere over the last 100 years. According to the Algaebase website, *A. vermiculophyllum* is currently accepted as a synonym of *Gracilariopsis vermiculophylla* Ohmi, 1956, and it is considered the basionym.

Gracilaria agar with low agarose content and high sulfate-group concentrations affects quality; one solution is to apply an alkaline treatment with sodium hydroxide [10,11]. The biotic and abiotic conditions, the physiological state of the seaweed, the agar extraction process, and the storage of the seaweed represent some of the variables that influence agar production and gelling qualities [12]. Prior to contemplating commercial applications, it is crucial to optimize alkali concentrations and extraction variables. The agar yield of *Gracilaria* sp. decreases as the NaOH concentration increases, and this variation can range from 10% to 50% depending on the species and season [13]. The abundance of seaweed resources has prompted researchers to explore various methods of utilizing them, including the extraction of a commercially valuable biopolymer and its characterization using diverse techniques. This is the first study carried out in Morocco regarding the extraction of agar from the invasive species *A. vermiculophyllum*. Other studies concerning other species of red seaweed have been conducted along the Moroccan coast dealing with the biochemical composition and seasonal variations in agar from *Gelidium latifolium* and *G. sesquipedale* [14,15];the seasonal variations in growth and composition of agar from *Gracilaria multipartita* [16]; and agar from *Gracilaria gracilis*, *Gelidium corneum*, and *G. microdon* and its properties [17,18]. The properties of agar derived from the invasive marine seaweed *A. vermiculophyllum*,the effects of the alkaline treatment and extraction times on the agar, the seasonal variations in agar extracted from this red seaweed, and the stability of its colagar have been studied by many authors worldwide [19–22]. The present study hypothesizes that alkaline pretreatment conditions significantly influence the gel quality, yield, and physicochemical and gelling properties of agar from the seaweed *A. vermiculophyllum*.

The research was carried out with the purpose of determining the potential application of the invasive species *A. vermiculophyllum* from Morocco as a commercial agar producer by evaluating the yield and physicochemical, rheological, and spectroscopic characteristics of agar under two types of extraction.

2. Materials and Methods

2.1. Algal Material

The thalli of *A. vermiculophyllum* were collected in early spring (March) from muddy substrates during low tides at Oualidia Lagoon (northwestern Atlantic coast of Morocco). Samples of *A. vermiculophyllum* were washed and cleaned with fresh tap water to remove sand, debris, and extraneous epiphytes and then cleaned with distilled water. Seaweed specimens were air-dried, sun-dried, and put in an oven at 50 °C until reaching a stable weight and stored for further agar extraction.

2.2. Agar Extractions

2.2.1. Native Agar Extraction

The agar extraction was performed according to the technique reported by Arvizu-Higuera et al. [19] and Villanueva et al. [21], with minor adjustments. Dry seaweed material (30 g) was added to distilled water with a ratio of 1:50 for 30 min at room temperature; soaking water was discarded; and then, the agar extraction was performed using hydrated material heated for 2 h at 85 °C in a water bath (Gesellschaft Für Labortechnik mbH, Burgwedel, Germany). The mixture was filtered through layers of cheesecloth. The filtrates were frozen for 24 h and then thawed. The resulting agar was dehydrated with ethanol and dried in an oven at 50 °C until it reached a constant weight.

2.2.2. Alkaline Pretreatment for Agar Extraction

In total, 30 g of the seaweed *A. vermiculophyllum* was soaked for 30 min in 7% NaOH (Sigma-Aldrich, St. Louis, MO, USA). The solution was heated at 85 °C for 30 min. After alkali treatment, the samples were washed completely with tap water and then distilled water, and then, they were neutralized with 0.5% acetic acid (Sigma-Aldrich, St. Louis, MO, USA). The acetic acid solution was eliminated to remove the excess acid, and the seaweed was washed again with tap water and then distilled water. The following steps are similar to native extraction.

For the two agar extraction conditions, extractions were conducted in triplicate.

2.3. Spectroscopic Characterization

The Fourier transform infrared (FT-IR) spectra of the selected native, alkali-treated, and commercial agar samples (Bacteriological agar type E, Biokar diagnostics, Beauvais, France) were performed using a Thermo Scientific Nicolet Impact 400D FT-IR Spectrometer (Nicolet Instrument Co., Madison, WI, USA). The FT-IR spectra were scanned in attenuated total reflectance (ATR) mode and were recorded in a wavelength between 4000 and 500 cm^{-1} with an average of 32 scans at a 4 cm^{-1} resolution, and eventually, the OMNIC software was used to process the IR spectra (Nicolet, Madison, WI, USA).

Nuclear magnetic resonance (^{13}C NMR) spectroscopic measurements of agar samples were carried out at 353 K on an AV II spectrometer (Proton Larmor frequency of 400.33 MHz, Bruker Corporation, Billerica, MA, USA) operating at a frequency of 400 MHz and equipped with pulsed gradient by using a 5 mm Triple Resonance Broadband Inverse probe (Bruker Corporation, Billerica, MA, USA) at a base frequency of 100.62 MHz. During the relaxation delay and mixing period, presaturation was applied. The exponential multiplication apodization functions were performed in one dimension at 0.5 for line broadening before the Fourier transformation.

2.4. Rheological Analyses

The gel strength of the agars was analyzed using a texture analyzer (CT3, AMETEK Brookfield, Middleboro, MA, USA). A 1.5% agar solution was poured into an AMETEK Brookfield gelatin bloom bottle following gel formation at room temperature, and it was subsequently refrigerated at 5 °C overnight to achieve stabilization. Afterward, the gel strength of the agar was assessed at room temperature using a texture analyzer probe (TA10 Cylinder; 12.7 mm diameter, 35 mm length); to measure the load in compression mode, the probe reached a depth of 4 mm during one cycle.

The melting and gelling temperatures of the agars were measured according to Freile–Pelegrin and Robledo's protocol [23], slightly modified. The gelling temperature was recorded by adding hot agar solution and a glass bead (5 mm diameter) to the test tubes. Then, the tubes were tilted up and down until the bead stopped moving, and the gel temperature was directly recorded. The melting temperature was tested using the same tubes by placing them in a water bath and increasing the temperature from 50 to 100 °C at 0.5 °C per min; as the bead dropped into the solution, the melting temperature was measured with a precision digital thermometer.

Hysteresis was calculated as the difference between the melting and gelling temperatures.

2.5. Chemical Properties

The 3,6-anhydro-galactose (3,6-AG) content was calorimetrically measured using the resorcinol–acetal technique [24], using fructose as a standard. The reagent was prepared using resorcinol (Sigma-Aldrich, St. Louis, MO, USA), 1,1-diethoxyethane (Sigma-Aldrich, St. Louis, MO, USA), and concentrated HCl (Scharlau, Sentiment, Spain). The absorbance of 3,6-AG was measured at a wavelength of 555 nm. D-fructose (Sigma-Aldrich, St. Louis, MO, USA) was used as a standard. The 3,6-AG content was calculated and expressed as a percentage (based on dry weight).

The sulfate content was determined following the method of Craigie et al. [25] by hydrolyzing the extracted agar powder in hydrochloric acid HCl (2 N). The dosage was made using a $BaCl_2$-gelatin reagent that contained gelatin (Sigma-Aldrich, St. Louis, MO, USA) and barium chloride (Sigma-Aldrich, St. Louis, MO, USA). The sulfate content was estimated using the $BaCl_2$ turbidimetric method [25], and the turbidity was measured at a wavelength of 550 nm in opposition to the blank. The standard used was K_2SO_4 (Sigma-Aldrich, St. Louis, MO, USA).

2.6. Data Analysis

A comparison of means was carried out using Student's *t*-test at a significance level of $p < 0.05$ to find significant differences between the native and alkali-modified agars in order to test whether alkali pretreatment affects agar quality. Pearson's correlation analysis was used to investigate the relationship between agar characteristics for both treatments. Before conducting the above analysis, the data were subjected to a normality assessment using the Kolmogorov–Smirnov test and verification of variance homogeneity via Levene's test. The statistical analysis was performed using the SPSS Statistics software version 26 for Windows.

3. Results

3.1. Agar Yield

The methods of extraction used had a direct impact on the agar yield. The agar yield obtained from the invasive species *A. vermiculophyllum* (Table 1) via native extraction (28.4 ± 0.9%, dry weight basis) differed significantly ($p < 0.000$) from that obtained with the alkali pretreatment (20.4 ± 0.8%, dw) (Table 2). The yield and the composition of agars differ depending on species, collection season, environmental factors, extraction process conditions, etc.

Table 1. Yield of native and alkali-treated agars from *A. vermiculophyllum* harvested from Morocco compared with *A. vermiculophyllum* collected around the world.

Agar Yield (%) dw	Native Extraction	Alkali Pretreatment	References
A. vermiculophyllum (Morocco)	28.4 ± 0.9	20.4 ± 0.8	Current study
A. vermiculophyllum (Portugal)	18.44	10.8	[20]
A. vermiculophyllum (Portugal)	15	29.4	[21]
A. vermiculophyllum (Mexico)	34.6	15.3	[19]
A. vermiculophyllum (Mexico)	45.7	-	[26]
A. vermiculophyllum (Mexico)	-	11.4 ± 0.4	[27]

(Mean ± SE, n = 3). SE: standard error, dw: dry weight.

Table 2. *t*-test for Equality of Means of agar properties between the alkali and native treatments.

Agar Properties	t	*p*-Value
Agar yield	−10.687	**0.000**
Melting temperature	17.117	**0.000**
Gelling temperature	8.890	**0.001**
Gel strength	16.257	**0.000**
Sulfate content	−7.491	**0.002**
3–6 AG content	10.251	**0.001**

t: t-value. The significant values are in bold.

3.2. FT-IR Spectroscopy

The Fourier-transformed infrared spectra of the native and alkali-pretreated agars showed similar FTIR spectra (Figure 1) with characteristic peaks compared with the commercial agar (Biokar Diagnostics). The majority of certain agarocolloid-type bands were located between 700 and 1600 cm^{-1} [28]. The spectra displayed major bands at 1035 and 1149 cm^{-1} corresponding to C-O and C-C stretching vibrations in the pyranose ring typical of all polysaccharides [17,29]; bands at 1241 and 1368 cm^{-1} have been recognized in ester–sulfate groups [29,30]. The band located around 715 cm^{-1} is attributed to the C-O-C bending mode in glycosidic linkages [28]. The band at 928 cm^{-1} is assigned to the C-O vibration of 3,6-AG [18,30]. A minor band at 845 cm^{-1} is assigned to the sulfate groups at the C-4 of D-galactose [13,31]. Finally, the band situated at 886 cm^{-1} is linked to the C-H bending at the C-1 of β galactopyranosyl [32].

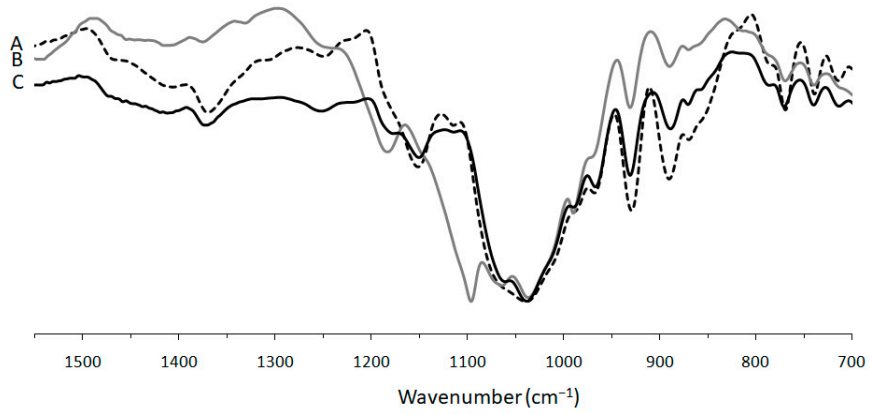

Figure 1. FTIR spectra of the agars extracted from *Agarophyton vermiculophyllum*: commercial agar (A), native agar (B), and alkaline pretreated agar (C).

3.3. ^{13}C NMR Spectroscopy

^{13}C NMR spectroscopy (nuclear magnetic resonance spectroscopy) is used as an efficient technique to analyze red seaweed polysaccharides. ^{13}C NMR spectroscopy provides information on the environment of every carbon atom in the molecule. The ^{13}C NMR spectra of the native and the alkali extractions, as well as the commercial agar, showed 12 major

signals (Figure 2). The signals for 3,6-anhydro-α-L-galactopyranose were identified at 98.5, 80.3, 77.5, 75.8, 70.07, and 69.6 ppm, linked to C1, C3, C4, C5 C6, and C2, sequentially [33,34]. The signal noticed at 102.6 ppm conformed to the C1 of β-D-galactopyranose. In addition, the signals registered at 70.4, 82.4, 68.9, 75.5, and 61.6 ppm corresponded to C2, C3, C4, C5, and C6 and, subsequently, a three-linked β-D-galactopyranosyl unit [33]. The non-appearance of the peak at 59.0 ppm coinciding with the O-methyl group showed a poorly methylated agarose structure.

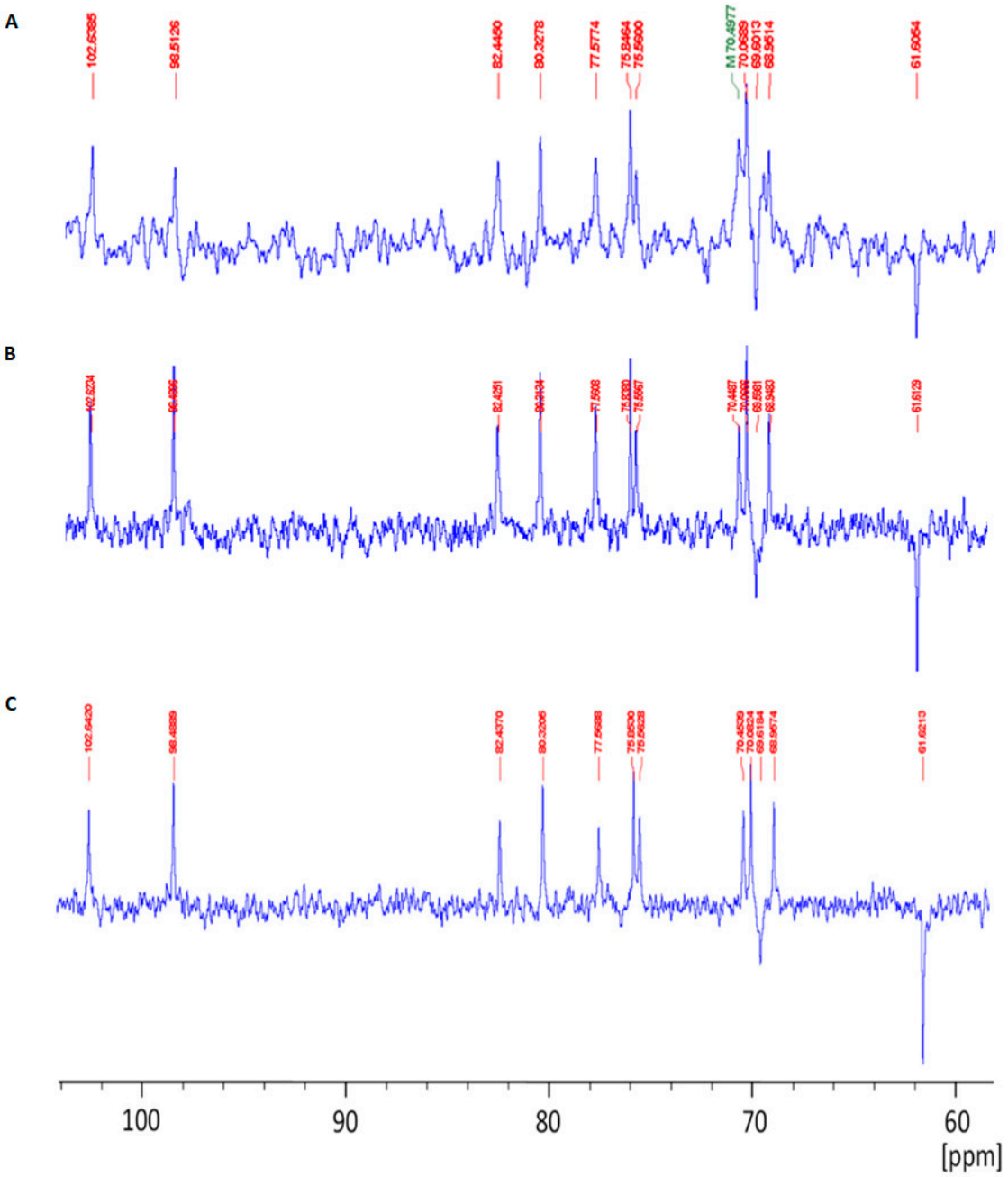

Figure 2. ^{13}C NMR spectra of agar extracted from *Agarophytonvermiculophyllum*: (**A**) native agar; (**B**) agar pretreated with 7% NaOH; (**C**) commercial agar.

3.4. Determination of Gel Properties

The gel strength of the extracted agar revealed a significant difference between both treatments (*t*-test, $p < 0.001$). The highest agar gel strength (763.8 ± 57.04 g cm^{-2}) was obtained during the alkali pretreatment (Table 3), while the lowest gel strength was recorded

for the native extraction sample (204.8 ± 17.10 g cm^{-2}). The gel strength of the tested commercial agar was 909.8 ± 187.60 (Figure 3). The gelling and melting temperatures of the agar gel samples in this study were significantly influenced by both extraction conditions (*t*-test, $p \leq 0.001$), with alkaline pretreatment showing the most elevated values (36.5 ± 0.9 °C and 83.2 ± 0.6 °C, respectively), while the lowest values were obtained through the native treatment (29.5 ± 0.9 and 73.4 ± 0.7 °C, respectively).

Table 3. Rheological properties of agars from *A. vermiculophyllum* compared with some important agarophyte species.

Species	Treatment	Gel Strength (g/cm^2)	Gelling T° (°C)	Melting T° (°C)	References
A. vermiculophyllum (Morocco)	Native Alkali	204.8 ± 17.10 763.8 ± 57.04	29.5 ± 0.9 36.5 ± 0.9	73.4 ± 0.7 83.2 ± 0.6	Current study Current study
Gracilaria gracilis	Native Alkali	105.3 ± 6.0 377.3 ± 19.7	31.7 ± 0.2 35.4 ± 0.3	78.5 ± 0.4 82.1 ± 0.1	[17]
Gelidium microdon	Native Alkali	350.0 ± 17.6 489.0 ± 19.4	35.1 ± 0.7 38.3 ± 0.8	85.6 ± 0.2 88.3 ± 0.7	[18]
Gelidium corneum	Native Alkali	341.0 ± 16.9 528.5 ± 11.0	36.0 ± 0.9 39.2 ± 1.0	86.7 ± 0.6 89.8 ± 0.1	[18]
Gracilaria fisheri	Native Alkali	145.6 ± 34.5 228.2 ± 48.1	49.25 ± 0.9 47.0 ± 0.0	72.4 ± 0.1 72.3 ± 0.0	[35]
Gracilaria edulis	Native Alkali	197.0 ± 72.8 239.9 ± 28.3	60.2 ± 0.4 61.0 ± 1.0	92.6 ± 0.3 87.6 ± 0.0	[35]
Gracilaria cliftonii	Native	133.0 ± 9	34.7 ± 0.7	85.7 ± 1.2	[36]
Gracilaria veleroae	Alkali	343.5 ± 1.4	43.9 ± 0.3	86.0 ± 0.9	[27]
Gracilaria tenuistipitata	Alkali	624.4–1250.8	28.1–43.2	96.4–99.6	[37]
Gracilaria tenuistipitata	Alkali	482	35.5	103.3	[38]
Gracilaria crassissima	Alkali	1390	50	93	[39]
Gracilaria lemaneiformis	Alkali	1908	41.4 ± 0.5	-	[40]
Gracilaria gracilis	Native	437	31	85	[41]
Gracilaria salicornia	Native Alkali	103.3 ± 6.0 210 ± 7.2	- -	- -	[42]
A. vermiculophyllum (Mexico)	Alkali	1064	35.7–39.6	92.4–99.7	[19]
A. vermiculophyllum (Mexico)	Native	85	27.7–36.5	73.9–53.5	[26]
A. vermiculophyllum (Mexico)	Alkali	644.0 ± 49.0	38.3 ± 0.6	99.7 ± 0.3	[27]
A. vermiculophyllum	Alkali	1331	40.7	93.1	[43]
A. vermiculophyllum (Portugal)	Alkali	614	35.4	79.1	[21]
A. vermiculophyllum (Portugal)	Native Alkali	247 348			[20]

3.5. Determination of Chemical Properties

The chemical properties of the agar were influenced by the alkaline pretreatment. The 3,6-AG content was negatively linked to the sulfate content. In general, the increase in 3,6-AG content coincided with the decline in sulfate content. There was a significantly negative correlation between the sulfate content and the 3,6-AG content because of the L-galactose-6-sulfate, which was susceptible to converting into 3,6-anhydro-L-galactose under alkali

treatments [26,37,39]. The native agar showed significantly higher sulfate content (10.5 ± 0.5%) than that of the alkaline pretreatment (6.2 ± 0.8%) ($p < 0.01$; Table 2). Likewise, the 3,6-AG content differed significantly ($p < 0.01$) between both studied treatment conditions. The alkali-pretreated agar from *A. vermiculophyllum* had the greatest 3,6-AG content (26.2 ± 1.9%), whereas the native extraction showed the lowest values (13.8 ± 0.7%) (Table 4).

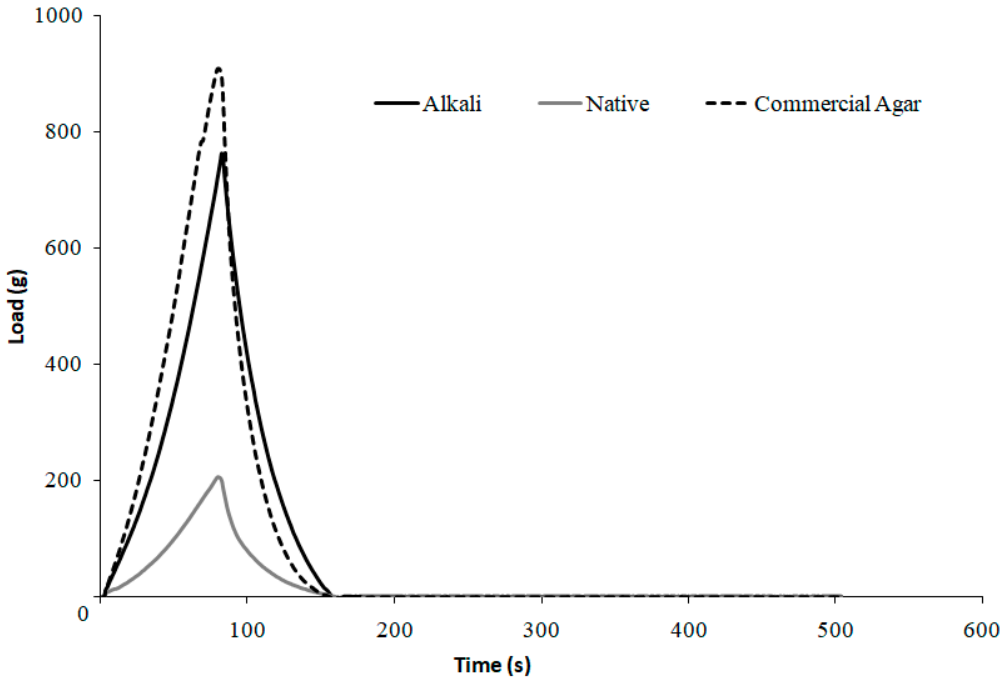

Figure 3. Comparison of texture analysis between the native, alkali-treated, and commercial agars using a texture analyzer.

Table 4. Agar physicochemical properties from *A. vermiculophyllum* and some agarophyte species.

Species	Treatment	Sulfate Content	3,6-AG Content	References
A. vermiculophyllum (Morocco)	Native Alkali	10.5 ± 0.5 6.2 ± 0.8	13.8 ± 0.7 26.2 ± 1.9	Current study
A. vermiculophyllum (Mexico)	Alkali	1.86	42.5	[21]
A. vermiculophyllum	Alkali	-	38.9 ± 0.9	[27]
Gracilaria gracilis	Native	0.65 ± 0.0	5.6 ± 0.4	[17]
Gracilaria edulis	Native Alkali	7.5 ± 0.1 4.9 ± 0.0	37.0 ± 2.8 43.0 ± 0.4	[35]
Gracilaria fisheri	Native Alkali	4.5 ± 0.1 2.8 ± 0.0	35.1 ± 0.0 38.8 ± 1.8	[35]
Gracilaria cliftonii	Native	5.9 ± 1.5	-	[36]
Gracilaria veleroae	Alkali	-	41.6 ± 0.3	[27]
Gracilaria tenuistipitata	Alkali	7.7–3.4	38.9–44.7	[37]
Gracilari atenuistipitata	Alkali	1.6	-	[38]
Gracilaria crassissima	Alkali	-	43	[39]
Gracilaria lemaneiformis	Alkali	0.7 ± 0.0	41.3 ± 0.8	[40]
Gracilaria gracilis	Native	6.8	-	[41]
Gracilaria salicornia	Native Alkali	1.45 1.15	19.4 20.9	[42]
Gracilaria crassa	Native Alkali	2.2 1.9	16.9 18.2	[42]

4. Discussion

The present study demonstrated that all the properties of the agar extracted from *A.vermiculophyllum* varied between both treatments. Other studies have confirmed the results accomplished using *A. vermiculophyllum*, with a higher agar yield during the native extraction (18%) compared with that attained using the alkaline treatment (10%) on *A. vermiculophyllum* from Portugal [20]. Belattmania et al. [17] reported that the yields of agar extracted from the red seaweed *Gracilaria gracilis* from the southern Moroccan Atlantic coast were 20.5 ± 1.3 and 15.16 ± 2.5% dw from the alkaline pretreatment (NaOH) and native extraction, respectively. The decrease in the agar yield was induced by subjecting *Gracilaria* to the alkaline treatment at high temperatures (80–100 °C) before the extraction process, resulting in the degradation of polysaccharides and agar failure due to diffusion during the pretreatment process [2,39]. On the other hand, an opposite effect for alkaline treatments has been demonstrated by other studies. Praiboon et al. [35] reported that agars obtained from pretreated *Gracilaria fisheri* and *G. edulis* using 5% NaOH exhibited a greater yield (34.3–39.6%) in comparison with native agars (10.9–13.3%). Alkaline pretreatment, using either NaOH or KOH within a concentration range of 3–7%, had the potential to enhance the agar yield [38]. In a study conducted by Vuai in 2022 [44], it was observed that different treatments had varying effects on the agar yield. The agar yield from *G. Salicornia*-treated agar was found to be 21.9 ± 0.7%, which was higher compared with an untreated agar yield (15.8 ± 0.7). Additionally, agar treated with *G. corticata* resulted in a yield of 27 ± 0.7, slightly higher than the yield of untreated agar at 26.2 ± 1.3%. *G. edulis*-treated agar had a yield of 17.2 ± 1.6%.

Concerning gel strength, Orduña-Rojas et al. [26] indicated a gel strength value of 158.0 g cm^{-2} for 7% alkali-pretreated agar extracted from *A. vermiculophyllum* (previously called *Gracilaria vermiculophylla*) from Mexico. According to Arvizu-Higuera et al. [19], the alkali-pretreated agar gel strength accounted for 1064 g cm^{-2}. The breaking gel strength of alkali-pretreated agar from *G. vermiculophylla* is higher (348 g cm^{-2}) than that of native agar (247 g cm^{-2}) [20]. Rodríguez-Montesinos et al. [27] reported a seasonal pattern for agar gel strength from *G. vermiculophylla*, with the lowest value recorded in the summer (524 g cm^{-2}) and the highest gel strength value in winter (793 g cm^{-2}). This current study supports findings from previous research conducted on *Gracilaria tenuistipitata* from Bangladesh according to Mohibbullah et al. [45]. It was found that the gel strength was lowest (132.78 ± 2.99 g cm^{-2}) in native agar without any treatment. However, when the alkaline pretreatment was applied, there was an increase in gel strength (201.33 ± 5.44 g cm^{-2}).

Concerning gelling and melting temperatures, other studies on the invasive seaweed *G. vermiculophylla* [26] have reported different gelling and melting temperatures during alkaline pretreatment (7%), which were 41.8 and 81.4 °C, respectively. The melting temperatures for an alkali pretreatment time of 0.5 h and a 2 h extraction time for alkali-treated agar were, respectively, 94.2 and 98.1 °C. However, the gelling and melting temperatures for the native extraction were 30.5 and 22.3 °C, respectively [19]. Agar from integrated, multitrophic, aquacultured *G. vermiculophylla* from Ria de Aveiro (northwestern Portugal) showed 40.7 ± 0.2 °C as the gelling temperature and 93.1 ± 0.5 °C as the melting temperature [43]. Some works have indicated that applying an alkaline pretreatment prior to agar extraction increases the agar's gelling and melting temperatures. For example, Villanueva et al. [21] mentioned that the gelling and melting temperatures of *G. vermiculophylla* agar exhibit the lowest values (21.6–26.4 and 62.7–70.0 °C, respectively), yet pretreatment with NaOH concentrations of up to 4% lead to improvements in both gelling and melting temperatures (31.0–35.8 and 73.6–80.4 °C, respectively). Mohibbullah et al. [45] reported that the melting temperatures of water- and NaOH-pretreated agars were 85.93 ± 0.34 °C and 86.00 ± 0.25 °C, respectively, and the gelling temperatures were recorded as 37.67 ± 0.22 °C, and 37.97 ± 0.12 °C.

Regarding the chemical characteristics, Orduña-Rojas et al. [26] indicated that minimal 3,6-AG content in *G. vermiculophylla* was found in native agars (6.7%), while 7% NaOH-

pretreated samples showed the greatest 3,6-AG content (33.8%). According to Yarnpakdee et al. [38], the 3,6-AG content for agars with NaOH pretreatments increased with an increasing alkaline concentration of up to 5%. The employment of an alkaline treatment before agar extraction was able to cleave the sulfate ester bond at the C-6 of L-galactose with the simultaneous formation of 3,6-AG (Figure 4), resulting in the enhancement of the gel-forming characteristics [46]. The gel strength enhancement after alkaline treatments is related to the elimination of a less energetically stable axial sulfate ester positioned at the C-6 of the L-galactopyranose unit, resulting in the production of more stable 3,6-anhydro-L-galactose [2]. Agar with 3,6-AG content produces a strong gel and, conversely, agar with low 3,6-AG content produces a weak gel [11,47,48]. A comparison of the mean (*t*-test) between the nature of the treatment and the chemical properties (sulfate and 3,6-AG content) has shown a significance level of $p < 0.01$, which means the relationship is highly significant; therefore, it is likely that there is a relationship between the two variables, which shows that the treatment affects the sulfate and 3,6-AG contents (Table 4). Pearson's correlation analysis (test of significance, two-tailed) was used to determine the correlation between the studied parameters previously cited. Pearson's correlation analysis of the native extraction showed a strong positive correlation (r = 1.000, $p = 0.000$) between the agar yield and the gelling temperature. The melting temperature also correlated positively with the 3-6 AG content (r = 1.000, $p = 0.013$), which is in agreement with the study by Villanueva et al. [21]. The agar yield did not show any significant correlation with either gel strength or sulfate content, which is in accordance with the study by Yousefi et al. [49], who worked on *Gracilaria corticata* from the Persian Gulf. In the same context, Wang et al. [37] confirmed the presence of a positive correlation between 3,6-anhydrogalactose content and the melting temperatures of agar from *Gracilaria tenuistipitata*. However, no significant correlations were obtained among any of the agar properties for the alkaline pretreatment condition. In contrast with these observations, according to Marinho-Soriano and Bourret [50], all gel properties are significantly positively correlated with 3,6-AG content and negatively with sulfate content. Concerning the species *Gracilaria dura*, the 3,6-AG content correlated positively with gel strength, but there was no significant correlation between sulfate content and gel strength. In the report of Orduña-Rojas et al. [26], who worked on the two agarophyte species *A. vermiculophyllum* and *Gracilariopsis longissima*, the sulfate content showed a negative correlation with gel strength (r = −0.95) for *G. longissima*. Also, the sulfate content for *A. vermiculophyllum* did not show a statistically significant difference compared with the low-alkali treatments, but it did show the opposite with the high-alkali treatments. The 3,6-AG content revealed a significant correlation with gel strength for *A. vermiculophyllum* (r = 0.91) and *G. longissima* (r = 0.89). This result was not in agreement with our obtained results.

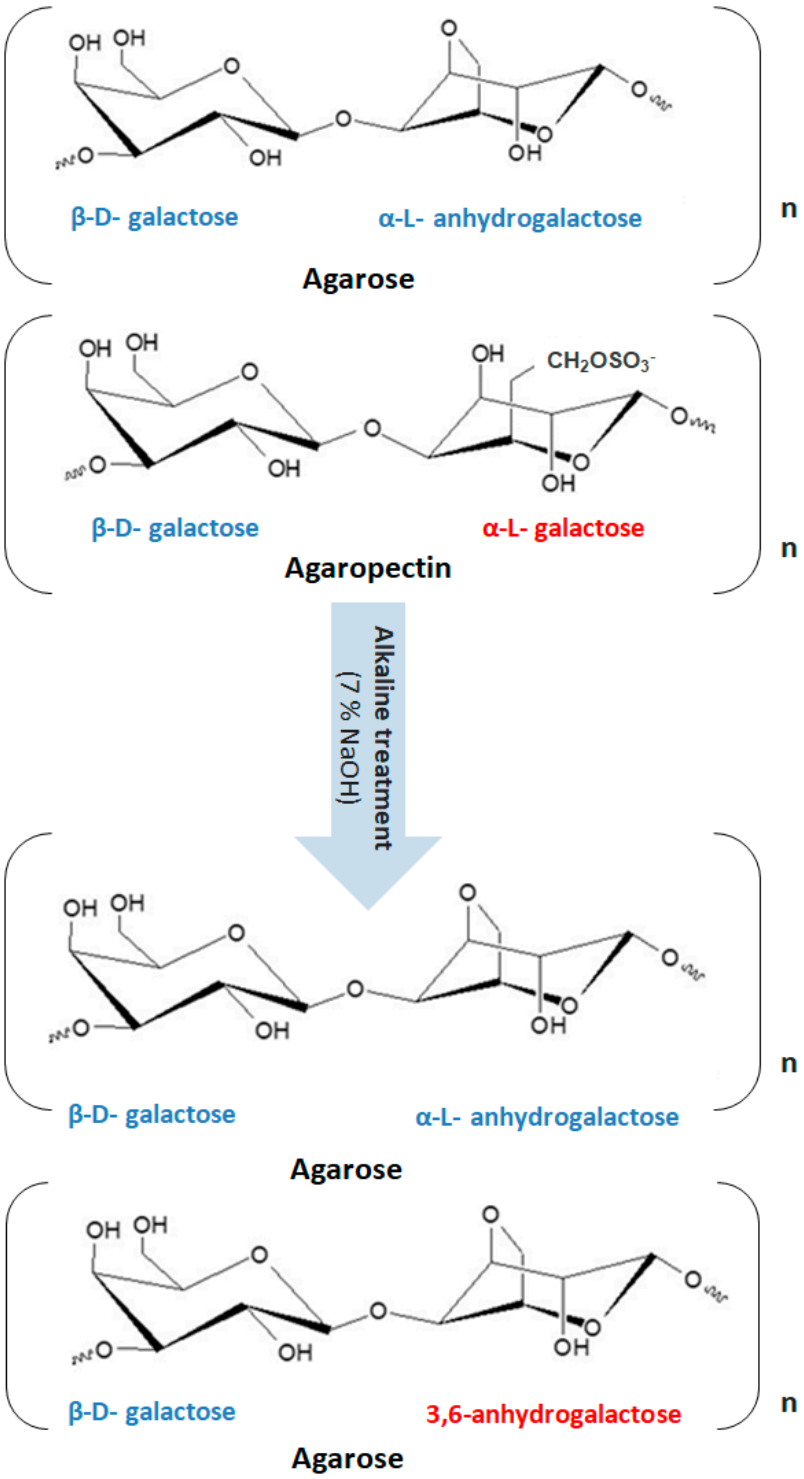

Figure 4. Illustration of the chemical transformation of the agar structure during alkaline treatment.

5. Conclusions

Agar extracted from the invasive red seaweed *A. vermiculophyllum* was characterized through spectroscopic and rheological analyses. The influence of alkaline pretreatment with 7% NaOH on the agar yield and properties was also investigated. Based on the findings of this study, the native extracted agar yield (28.4 ± 0.9%, dry weight basis) was higher than that of the alkaline-pretreated agar samples (20.4 ± 0.8%). The physical and chemical characteristics of the extracted agar were improved by the alkaline pretreatment in comparison with the native extraction process. The 7% NaOH-pretreated samples revealed

higher gel strength, which is considered a crucial parameter for agar applications. It was observed that the alkali pretreatment had a positive effect on gel strength, leading to an improvement in its value, increased gelling and melting temperatures with a rise in 3,6-AG content, and a reduction in sulfate levels. Overall, the satisfying yield, as well as the physical and chemical characteristics of the extracted agar, makes this invasive species a possible candidate for commercial purposes as a local agar source. Furthermore, this study provides new possibilities for future research, such as the optimization of extraction conditions and the seasonality and improvement of seaweed storage techniques.

Author Contributions: Conceptualization, B.S., F.B. and Z.B.; methodology, A.N., Z.B., B.S., A.R. and A.C.; software, C.J. and F.B.; validation, C.J., F.B. and B.S.; formal analysis, C.J., F.B. and A.N.; writing—original draft preparation, A.N.; writing—review and editing, B.S.; Z.B. and A.C.; visualization, C.J., F.B., A.N., F.H. and Z.B.; supervision, B.S. and A.C.; project administration, B.S. and C.J.; funding acquisition, B.S. and C.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the VPMA3/DESRS-ANPMA-CNRST project,"Exploitation de la diversité spécifique et génétique pour une bioraffinerie innovante des algues marines de la côte atlantique marocaine".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are reported within this manuscript.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of the data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Liao, Y.C.; Chang, C.C.; Nagarajan, D.; Chen, C.Y.; Chang, J.S. Algae-derived hydrocolloids in foods: Applications and health-related issues. *Bioengineered* **2021**, *12*, 3787–3801. [CrossRef]
2. Zhang, H.; Zhang, F.; Yuan, R. Applications of natural polymer-based hy-drogels in the food industry. In *Hydrogels Based on Natural Polymers*; Chen, Y., Ed.; Elsevier: London, UK, 2020; pp. 357–410. [CrossRef]
3. Mostafavi, F.S.; Zaeim, D. Agar-based edible films for food packaging applications—A review. *Int. J. Biol. Macromol.* **2020**, *159*, 1165–1176. [CrossRef]
4. Lomartire, S.; Gonçalves, A.M.M. Algal Phycocolloids: Bioactivities and Pharmaceutical Applications. *Mar. Drugs* **2023**, *21*, 384. [CrossRef]
5. FAO. *The State of World Fisheries and Aquaculture: Sustainability in Action*; FAO: Rome, Italy, 2020; pp. 1–206.
6. Mantri, V.A.; Shah, Y.; Thiruppathi, S. Feasibility of farming the agarose-yielding red alga *Gracilaria dura* using tube-net cultivation in the open sea along the Gujarat coast of NW India. *J. Appl. Phycol.* **2020**, *1*, 12–19. [CrossRef]
7. Porse, H.; Rudolph, B. The seaweed hydrocolloid industry: 2016 updates, requirements, and outlook. *J. Appl. Phycol.* **2017**, *29*, 2187–2200. [CrossRef]
8. McHugh, D.J. Worldwide distribution of commercial resources of seaweeds including *Gelidium*. *Hydrobiologia* **1991**, *221*, 19–29. [CrossRef]
9. Kim, J.K.; Yarish, C.; Hwang, E.K.; Park, M.; Kim, Y. Seaweed aquaculture: Cultivation technologies, challenges and its ecosystem services. *Algae* **2017**, *32*, 1–13. [CrossRef]
10. Kim, S.Y.; Weinberger, F.; Boo, S.M. Genetic Data Hint at a Common Donor Region for Invasive Atlantic and Pacific Populations of *Gracilaria Vermiculophylla* (gracilariales, Rhodophyta). *J. of Phycol.* **2010**, *46*, 1346–1349. [CrossRef]
11. Armisen, R. World-wide use and importance of *Gracilaria*. *J. Appl. Phycol.* **1995**, *7*, 231. [CrossRef]
12. Lee, W.-K.; Lim, Y.-Y.; Leow, A.T.-C.; Namasivayam, P.; Abdullah, J.O.; Ho, C.-L. Factors affecting yield and gelling properties of agar. *J. Appl. Phycol.* **2017**, *29*, 1527–1540. [CrossRef]
13. Martinez-Sanz, M.; Gomez-Mascaraque, L.G.; Ballester, A.R.; Martinez-Abad, A.; Brodkorb, A.; Lopez-Rubio, A. Production of unpurified agar-based extracts from red seaweed *Gelidium sesquipedale* by means of simplified extraction protocols. *Algal Res.* **2019**, *38*, 101420. [CrossRef]
14. Mouradi-Givernaud, A.T.; Givernaud, Y.; Morvan, H.; Cosson, J. Agar de *Gelidium latifolium* (Rhodophyceae, Gelidiales), composition biochimique et variations saisonnières. *Bot. Mar.* **1992**, *35*, 153–159. [CrossRef]
15. Mouradi Givernaud, A.; Hassani, L.A.; Givernaud, T.; Lemoine, Y.; Benharbet, O. Biology and agar composition of *Gelidium sesquipedale* harvested along the Atlantic coast of Morocco. *Hydrobiologia* **1999**, *398*, 391–395. [CrossRef]
16. Givernaud, T.; El Gourji, A.; Mouradi-Givernaud, A.; Lemoine, Y.; Chiadmi, N. Seasonal variations of growth and agar composition of *Gracilaria multipartita* harvested along the Atlantic coast of Morocco. *Hydrobiologia* **1999**, *398*, 167–172. [CrossRef]

17. Belattmania, Z.; Bhaby, S.; Nadri, A.; Khaya, K.; Bentiss, F.; Jama, C.; Reani, A.; Vasconcelos, V.; Sabour, B. *Gracilaria gracilis* (Gracilariales, Rhodophyta) from Dakhla (southern Moroccan Atlantic coast) as source of agar: Content, chemical characteristics, and gelling properties. *Mar. Drugs* **2021**, *19*, 672. [CrossRef] [PubMed]
18. Belattmania, Z.; Bentiss, F.; Jama, C.; Nadri, A.; Reani, A.; Sabour, B. Spectroscopic characterization and gel properties of agar from two *Gelidium* species from the Atlantic coast of Morocco. *Biointerface Res. Appl. Chem.* **2021**, *11*, 12642–12652. [CrossRef]
19. Arvizu-Higuera, D.L.; Rodríguez-Montesinos, Y.E.; Murillo-Álvarez, J.I.; Muñoz-Ochoa, M.; Hernández-Carmona, G. Effect of alkali treatment time and extraction time on agar from *Gracilaria vermiculophylla*. *J. Appl. Phycol.* **2008**, *20*, 515–519. [CrossRef]
20. Pereira, S.G.; Teixeira-Guedes, C.; Souza-Matos, G.; Maricato, É.; Nunes, C.; Coimbra, M.A.; Rocha, C.M. Influence of ohmic heating in the composition of extracts from Gracilaria vermiculophylla. *Algal Res.* **2021**, *58*, 102360. [CrossRef]
21. Villanueva, R.D.; Sousa, A.M.M.; Gonçalves, M.P.; Nilsson, M.; Hilliou, L. Production and properties of agar from the invasive marine alga, *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta). *J. Appl. Phycol.* **2010**, *22*, 211–220. [CrossRef]
22. Afonso, C.; Correia, A.P.; Freitas, M.V.; Baptista, T.; Neves, M.; Mouga, T. Seasonal Changes in the Nutritional Composition of *Agarophyton vermiculophyllum* (Rhodophyta, Gracilariales) from the Center of Portugal. *Foods* **2021**, *10*, 1145. [CrossRef]
23. Freile-Pelegrín, Y.; Robledo, D. Influence of alkali treatment on agar from *Gracilaria cornea* from Yucatan, Mexico. *J. Appl. Phycol.* **1997**, *9*, 533–539. [CrossRef]
24. Xie, X.-T.; Zhang, X.; Liu, Y.; Chen, X.-Q.; Cheong, K.-L. Quantification of 3,6-anhydro-galactose in red seaweed polysaccharides and their potential skin-whitening activity. *Biotechnol.* **2020**, *10*, 189. [CrossRef] [PubMed]
25. Craigie, J.S.; Wen, Z.C.; Van der Meer, J.P. Interspecific, intraspecific and nutritionally determined variations in the composition of, agars from *Gracilaria* spp. *Bot.* **1984**, *27*, 55–61. [CrossRef]
26. Orduña-Rojas, J.; Suárez-Castro, R.; López-Álvarez, E.S.; Riosmena-Rodríguez, R.; Pacheco-Ruiz, I.; Zertuche-González, J.A.; Meling-López, A.E. Influence of alkali treatment on agar from *Gracilariopsis longissima* and *Gracilaria vermiculophylla* from the Gulf of California, Mexico. *Cienc. Mar.* **2008**, *34*, 513–521. [CrossRef]
27. Rodríguez-Montesinos, Y.E.; Arvizu-Higuera, D.L.; Hernández-Carmona, G.; Muñoz-Ochoa, M.; Murillo-Álvarez, J.I. Seasonal variation of the agar quality and chemical composition of *Gracilaria veleroae* and *Gracilaria vermiculophylla* (Rhodophyceae, Gracilariaceae) from Baja California Sur, Mexico: Seasonal variation of two *Gracilaria*. *Phycol. Res.* **2013**, *61*, 116–123. [CrossRef]
28. Melo, M.R.S.; Feitosa, J.P.A.; Freitas, A.L.P.; de Paula, R.C.M. Isolation and characterization of soluble sulfated polysaccharide from the red seaweed *Gracilaria cornea*. *Carbohydr. Polym.* **2002**, *49*, 491–498. [CrossRef]
29. Guerrero, P.; Etxabide, A.; Leceta, I.; Peñalba, M.; de la Caba, K. Extraction of agar from *Gelidiumsesquipedale* (Rodhopyta) and surface characterization of agar based films. *Carbohydr. Polym.* **2014**, *99*, 491–498. [CrossRef]
30. Sousa, A.M.M.; Morais, S.; Abreu, M.H.; Pereira, R.; Sousa-Pinto, I.; Cabrita, E.J.; Delerue-Matos, C.; Gonçalves, M.P. Structural, Physical, and Chemical Modifications Induced by Microwave Heating on Native Agar-like Galactans. *J. Agric. Food Chem.* **2012**, *60*, 4977–4985. [CrossRef]
31. Mollet, J.-C.; Rahaoui, A.; Lemoine, Y. Yield, chemical composition and gel strength of agarocolloids of *Gracilariagracilis*, *Gracilariopsislongissima* and the newly reported *Gracilaria* cf. *Vermiculophylla from Roscoff (Brittany, France). J. App. Phycol.* **1998**, *10*, 59–66. [CrossRef]
32. Gómez-Ordóñez, E.; Rupérez, P. FTIR-ATR spectroscopy as a tool for polysaccharide identification in edible brown and red seaweeds. *Food Hydrocoll.* **2011**, *25*, 1514–1520. [CrossRef]
33. Lahaye, M.; Rochas, C.; Yaphe, W. A new procedure for determining the heterogeneity of agar polymers in the cell walls of *Gracilaria* spp. (Gracilariaceae, Rhodophyta). *Canad. J. Bot.* **1986**, *64*, 579–585. [CrossRef]
34. Lahaye, M.; Yaphe, W.; Viet, M.T.P.; Rochas, C. 13C-n.m.r. Spectroscopic investigation of methylated and charged agarose oligosaccharides and polysaccharides. *Carbohydr. Res.* **1989**, *190*, 249–265. [CrossRef]
35. Praiboon, J.; Chirapart, A.; Akakabe, Y.; Bhumibhamon, O.; Kajiwara, T. Physical and chemical characterization of agar polysaccharides extracted from the Thai and Japanese species of *Gracilaria*. *Sci. Asia* **2006**, *32*, 11–17. [CrossRef] [PubMed]
36. Kumar, V.; Fotedar, R. Agar extraction process for *Gracilaria cliftonii* (Withell, Miller & Kraft, 1994). *Carbohydr. Polym.* **2009**, *78*, 813–819. [CrossRef]
37. Wang, L.; Shen, Z.; Mu, H.; Lin, Y.; Zhang, J.; Jiang, X. Impact of alkali pretreatment on yield, physico-chemical and gelling properties of high quality agar from *Gracilaria tenuistipitata*. *Food Hydrocoll.* **2017**, *70*, 356–362. [CrossRef]
38. Yarnpakdee, S.; Benjakul, S.; Kingwascharapong, P. Physico-chemical and gel properties of agar from *Gracilaria tenuistipitata* from the lake of Songkhla, Thailand. *Food Hydrocoll.* **2015**, *51*, 217–226. [CrossRef]
39. Freile-Pelegrín, Y.; Murano, E. Agars from three species of *Gracilaria* (Rhodophyta) from Yucatan Peninsula. *Bioresour. Technol.* **2005**, *96*, 295–302. [CrossRef] [PubMed]
40. Li, H.; Huang, J.; Xin, Y.; Zhang, B.; Jin, Y.; Zhang, W. Optimization and scaleup of a new photobleaching agar extraction process from *Gracilaria lemaneiformis*. *J. Appl. Phycol.* **2009**, *21*, 247–254. [CrossRef]
41. Rodríguez, M.C.; Matulewicz, M.C.; Noseda, M.D.; Ducatti, D.R.B.; Leonardi, P.I. Agar from *Gracilariagracilis* (Gracilariales, Rhodophyta) of the Patagonic coast of Argentina—Content, structure and physical properties. *Bioresour. Technol.* **2009**, *100*, 1435–1441. [CrossRef] [PubMed]
42. Oyieke, H.A. The yield, physical and chemical properties of agar gel from Gracilaria species (Gracilariales, Rhodophyta) of the Kenya coast. *Hydrobiologia* **1993**, *260–261*, 613–620. [CrossRef]

43. Sousa, A.M.M.; Alves, V.D.; Morais, S.; Delerue-Matos, C.; Gonçalves, M.P. Agar extraction from integrated multitrophic aquacultured *Gracilaria vermiculophylla*: Evaluation of a microwave-assisted process using response surface methodology. *Bioresour. Technol.* **2010**, *101*, 3258–3267. [CrossRef]
44. Vuai, S.A.H. Characterization of agar extracted from Gracilaria species collected along Tanzanian coast. *Heliyon* **2022**, *8*, e09002. [CrossRef]
45. Mohibbullah, M.; Talha, M.A.; Baten, M.A.; Newaz, A.W.; Choi, J.-S. Yield optimization, physicochemical characterizations, and antioxidant properties of food grade agar from *Gracilaria tenuistipitata* of Cox's Bazar coast, Bangladesh. *Food Sci. Nutr.* **2023**, *11*, 2852–2863. [CrossRef]
46. Murano, E.; Toffanin, R.; Zanetti, F.; Knutsen, S.H.; Paoletti, S.; Rizzo, R. Chemical and macromolecular characterisation of agar polymers from *Gracilaria dura* (C. Agardh) J. Agardh (Gracilariaceae, Rhodophyta). *Carbohydr. Polym.* **1992**, *18*, 171–178. [CrossRef]
47. Duckworth, M.; Hong, K.C.; Yaphe, W. The agar polysaccharides of Gracilaria species. *Carbohydr. Res.* **1971**, *18*, 1–9. [CrossRef]
48. Montaño, N.E.; Villanueva, R.D.; Romero, J.B. Chemical characteristics and gelling properties of agar from two Philippine *Gracilaria* spp. (Gracilariales, Rhodophyta). *J. App. Phycol.* **1999**, *11*, 27–34. [CrossRef]
49. Yousefi, M.K.; Islami, H.R.; Filizadeh, Y. Effect of extraction process on agar properties of *Gracilariacorticata* (Rhodophyta) collected from the Persian Gulf. *Phycologia* **2013**, *52*, 481–487. [CrossRef]
50. Marinho-Soriano, E.; Bourret, E. Polysaccharides from the red seaweed *Gracilaria dura* (Gracilariales, Rhodophyta). *Bioresour. Technol.* **2005**, *96*, 379–382. [CrossRef] [PubMed]