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Seaweed Fiber Fabricated with Agar Alkali-Free Extracted from *Gracilaria lemaneiformis*

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ABSTRACT

The sulfate groups in agar structure played a good role in the formation of fiber. However, commercially available agar is usually extracted from red algae by alkali treatment to decrease the content of sulfate group for the purpose of high gel strength. In this paper, an alkali-free method of agar extraction from *Gracilaria lemaneiformis* was proposed for the wet-spinning purpose. This method is environmentally friendly, reduces the extraction steps, saves energy, and reduces the production cost of agar fiber. The improved agar preparation process not only has higher agar yield, but also has higher molecular weight and sulfate group content, which is beneficial to the preparation and forming of fiber and makes the fiber have higher mechanical strength and elongation. Therefore, this extraction technology has broad application prospect in the textile field.

KEYWORDS

Seaweed fiber; *Gracilaria lemaneiformis*; agar; alkali-free treatment

1 Introduction

Agar is a natural polysaccharide containing a small amount of sulfate groups extracted from red algae [1]. It consists of 3, 6-anhydro-L-galactopyranose and D-galactose alternately linked via α-1, 3 and β-1, 4 glycosidic bonds (Fig. 1 left) [2]. Agar can be fractionated into two components. The fraction with the greatest gelling capability is termed agarose, which is the neutral polysaccharide [3]. The remaining fraction is termed agaropectin and contains all the charged polysaccharide components [4]. In a previous report, Sousa et al. [5] found that in order to obtain high strength agar gel, the charged components, especially sulfate group content, should be reduced as much as possible. Meanwhile, Sousa et al. [6] reported that the high molecular weight and 3, 6-anhydro-L-galactopyranose content of the agar could reduce the loss of viscoelastic modulus and showed a compact and regular polymer network, which had a positive effect on the increase of gel strength.

Agar is traditionally utilized in pharmaceutical, food and beverage industries, owing to its gelation properties and abundant bioactivities, such as hypolipidemic, immune regulation and so on [7,8]. In recent years, seaweed fibers have made great progress in textiles [9], sensors [10], wound dressing [11], etc. [12–14]. Agar has also been developed for wet spinning [15].
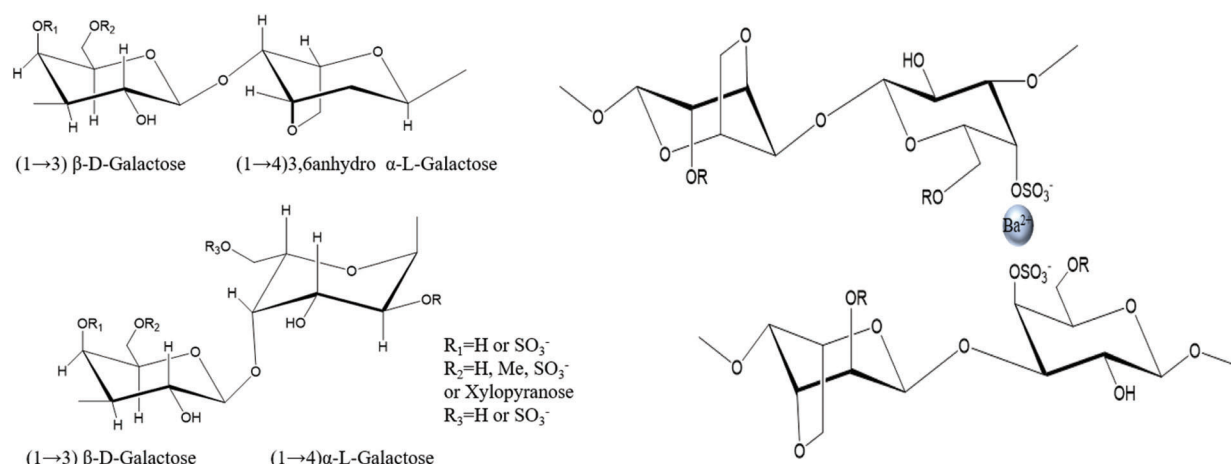
Figure 1: The chemical structure of agar (left) and the schematic diagram of formation of agar fiber (right)

Gracilaria lemaneiformis is a kind of abundant, renewable and inexpensive red algae, mainly distributed in China's Shandong, Fujian and other coastal provinces [16]. From the China Fishery Statistical Yearbook 2020, seaweed aquaculture in Chinese coastal regions had accumulated 2.5 million tones, in which *Gracilaria lemaneiformis* accounted for the second highest production. *Gracilaria lemaneiformis* contains a lot of carbohydrates, protein, dietary fiber and other active ingredients and have high economic benefits [17]. *Gracilaria lemaneiformis* is mainly used as abalone feed and main raw material for agar extraction [18]. In addition, the vast majority of agar is extracted from *Gracilaria lemaneiformis* [19].

As mentioned above, due to the pursuit of high gel strength, commercially available agar is usually extracted from red algae by alkali treatment to decrease the content of sulfate group [20,21]. Wang et al. [22] found that alkali treatment could increase the gel strength of agar and reduce the content of sulfate group and yield of agar, and the highest yield (23.14%) and gel strength (1068.15 g/cm^2) were obtained when alkali concentration was 6% and treated at 80°C for 1.5 h. However, there are few studies on improving agar extraction processes for the preparation of wet-spun fiber. Meanwhile, the conventional extraction processes with alkali-treatment have several disadvantages, including consuming a lot of time, energy and low yield [23–25].

In our previous studies, we have successfully prepared agar fiber with commercial agar (i.e., C-agar in this study). Meanwhile, we speculated that the interplay between barium cation and sulfate anion could be exploited to improve the mechanical properties of agar fiber (Fig. 1 right) [15,26]. The sulfate groups in agar structure played a good role in the formation of fiber. Hence, in order to increase the content of sulfate in agar molecules, to benefit wet spinning and to reduce the economic cost, we came up with the idea of alkali-free treatment to extract agar from *Gracilaria lemaneiformis* for the wet-spinning of agar fiber.

In this paper, the molecular structure and solution properties of alkali-extracted and alkali-free agar from *Gracilaria lemaneiformis* were compared. The effect of alkali pretreatment on the molecular structure of agar was investigated. The properties of agar fibers fabricated by alkali-free agar extracted from *Gracilaria lemaneiformis* and commercial agar were examined.

2 Material and Methods

2.1 Material

Gracilaria lemaneiformis was harvested between September and October in 2021 at Putian, Fujian Province of China (25°25' N 119°0' E). Commercial agar (C-agar) was purchased from Lanji Technology

Development Co., Ltd. (Shanghai, China). All the other reagents used in this article were of reagent grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2 Agar Extraction and Agar Fiber Preparation

The traditional extraction method was depicted as path A in Fig. 2. The *Gracilaria lemaneiformis* was pretreated using NaOH with various concentrations (2 wt%, 4 wt%, 6 wt% and 8 wt%) at 80°C for 2 h. The treated *Gracilaria lemaneiformis* was washed to neutral pH, and then autoclaved (BXM-30R Steam Sterilizer, China) at 120°C for 120 min. Agar was obtained after immersing in ethanol, centrifugation at 5000 rpm for 10 min and freeze-drying to constant weight, and then weighed. The resulting products were labelled as A_n-agar, where n was the mass fraction of NaOH.
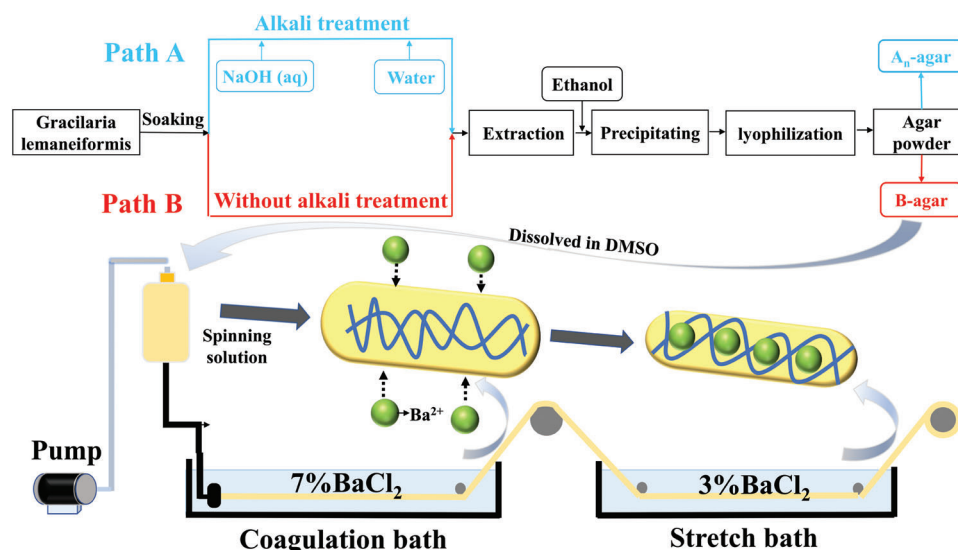

Figure 2: Steps of agar extracting (Path A. Traditional agar extracting process; Path B. Improved agar extracting process) and wet spinning process

The alkali treatment step was omitted in new extraction procedure (Fig. 2, path B). The other steps were the same as that of path A. The obtained agar via path B was labelled B-agar.

A_n-agar, B-agar and C-agar powders were dissolved in DMSO to obtain agar solution (12 wt%), and were referred to as A_n-agar-sol, B-agar-sol and C-agar-sol.

Agar fiber was spun via a wet-spinning device in Fig. 2. B-agar-sol and C-agar-sol were extruded under the pressure of 0.2 MPa and through a spinneret (60 holes, diameter = 0.08 mm × 60 mm) into the coagulation and stretch bath containing 7 wt% and 3 wt% $BaCl_2$ solution, separately. The fibers fabrication with B-agar and C-agar were labelled agar fiber B (AGF-B) and agar fiber C (AGF-C).

2.3 Fourier Transform Infrared Spectroscopy (FTIR)

The Infrared spectra of agar and agar fibers were recorded using a Nicolet iS50 FTIR spectrometer (Thermo Fisher Scientific Co, USA). The samples were scanned 32 times with a resolution of 4 cm^{-1} over the scanning range of 500 to 4000 cm^{-1}.

2.4 Zeta Potential

Zeta potentials of the agar were recorded at 25°C using a Malver Mastersizer 3000 analyzer (Malvern Instruments, Ltd., UK). 20 mg agar was added to 50 ml distilled water, which was stirred for 24 h at 95°C to form a homogeneous solution.

2.5 Gel Permeation Chromatography (GPC)

The average molecular weight (Mw) of agar was determined using a GPC machine (Malvern Instruments, Ltd., UK). The standards and RPCs were injected on A6000 M GPC column and eluted in 0.1 mol/L $NaNO_3$ with the temperature maintained at 40°C.

2.6 Elemental Analyses (EA)

Elemental analyses (EA) were conducted in elemental analyzer (Elemental vario El cube, Germany) to determine sulfur content in nascent fibers. Before this, the samples were dried at 60°C for an hour by desiccator.

2.7 Dynamic Light Scattering (DLS)

The DLS of the agar solution was performed with a Malver Mastersizer 3000 analyzer (Malvern Instruments, Ltd., UK). A_n-agar-sol, B-agar-sol and C-agar-sol with the volume of 0.5 ml were added to 50 ml DMSO at 95°C under stirring for 24 h, respectively. Tests were performed at 25°C with a 173° scattering angle.

2.8 Scanning Electron Microscopy (SEM)

The surface morphology of the agar fiber was observed by SEM (Quanta 250 FEG, USA). The agar fiber was coated with gold and observed at 15 kV acceleration voltage.

2.9 X-Ray Diffraction (XRD)

The crystallization characteristics were characterized with an X-ray diffractometer (Bruker D8 Advance, Germany) with 2°/min scanning rate in a 2θ range from 5° to 60° at 40 kV and 30 Ma.

2.10 Tensile Test

The mechanical properties of the agar fibers were examined using Single Fiber Strength instrument (Instron 5565 Mechanical Tester, USA) at a test speed of 20 mm/min. Test conditions: temperature: 25 ± 1°C, humidity: 60% ± 5%, gauge length: 20 mm.

2.11 Statistical Evaluation

The extraction experiment, zeta potential, GPC and DLS test were repeated five times. The mechanical properties of the fibers were obtained from an average of 30 samples. The data of agar yield, the zeta potential, the Mw of agar and mechanical strength of agar fibers were presented as means ± standard deviation (SD). One-way analysis of variance (ANOVA) and least significant difference (LSD) method tests were performed on the tensile data of agar fibers using the SPSS statistical analysis software to analyze the significant differences between the mean values of fiber properties, and values of $p < 0.05$ were considered significant.

3 Results and Discussions

3.1 Effect of Alkali Treatment on A_n-Agar and A_n-Agar-Sol

The FTIR spectra of A_n-agar, B-agar and C-agar are shown in Fig. 3. The characteristic peaks of A_n-agar, B-agar and C-agar were semblable. A stretching vibration band of O-H at about 3362 cm^{-1} could probably be attributed to isolated O-H moieties [27]. The bands at 2920 cm^{-1} corresponded to asymmetric stretching vibration band of CH_2 [28], and it was the characteristic peak of carbohydrate. The weak peak at 1250 cm^{-1}

was related to the sulfate group stretching vibration [29]. The signal of sulfate group stretching vibration for A_n-agar decreased with increasing alkali concentration, which indicated that alkali pretreatment affected the content of sulfate group.
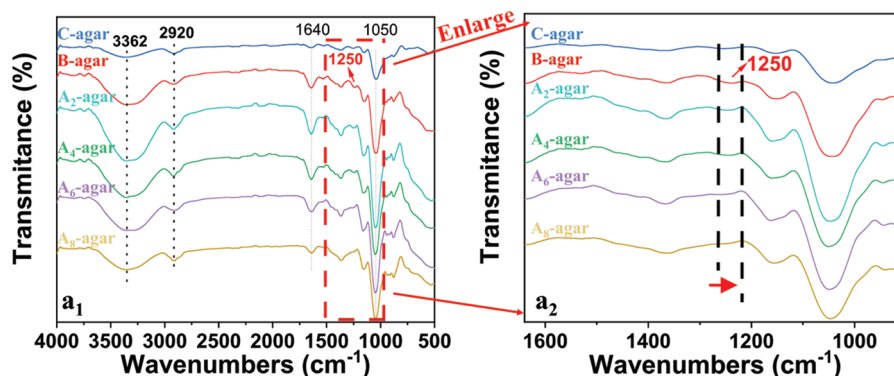

Figure 3: FTIR spectra of A_n-agar, B-agar and C-agar

According to references [30], the yield of agar extracted from the *Gracilaria lemaneiformis* was defined as the mass of the extracted agar divided by the mass of dry *Gracilaria lemaneiformis*. As shown in Fig. 4a, the yield of B-agar was 37.5% ± 1.4%, which was much higher than that of A_n-agars. The yields were 20.4 ± 1.2%, 13.4 ± 1.0%, 11.1 ± 0.7% and 8.7 ± 0.9% for A_2-agar, A_4-agar, A_6-agar and A_8-agar, respectively. Furthermore, agar yields decreased with increasing alkali concentration. We preliminarily speculated that this result was due to the alkali pretreatment disrupting the molecular structure.
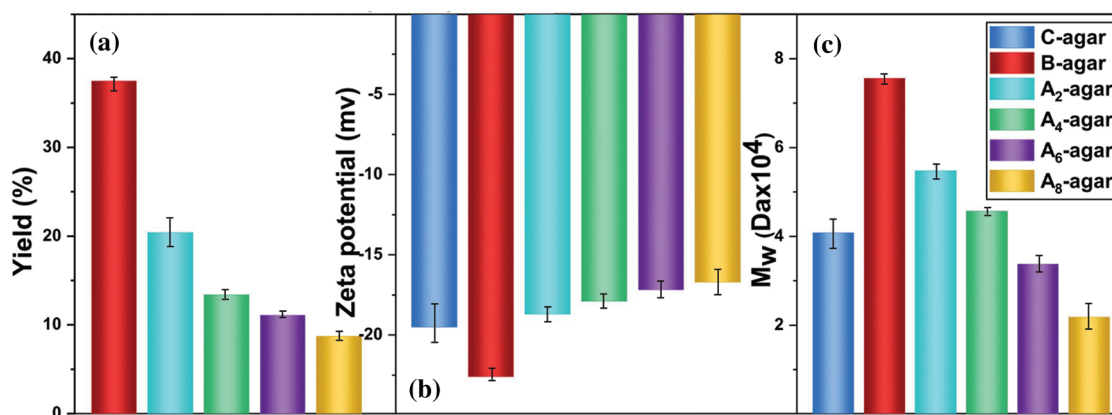

Figure 4: The yield (a) Zeta potential (b) and the Mw (c) of A_n-agar, B-agar and C-agar

As shown in Fig. 4b, the Zeta potential of B-agar was -22.6 mV, while that of A_n-agar was smaller in value than that of B-agar, and decreased with the increase of alkali concentration. Namely, B-agar had more sulfate group than A_n-agar and C-agar.

As shown in Fig. 4c, the Mw of B-agar was 7.5×10^4 Da, which was larger than that of A_n-agar (5.5×10^4 Da, 4.6×10^4 Da, 3.4×10^4 Da, 2.2×10^4 Da) and C-agar (4.1×10^4 Da). The result further confirmed that the alkali pretreatment could destroy the molecular structure of agar with low yield and low Mw [31].

The sulfur content of A_n-agar, B-agar and C-agar is shown in Table 1. In a sense, the sulfur content in agar reflected the content of sulfate group because only sulfate group contained sulfur element. The results showed that the sulfur content of B-agar was the highest, much higher than that of C-agar, and the sulfur content gradually decreased with the increase of alkali pretreatment concentration, which was consistent with the above FTIR spectra and Zeta potential test results.

Table 1: Elemental analyses of A_n-agar, B-agar and C-agar

Samples	C (%)	H (%)	N (%)	S (%)
C-agar	41.26	7.29	0.18	0.32
B-agar	35.78	6.45	0.48	1.26
A_2-agar	36.91	6.72	0.49	1.18
A_4-agar	36.71	6.55	0.42	0.66
A_6-agar	37.23	6.59	0.45	0.42
A_8-agar	38.44	7.03	0.19	0.16

The particle size distributions of A_n-agar-sol, B-agar-sol and C-agar-sol were shown in Figs. 5a and 5b. The average particle sizes were 547.3 nm, 384.6 nm, 340.0 nm, 259.2 nm, 192.5 nm and 432.9 nm for B-agar, A_2-agar, A_4-agar, A_6-agar, A_8-agar and C-agar, respectively. The mean particle size of the A_n-agar-sol decreased with increasing alkali concentration, which indicated the degradation of the A_n-agar molecular chains. The three peaks of A_n-agar-sol and only two peaks of B-agar-sol in the size distribution by intensity indicated that the particle size of A_n-agar-sol was not uniform. Meanwhile, in the size distribution by volume, the size of the agar solution decreased gradually with increasing alkali concentration and the particle size of A_n-agar-sol was too small to make the solution viscous. The results further confirmed that A_n-agar molecular chains were degraded and B-agar had larger molecular chains with good solution properties for the preparation of fiber than A_n-agar and C-agar.
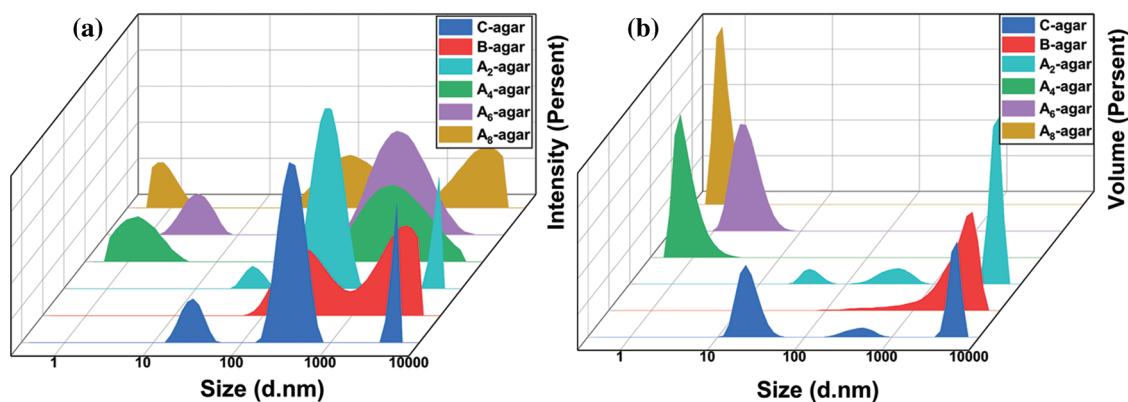

Figure 5: Size distribution by volume of agar solution

Due to the low yield, low molecule weight, low viscosity of agar alkali-extracted from *Gracilaria lemaneiformis*, it proved that A_n-agars were not suitable for spinning. Therefore, in the following studies, we only compared the spinning properties of B-agar and C-agar.

3.2 Properties of Agar Fiber

As we all know, the properties of polysaccharide fibers depend largely on the molecular weight and structure of polysaccharides, but at the same time, spinning parameters such as temperature, drawing ratio and spinning process also affect the properties of the fibers [32,33]. In this paper, the environmental temperature is 25°C, the tensile ratio is 1:1.2, and the parameters are controlled at the same level. Only the influence of polymer molecules on the fiber is studied.

Fig. 6a–6d showed the good morphology of AGF-B and AGF-C. The shape and structure of the fibers were determined by the gelation process of the spinning solution and the interaction between Ba^{2+} and sulfate group played an important role in it [34]. Moreover, AGF-B was more regular and softer than AGF-C.

FTIR spectra of AGF-C and AGF-B were presented in Fig. 6e. The result showed that the peak of the sulfate group at 1250 cm^{-1} of AGF-B was slightly stronger than that of AGF-C, indicating that there were more sulfate groups in AGF-B and AGF-B was easier to bind to Ba^{2+}.

As shown in Fig. 6f, XRD patterns of agar fiber displayed a broad and higher intensity diffraction region at 2θ-13.5° beside the typical amorphous peak at 2θ-18.4°, which indicated an orderly structure and semi-crystalline nature of agar (as a copolymer of galactose and 3, 6-anhydro-galactose) [35]. Similar results were reported by Arfat et al. [36]. However, the diffraction pattern of AGF-C exhibited higher crystallinity than AGF-B, which may be the reason why AGF-B felt softer and had higher elongation at break (Table 2) compared to AGF-C.
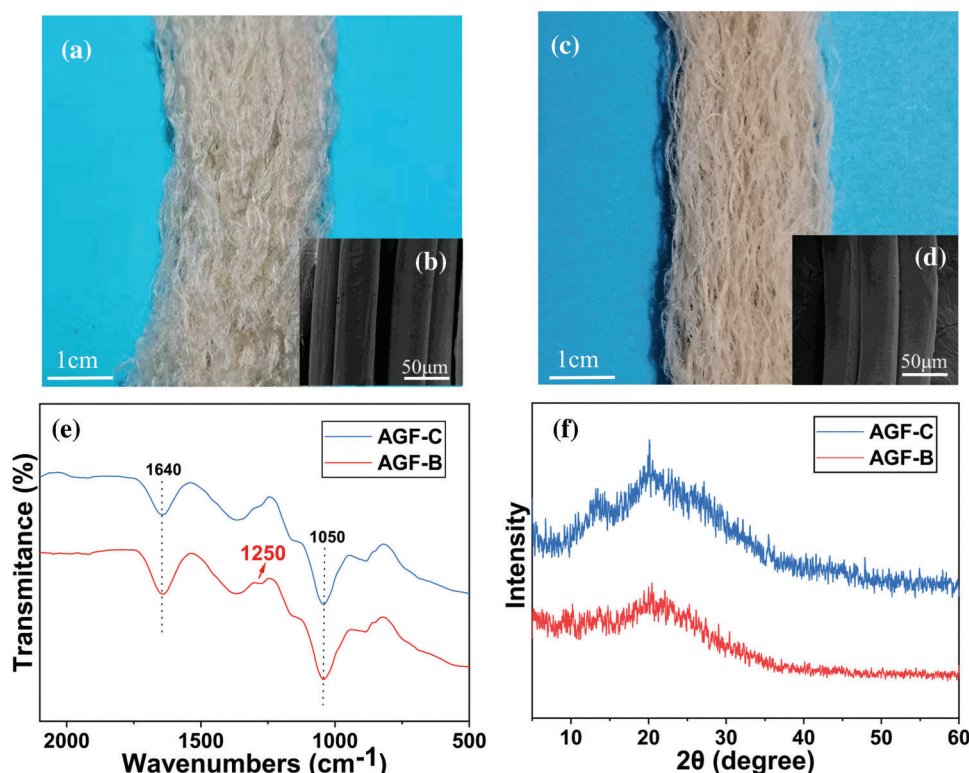

Figure 6: Morphology and structure of agar fibers: a. Optical image of AGF-C; b. SEM of surface of AGF-C; c. Optical image of AGF-B; d. SEM of surface of AGF-B; e. The FTIR spectra of agar fiber; f. The XRD spectra of agar fiber

Table 2: The mechanical data of AGF-C and AGF-B

Samples	Linear intensity (cN/dtex)	Breaking force (cN)	Linear density (dtex)	Elongation at break (%)	Breakdown time (s)
AGF-C	0.61 ± 0.18	16.04 ± 3.33	27.41 ± 5.67	18.43 ± 12.93	22.55 ± 15.56
AGF-B	0.63 ± 0.06	15.36 ± 2.61	24.42 ± 4.10	38.89 ± 13.90	24.23 ± 8.09

Note: Value (means ± stand deviation).

As shown in Table 2, AGF-B not only exhibited slight improvement of the linear intensity but also significantly increased elongation at break. The breaking elongation of the fibers increased from $18.43 \pm 12.93\%$ to $38.89 \pm 13.90\%$. Obviously, the molecular structure of the agar seemed to directly affect the mechanical properties of the fiber, which was also proved by the tensile test results.

In general, high crystallinity results in high strength. But the high molecular weight of AFG-B made up for its weakness in strength. The relatively high sulfate group content in B-agar also improved the strength of AGF-B by combining sulfate group with barium ions. At the same time, the high molecular weight of agar ensured the integrity of the molecular chain and the amorphous structure of AGF-B provided more space for the rotation of its molecular chain, so the elongation at break increased significantly.

4 Conclusions

In this study, agar extracted from *Gracilaria lemaneiformis* without alkali pretreatment was adopted to prepare agar fiber. The new extraction procedure by eliminating the step of alkali treatment displayed higher yield ($37.5\% \pm 1.4\%$) and molecular weight (75, 567 Da) and the size distribution of spinning solution was homogeneous and suitable for wet spinning. Furthermore, the morphology of agar fiber was improved and the elongation at the break of agar fiber was increased to $38.89\% \pm 13.90\%$. Therefore, the developed extraction procedure has promising application prospect in textile fields.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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