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Introduction of alkali soaking and microwave drying processes to improve agar quality of *Gracilaria verrucosa*

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Abstract−*Gracilaria* species produce agars with low quality due to their high sulfate concentrations. For this reason, the gel properties of many *Gracilaria* agars must be improved by appropriate processes. In this study, we developed an improved agar extraction process for *Gracilaria verrucosa* by alkali soaking extraction and microwave thawing and drying. Variables such as the seaweed to alkali volume ratio, extraction time, and alkali concentration were adjusted to optimize the yield and agar quality. The yield of the agar was maximized (34.5±0.6%) using 3% alkali concentration; the lowest yield (17.0±1.7%) was obtained with 1% alkali concentration. Agar gel strengths ranged from 462.0±7 to 627.3±11 $g \cdot cm^{-2}$. We have developed a process for quickly producing an agar gel through thawing and drying using microwave radiation. This study shows the possibility of producing a high-value agar using alkali soaking extraction methods for nonedible *G. verrucosa.*

Keywords: *Gracilaria verrucosa*, Agar, Alkali Soaking, Seaweed-alkali Solution Ratio, Microwave Drying

INTRODUCTION

Marine algae are harvested or cultivated for the extraction of hydrocolloids and production of bioethanol, food, and fertilizer [1]. The hydrocolloids can be divided into three major categories: carrageenan, alginate, and agar gelatinous materials. Of particular importance, red algae are classified as a producer of a hydrocolloid agar. Agar has various applications, such as in the manufacture of foodstuffs, pharmaceuticals, and cosmetic products, as well as for biological and medical research [2]. Agar is a complex mixture of polysaccharides, consisting of two major components, agarose and agaropectins, obtained from the cell walls of certain marine red algae known as agarophytes. Agarose is a neutral, marine polysaccharide with a linear structure of repeated units of the disaccharide agarobiose, which consists of D-galactose and 3,6-L-galactose. Agaropectin is an acid polysaccharide, containing a sulfate ester, pyruvic acid, and D-glucuronic acid, in addition to agarobiose [3]. Commercial grade agar is obtained primarily from the red algae *Gelidium* and *Gracilaria* spp. Generally, *Gelidium* species have been, and are continuing to be, exploited because of the high quality of their agarocolloids, while *Gracilaria* species produce agars with lower quality due to their high sulfate concentrations. For this reason, the gel properties of many *Gracilaria* agars are improved by alkali pre-treatment. An alkaline pre-treatment is usually applied to convert the L-galactose-6-sulfate residue into the 3,6-anhydroform. It has been extensively used to improve the gel-forming ability of agar and for quantitative determination of galactose-6-sulfate residues in commercial and laboratory-scale processes [4]. Likewise, the physicochemical properties of agar can be improved through microwave assisted extraction, ionic liquid, and enzymatic desulfation [5-7]. According to a recent study, the use of microwave irradiation is beneficial for chemical reaction rate enhancement during the synthesis of polysaccharides [8]. Microwave-assisted extraction (MAE) has also been shown to promote increased extraction and recovery separation efficiency for biomass [9,10].

In this study, we investigated the influence of alkaline soaking with various concentrations of NaOH on the yield and physicochemical properties of the hydrogels produced from *G. verrucosa*. Also, a microwave drying process was introduced to improve the agar quality. The development of a simplified work-flow for the alkaline soaking and microwave drying processes has the potential to allow the *G. verrucosa* derived hydrogels to alleviate the shortage of *G. amansii* derived material for the agar industry.

EXPERIMENTAL

1. Materials

Gracilaria verrucosa samples were collected at Wando, South Korea from April to June 2014.

The collected samples were transported to the laboratory, where they were washed in filtered tap water to remove sand and excess salt and dried in an oven at 60 °C. The samples were stored in plastic bags until agar extraction.

2. Proximate Composition Analysis

The moisture content of the sun-dried algae samples was determined by the heat drying method [11]. Ash content was deter-

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mined after being placed in an electric oven for 5 h at 525 °C [11]. Crude lipids were extracted in a Soxhlet extractor using a chloroform-methanol (2 : 1, v/v) mixture and then purified according to the method of Folch et al. [12]. The purified lipid extract was evaporated to dryness in a vacuum dry oven. Protein content was calculated from %N determined by an elemental analyzer (Thermo Quest, Flash 2000), using the correction factor of 6.25, as proposed by Marks et al. [13]. Carbohydrate content was determined as the weight difference after accounting for lipid, protein, moisture, and ash contents.

3. Mineral Composition Analysis

Mineral composition was analyzed by using an inductively coupled plasma mass spectrometer (Perkin-Elmer, Nexion 300X, USA). Dried algae powder was separated into 1 g samples, treated with 10 mL of concentrated HNO_3 and left to stand overnight. Then, 2.5 mL of concentrated $HClO_4$ and 0.25 mL of concentrated H_2SO_4 were added to the samples. The digested samples were then treated with 100 mL of 2% HCl and filtered with a 0.22 μm membrane filter. Acid digestion of the seaweed samples was performed using a microwave digestion system (Milestone, ETHOS 1, USA) [14]. All determinations were performed in triplicate and the data are represented on dry weight basis as mean values±standard deviation.

4. Agar Extraction

4-1. Effect of Algae to Water Ratio

Samples of 15 g each were soaked for 30 min at 25 °C (room temperature) in different volumes of water to obtain different algae-solvent ratios. These four ratios between algae and water were 1 : 25, 1 : 30, 1 : 35 and 1 : 40. All samples were extracted using 2 L Erlenmeyer flasks with a 3% NaOH concentration for 1.5 h.

4-2. Effect of Alkali Concentration

Alkali soaking was performed using four concentrations of NaOH: 0, 1, 3, and 5% (w/v). The 15 g dried samples of algae at each algae-to-water ratio were soaked in each alkali concentration for 0.5 h at room temperature in a 2 L Erlenmeyer flask.

4-3. Effect of Extraction Time

Samples of 15 g each were soaked for 0.5 h at 25 °C with an algae-water ratio of 1 : 35. Sample extraction was performed at 121 °C at 0.5, 1, 1.5, and 2 h in triplicate in an autoclave (Dasol Scientific Co., Ltd., DS-80A).

4-4. Post-extraction Process

The extracts were filtered under pressure with filter paper (Whatman, 90 mm) and were neutralized to pH 7 with H_2SO_4. The filtrate was allowed to gel (agar-agar) at room temperature and then stored at −20 °C overnight. The frozen gel was thawed by microwave, washed with distilled water, dehydrated using 95% alcohol, and then dried for 24 h at 55 °C.

5. Analysis of Physicochemical Properties of Agar

To determine the gel strength of each dried agar sample (1.5 wt%), they were first heated for 20 min at 121 °C in an autoclave. The hot agar gel was poured into a 50 mL plastic cylindrical can (2 cm diameter, 2 cm height) and examined by a texture analyzer to determine gel strength. A 10 mm diameter cylindrical plunger was used and operated at a cross-head speed of 1 mm s^{-1} [15]. The melting point of the gel in the test tubes was measured by first placing a glass bead (5 mm diameter) on the gel surface. The test tube rack with agar filled test tubes was then transferred to a boiling water bath [16]. The melting point was recorded with a digital thermometer when the bead sank into the solution [16]. The same test tubes were then brought to room temperature (20 °C) to measure the gelling temperature. The tubes were tilted up and down in a water bath at room temperature until the glass bead ceased moving. The gel temperature in the tube was immediately measured by introducing a digital thermometer into the agar gel.

Sulfate content was determined by the gelatin-$BaCl_2$ turbidimetric assay [17]. In brief, agar powder (50 mg) was first hydrolyzed in 6 mL of 1 M HCl at 105-110 °C for 12 h. After cooling to room temperature, the solutions were passed through a Whatman GF/A glass microfiber filter. The filtrate (0.2 mL) was transferred to a 15 mL conical tube containing 3.8 mL of 3% trichloroacetic acid and 1 mL of gelatin-$BaCl_2$ reagent. After 20 min, the absorbance at 360 nm was measured with a UV-spectrophotometer. The sulfate content in the samples was calculated with a calibration curve of K_2SO_4.

Fourier-transform infrared (FT-IR) spectra were measured on a KBr-pellet using a Nicolet 6700 FT-IR (Scinco, USA). The range of analysis was from 500 to 1,500 cm^{-1}.

RESULTS AND DISCUSSION

1. Proximate Composition

Table 1 shows the proximate composition of the *G. verrucosa* and *G. Amansii* samples. On a dry weight basis, the composition of *G. verrucosa* was 22.46% ash, 12.46% moisture, 10.21% total proteins, 1.79% total lipids, and 53.08% total carbohydrates. In contrast, the composition of *G. Amansii* was 5.83% ash, 13.65% moisture, 11.03% total proteins, 0.50% total lipids, and 68.99% total carbohydrates. The results showed that *G. verrucosa* has high ash content when compared to *G. Amansii*. *G. Amansii* was found to have a total protein content higher than that of the *G. verrucosa*. The crude lipid content of *G. verrucosa* was found to be 1.79% of its dry weight. In general, marine algae contain only minimal amounts of lipids which range from 1% to 3% of algae dry weight [18,19]. However, the *G. Amansii* stands in contrast to typical algae in terms of lipid content. The varying proximate content observed in different algal species might be due to the influence of environmental factors like temperature, region, salinity, and sunlight intensity [20]. The present study indicates the possibility of algae to be used as a future food resource in food manufacturing to improve the nutritive value of lower quality foodstuffs for human consumption.

2. Mineral Composition

A total of twelve mineral elements were detected in the *G. verrucosa* and *G. amansii* samples (Table 2). Both *G. verrucosa* and *G. amansii* contained high amounts of the macro-minerals (Na, P, K,

Table 1. Proximate composition of *Gracilaria verrucosa* and *Gelidium amansii*

Content	*Gracilaria verrucosa* (%)	*Gelidium amansii* (%)
Ash	22.46	5.83
Moisture	12.46	13.65
Lipid	1.79	0.50
Protein	10.21	11.03
Carbohydrate	53.08	68.99

Table 2. Comparison of mineral composition mg/kg dry weight of *G. verrucosa* and *Gelidium amansii* (Mean±SD, n=3)

Species	Minerals												Ref.
	Na	P	K	Ca	Mg	Fe	Zn	Cu	Ni	Mn	Mo	Se	
G. verrucosa	4186.00 ±775	3700.33 ±28.25	1633.00 ±132.69	1356.25 ±159.98	1203.25 ±60	215.50 ±14.28	39.75 ±11.32	16.25 ±8.88	9.25 ±2.15	7.00 ±0.00	4.5 ±1.15	N.D.	This study
	4524.00 ±213.50	-	7924.00 ±317.80	305.90 ±5.50	498.30 ±41.00	8.80 ±2.50	4.05 ±0.10	0.40 ±0.00	-	7.40 ±0.40	1.10 ±0.10	2.25 ±0.30	[46]
	360.00 ±11.00	2293.20 ±40.70	316.0 ±10.6	2248.00 ±55.20	167.30 ±11.70	313.2 ±15.50	20.00 ±2.80	24.40 ±3.1	-	90.30 ±18.20	-	-	[47]
G. amansii	7212.59 ±467.57	4851.83 ±231.08	13110.57 ±889.83	32869.50 ±2634.32	16610.35 ±1273.34	1618.53 ±179.78	64.94 ±7.76	11.21 ±1.55	58.57 ±6.88	49.28 ±4.45	10.17 ±16.2	N.D.	This study

N.D.: not detected

Ca, Mg, and Cu) and appreciable amounts of trace elements (Fe, Zn, Ni, Mn, Mo, and Se) needed for human nutrition. For *G. verrucosa*, among the 12 elements detected, Na and Mo were found to be the most (4186±775) and least 4.5±1.15 abundant, respectively. By contrast, in the *G. amansii* Ca and Mo content was found to be the highest (32869.50±2634.32) and lowest 10.17±16.2), respectively. Overall, *G. amansii* was found to be rich in essential minerals, especially Ca, Mg, K, Na, and P. Previous reports on marine algae found that most of the trace elements present in algal biomass are heavy metals, but their content is generally below the toxic limits allowed by several countries [21]. Zn, Cu, Ni, Mn, and Se are elements that have potential toxicity for living organisms. Heavy metals that accumulate in the body are not easily eliminated but, in this study, only trace amounts of heavy metals were detected. The lack of harmful heavy metals solidifies algae as a safe and abundant marine food source. In summary, these results have shown that large amounts of important minerals are contained in edible red algae. *G. verrucosa* and *G. amansii* could potentially be used as a nutrient supplement to help meet the recommended daily intakes of some macro nutrients and trace elements.

3. Algae to Water Ratio

The agar characteristics were significantly influenced by the algae to water ratios (Table 3). The *G. verrucosa* agar yield was significantly higher with an algae-to-water ratio of 1 : 25, with the ratio of 1 : 35 resulting in particularly low yields. However, the gel strengths with algae to water ratios of 1 : 30 and 1 : 35 were significantly higher. Agar yield and gel strength differed significantly with different volumes of water for same amount of *G. verrucosa*. On the other hand, the *G. amansii* results showed that the agar yield with an algae-to-water ratio of 1 : 30 was significantly higher than other ratios. The lower yielding ratios had approximately the same yields. Larger volumes of water promote the swelling of the algae, thus allowing the agar to be extracted easily and ultimately influencing agar gel strength, especially that of *G. amansii* derived agar. The higher gel strength with an algae-to-water ratio of 1 : 30 can be attributed to the low sulfate content, the most likely reason for changes in the agar structure [3,22].

4. Extraction Time and Microwave Thawing Effects

The effect of alkali concentration and extraction time on agar yield and properties is shown in Table 4. Maximum agar yield was obtained at an extraction temperature of 121 °C. The agar yield ranged from 17.0±1.4 to 34.5±0.6 depending on temperature. The highest gel strength (627.3±11 g cm^{-2}) was recorded for the agar extracted using 3% NaOH for 1.5 h. In contrast, the lowest gel strength (49.0±8 g cm^{-2}) was recorded for the agar extracted using 0% NaOH for 2 h. Both low agar yield and gel strength were recorded for agar extracted with non-alkali soaking. Different NaOH concentrations influenced the agar yield at most extraction times tested. The exception to this effect was at the extraction time of 0.5 h, where NaOH concentration did not affect the agar yields. At 1.5 h, the agar yields from *G. verrucosa* treated with 1 and 3% NaOH were significantly higher than at 1.0 h. At 1.0 h, the agar yield from *G. verrucosa* treated with 1% NaOH was significantly lower. The gel strength of agar from alkali treated *G. verrucosa* is shown in Table 4. Gel strength of agar extracted with 3% NaOH from *G. verrucosa* samples was significantly higher than at other NaOH

Table 3. Agar yield and physical properties from *Gracilaria verrucosa* and *Gelidium amansii* extracts treated with different solution volume ratios (Mean±SD, n=3)

	Gracilaria verrucosa				*Gelidium amansii*			
Ratio	1 : 25	1 : 30	1 : 35	1 : 40	1 : 25	1 : 30	1 : 35	1 : 40
Yield (%)	38.9±0.3	34.5±0.6	34.8±1.3	34.9±0.9	43.5±0.5	48.3±0.5	43.7±0.9	46.4±0.6
Gel strength (g·cm^{-2})	380.1±3	627.3±11	625.3±13	531.7±9	158.0±16	312.9±19	439.2±13	586.3±17
Melting temp (°C)	86.5±0.7	88.7±0.8	89.3±1.0	86.1±0.8	92.9±0.6	92.3±1.2	91.6±1.0	90.5±1.0
Gelling temp (°C)	42.1±0.8	42.5±0.7	43.1±0.7	41.8±0.9	33.5±0.6	32.1±0.5	31.5±0.5	30.8±0.8

G. verrucosa with 3% NaOH

G. amansii with 1 N H_2SO_4

Table 4. Agar yield and properties from *Gracilaria verrucosa* at different extraction times and alkali concentrations (Mean±SD, n=3)

	0.5 h			1.0 h			1.5 h			2.0 h		
Alkali con. (%)	0	1	3	0	1	3	0	1	3	0	1	3
Yield (%)	11.1 ±0.9	23.0 ±1.2	18.0 ±1.7	8.2 ±0.5	17.0 ±1.7	19.3 ±1.6	5.6 ±0.6	30.1 ±0.8	34.5 ±0.6	4.7 ±0.5	20.1 ±0.9	24.8 ±0.8
Gel strength ($g \cdot cm^{-2}$)	14.6 ±6	462.0 ±7	569.8 ±18	127.4 ±6	504.5 ±5	580.0 ±11	57.1 ±20	564.9 ±7	627.3 ±11	49.0 ±8	540.4 ±12	609.9 ±10
Melting temp (°C)	80.4 ±0.6	85.6 ±0.7	88.2 ±0.6	78.7 ±1.0	86.9 ±1.2	88.8 ±0.9	77.4 ±1.1	87.8 ±0.8	88.7 ±0.8	76.8 ±0.7	88.4 ±0.8	89.6 ±0.7
Gelling temp (°C)	38.7 ±1.0	40.3 ±1.2	41.8 ±0.8	38.2 ±0.8	44.1 ±0.9	42.6 ±0.8	37.2 ±0.7	42.1 ±0.8	42.5 ±0.7	36.6 ±0.9	40.7 ±0.9	42.1 ±0.7
Sulfate conc. (%)	7.0 ±0.9	7.5 ±1.2	7.1 ±0.6	7.2 ±1.3	7.0 ±0.6	7.1 ±0.7	7.2 ±1.1	6.9 ±0.3	6.8 ±0.7	7.1 ±1.7	7.6 ±0.6	7.3 ±0.9

concentrations. In addition, the gel strength of agar extracted for 1.5 h, from the 3% NaOH treated samples was significantly higher than all other conditions tested. Gel strength showed a strong positive correlation with alkali concentration. The sulfate content of the agar from alkali treated *G. verrucosa* at various alkali concentrations and extraction times ranged from 6.8±0.7 to 7.6±0.6% (Table 4). Alkali concentrations at different extraction times had no effect on the sulfate content of the agar. Gelling temperature and melting point of the agar was in the range of 40.3±1.2 to 44.1±0.9 °C and 85.6±0.7 to 89.6±0.7 °C, respectively. Minimum melting and gelling temperatures were observed for *G. verrucosa* treated with 1% NaOH for 0.5 h, and maximum temperatures were measured the for the 1.5 h 3% NaOH treatment (Table 4). Alkali treatment of *G. verrucosa* at different extraction times and NaOH concentrations did not greatly influence the gelling and melting point of the agar. Navarro et al. [23] reported that the desulfation process with microwave assistance results in the removal of 60-93% of the original sulfate. To investigate the effect on the removal of sul-

Table 5. Yield and physical properties of agar obtained from *Gracilaria* species

Species	Gel strength ($g \cdot cm^{-2}$)	Yield (%)	Melting temp. (°C)	Gelling temp. (°C)	Extraction method	Ref.
G. cornea	1,134±57	20.1±1.5	91.2±0.9	40.8±0.4	Alkali	27
G. crassa	800±15.40	16±0.86	-	-	Alkali	28
G. corticata	634.2±10.0	3.54±1.59	-	-	Alkali	29
G. verrucosa	627.3±11	34.5±0.6	88.7±0.8	42.5±0.7	Alkali	This study
G. verrucosa	562.1	12.2	86.9	42.1	Alkali	30
G. Gracilis	543.26±236.29	25.75±4.38	-	41.21±4.41	Native	31
G. edulis	490±8.16	16±0.87	-	-	Alkali	28
G. dura	455±97.85	34±1.00	-	39.78±2.07	Native	32
G. dura	390	21.2	82	31	Alkali	31
Gracilaria sp.	334.50±14.1	31.30±1.79	75.00±0.00	53.6±0.89	Alkali	33
G. crassa	250±15.20	23±0.86	-	-	Native	28
G. edulis	239.95±28.35	34.34±1.74	87.63±0.06	61.00±1.00	Alkali	33
G. fisheri	228.27±48.18	39.55±7.59	72.37±0.06	47.00±0.00	Alkali	33
Gracilaria sp.	202.31±7.39	39.42±0.71	82.00±0.00	53.40±0.55	Native	33
G. edulis	197.08±72.87	10.90±0.92	92.60±0.30	60.20±0.45	Native	33
G. dura	160	27.7	80	31	Native	34
G. cliftonii	147±17	56.7±0.4	82.0±1	33.1±1	Native	35
G. fisheri	145.61±34.55	13.33±1.78	72.40±0.10	49.25±0.96	Native	33
G. foliifera	135±7.63	15±0.73	-	-	Alkali	28
G. corticata	110±6.29	11±0.72	-	-	Alkali	28
G. bursa-pastoris	101.78±65.28	27.63±4.87	-	39.74±4.11	Native	31
G. corticata	100±6.19	16±0.77	-	-	Native	28
G. edulis	100±7.30	25±0.76	-	-	Native	28
G. foliifera	100±8.56	22±0.80	-	-	Native	28
G. longissima	91±2.6	46.2	75.5±1.1	34.9±2.6	Native	36
G. vermiculophylla	84.5±8.1	45.7	73.9±3.1	36.4±0.5	Native	36

fate, the frozen gels were thawed and dried by microwave radiation. Sulfate content of agar from alkali treated *Gracilaria* species was consistently under 7% (data not shown). Frozen agar thawing is not significantly affected by sulfate removal and the microwave shortened thawing times when compared with natural thawing, completing a fast agar production process.

5. Novel Extraction Process

The agar yields and gel strengths of *Gracilaria* species are summarized in Table 5. The agar gel strengths ranged from 84.5 to 1,134 g cm^{-2} while the agar yields ranged from 3.54 to 56.7% on a dry weight basis. The properties of the agar can differ due to regional differences in certain characteristics of *Gracilaria* species. Also, the extraction method changes the characteristics of the agar, whether the native extraction or the alkali extraction method is used. Alkali extraction results in better gel strength than native extraction, with similar yields. The general agar extraction process is based on the alkali treatment of *Gracilaria* spp. and the process flow diagram of *Gracilaria* agar production is shown in Fig. 1. The *Gracilaria* algae were treated with 0.5-10% NaOH solution for 0.5-2 h at 60-90 °C. After washing with water, the agar components were extracted with hot water to prepare an agar-agar solution. The agar-agar gel was then freeze-thawed and dehydrated [37]. However, these general processes are time consuming and can have problems with variability. For example, the quality of the agar can change due to the loss of seaweed during the pretreatment process and during drying after the freeze-thawing process. The process used in this work employed alkali soaking extraction instead of alkaline pretreatment with the goal of developing a fast agar manufacturing process by reducing thawing time by using microwaves. The alkali immersion method is similar to the alkali pretreatment method, but it requires shorter agar extraction times than alkali pretreatment. The sodium content can also be increased through neutralization after alkali extraction. The most common method of drying and thawing food is hot air drying. However, since hot air drying is not energy efficient and takes a long time, shrinkage occurs due to a rapid decrease of moisture on the surface [38]. Also, hot air drying has been reported to reduce quality characteristics such as color, nutrients, incense, and texture and cause severe shrinkage and reduced bulk density [39]. Especially in the case of polysaccharide gels, freeze-thaw treatments significantly affect the properties of the gels [40]. Thus, microwave thawing and drying offers a promising solution to reduce the drying time when compared to hot air methods, and is an effective way to reduce changes in the physicochemical properties of the manufactured agar.

Generally, agar extracted from *Gracilaria* species is of lower quality due to their high sulfate concentrations [29,41]. However, the gel properties of *Gracilaria* agar can be improved by alkali treatment, converting L-galactose-6-sulfate to 3,6-anhydro-L-galactose, which is responsible for the enhancement of gel-forming ability [2]. Alkali treatment variables like alkali concentration, heating time, and temperature have been reported to affect the yield and properties of agar from other species. Also, the gel strength of agar varies with the species, harvest location, and season [4,33,42]. The method used in this study gave results similar to the alkali-treatment method. The yield was maximized with increased extraction time. However, the agar yield was lower than that reported by Hurtado-Ponce et al. (23-48%) [43] for another species of *Gracilaria* from the Philippines. Alkali soaked extracted agar from *G. verrucosa* had a gel strength ranging from 462.0±7 to 627.3±11 g cm^{-2}, which is comparable to commercial agar (>800 g cm^{-2}). Low yield and poor physical properties were obtained by the native extraction of agar from *G. verrucosa*, suggesting that yield and gel strength improvement can be achieved by switching to an alkali soaking extraction method. The poor gelling ability of agarocolloids is due to

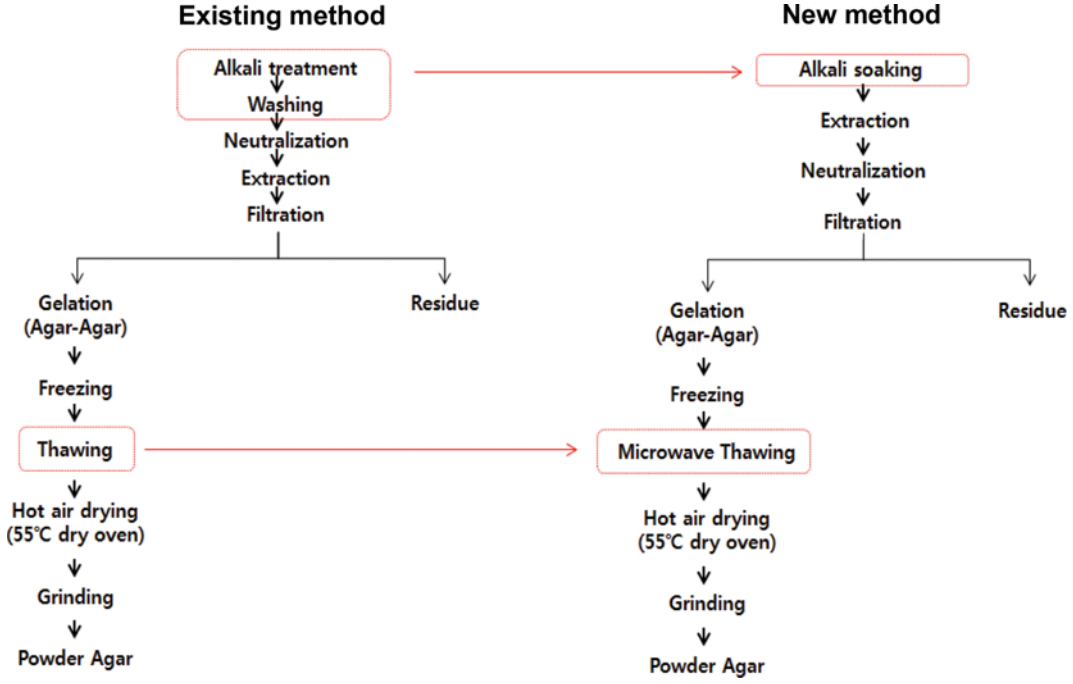

Fig. 1. Comparison of conventional and novel agar extraction processes for *Gracilaria verrucosa*.

the presence of D-galactose-6-sulfate residues and 4-0-methyl-α-L-galactose [44]. Murano et al. [33] suggested that the increased amount of 3,6-anhydro-L-galactose enhanced gelling properties by removing the sulfate ester from C-6 of the L-galactose of agar. Alkaline treatment improved the gel formation ability by converting L-galactose-6-sulfate into 3,6-anhydro-L-galactose. Freile-Pelegrin et al. [16] reported a strong correlation between gel strength and alkaline concentration. Kumar et al. [31] reported the changes in gel strength and yield of *G. cliftonii* agar according to the agar/water ratio. The structure might be changed by extraction conditions such as agar/water ratio and alkali concentration which would also affect other characteristics of the final agar product. Therefore, there is a strong relation between agar/water ratio (and alkali concentration) and yield, and a weak relation between the yield and gel strength. As a result, the gel strength increased by increasing the content of 3,6-anhydro-L-galactose in 3% alkali soaking compared to the 1% alkali treatment [45]. The alkali soaking extracted agar had a low melting temperature (85.6-89.6 °C) that falls in the range of the USP standard (>85 °C). The melting temperatures of native agar (0% NaOH) for all extraction times were found to be 76.8-80.4 °C.

Conventional alkali treatment is comprised of two steps, NaOH pretreatment (0.5-2 h using 1-10% NaOH at 60-80 °C) and the extraction process under various conditions with increased temperature and pressure. The agar soaking process performs the extraction process using a NaOH solution without pretreatment. This shortens the extraction time but causes some impurities to be mixed in the final agar product. In addition, the purity of the agar may fall because of the presence of chlorophyll, resulting in a slight green color. The yield of the new process was ca. 30% compared to the conventional yield in the range of 11-25%. The purity was comparable between the two processes because it was dependent on downstream purification steps. Extraction time was much shorter in the new process because the three steps of conventional process were changed to a single step. The specific time savings may vary depending on the process equipment applied.

6. Spectroscopic Analysis

The FT-IR spectra of the agar and its fractions are shown in Fig. 2. Inspection of the spectra in Fig. 2 and the data from Table 6 reveal the characteristic bands of agarocolloids [24]. Spectra of commercial agar (Bread Garden) from different origins, (a), bacto-agar (b), extracted from *G. verrucosa* (c) *G. amansii* and (d) algae are also shown. In the FT-IR spectra (Fig. 2(a), (b)) characteristic bands appear at 740, 770, 890, 930, 1,070 and 1,250 cm^{-1}. In the FT-IR spectra (Fig. 2(c), (d)) the characteristic bands appear at 740, 890, 930, 970, 1,070 and 1,250 cm^{-1}. The characteristic bands in the FT-IR spectrum arose from absorption by the ester sulfate (1,250 cm^{-1}) and 3, 6-anhydro-L-galactopyranose (930, 790, and 715 cm^{-1}), with the one latter peak designated as diagnostic bands for agarans [25]. According to Christiaen and Bodard [26], the 890 cm^{-1} band is specific for agar and can be attributed to the anomeric C-H of β-galactose residues. The alkali soaking agar from *G. verrucosa* exhibited a skeletal mode of the galactan peaks and was shifted to 1,100 cm^{-1}.

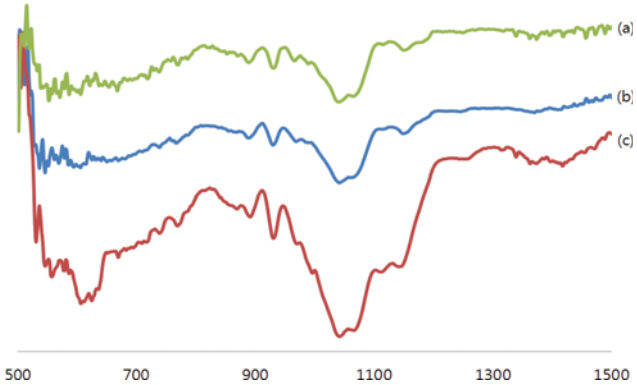

Fig. 2. FT-IR spectra of agar from *Gelidium amansii* (a), commercial agar (b), and agar from *Gracilaria verrucosa* (c).

Table 6. Assignment of the most important absorption bands in FT-IR spectra of *Gracilaria verrucosa* agar

Wavenumber (cm^{-1})	Assignment
1370	Ester sulfate
1250	ν_{as} S=O (ester sulfate)
1070	Skeletal mode of the galactan
930	Vibration of the C-O-C of 3,6-anhydro-L-galactose
890	Agar specific band
845	4-Sulfate galactose
830	2-Sulfate galactose
820	6-Sulfate galactose
805	Sulfate on C-2 of 3,6-anhydro-L-galactose
740	C-O-C bending mode in glycosidic linkages
716	C-O-C bending mode in glycosidic linkages

CONCLUSION

Agar was successfully extracted from *G. verrucosa* with newly developed alkali soaking extraction methods. Alkali soaking using NaOH, especially at a concentration of 3%, increased the agar yield and improved gelling properties. NaOH soaking extraction showed a higher efficacy in the improvement of agar properties when compared with native agar (0% NaOH) and *G. amansii* agar. It is recommended that dry *G. verrucosa* should be soaked with agitation for 1 h at 30 °C, with an algae-to-water ratio of 1 : 30, and subsequent extraction of agar in boiling water at 121 °C for 1.5 h to obtain maximum yield and higher quality. These results detail the optimum conditions to produce higher quality agar from *Gracilaria* species and demonstrate new extraction and drying processes to improve the agar quality with shorter process times.

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