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Effect of extraction process on agar properties of *Gracilaria corticata* (Rhodophyta) collected from the Persian Gulf

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?1 This study investigated the effects of different extraction variables including soaking times, soaking temperatures, alga-to-water ratios, extraction times and extraction temperatures on agar yield and other quality properties of *Gracilaria corticata* growing along northeastern coast of the Persian Gulf. An alkali treatment was also examined to optimise the extraction conditions. The results indicated that agar properties were significantly affected by all experimental variables; although, soaking treatment had less effects on agar yield compared to the other treatments. On the assumption that gel strength was the major criterion for processing of *Gracilaria*, a soaking time of 1 h at 40°C with an alga-to-water ratio of 1:200 followed by extraction for 2.5 h at 80°C was recommended for *G. corticata* to achieve agar with better quality. The gel strength of *G. corticata* in most treatments was more than 350 g cm^{-2} (established standard for food-grade agar), but this value is a considerable distance from the standard for commercial agar extraction processes (more than 800 g cm^{-2}) without using alkali treatment.

Key Words: Agar extraction, Alkali treatment, Gel strength, *Gracilaria corticata*, Persian Gulf

INTRODUCTION

Agar is a polysaccharide that is extracted from members of *Gracilaria* and *Gelidium* (Armisén & Galatas 1987; Marinho-Soriano *et al*. 2001; Meena *et al*. 2008), and agar is broadly applied as a gelling and stabilizing agent in the medical, cosmetics and food industries (Freile-Pelegrín & Robledo 1997; Freile-Pelegrín & Murano 2005; Villanueva *et al*. 2010). Agar comprises two different constituents: agarose, a neutral polysaccharide with a linear structure of the agarobiose units, and agaropectin, an acid polysaccharide conjugated to agarobiose (Araki 1966; Duckworth & Yaphe 1971; Yaphe 1984). *Gracilaria* is a diversified genus of Rhodophyta with more than 160 species, which are considered as the major sources (53%) of commercially valuable agar around the world (Marinho-Soriano & Bourret 2003; McHugh 2003).

The process of agar extraction from *Gracilaria* species has been clarified by many detailed studies (Marinho-Soriano *et al*. 2001; Freile-Pelegrín & Murano 2005; Andriamananatonio *et al*. 2007; Pereira-Pacheco *et al*. 2007; Arvizu-Higuera *et al*. 2008; Orduña-Rojas *et al*. 2008; Li *et al*. 2009). The extraction of agar is usually done by leaching the dry *Gracilaria* in boiling water, filtering the extract and separating the agar by freeze-and-thaw techniques (Armisén & Galatas 1987). Although the general steps of the extraction procedure from Gracilariales order are well established, the extraction method for each algal species needs to be optimised to improve agar yield and quality. Agar of some algae, such as *Gelidium*, is easily extracted with boiling water, while other species, like *Gracilaria*, need some pretreatment to increase agar yield (Arvizu-Higuera *et al*. 2008).

Agar quality can be affected by various factors, such as species, methods of extraction, postharvest storage, and some ecological parameters, such as season, life cycle and geographical features (Marinho-Soriano & Bourret 2003; Marinho-Soriano *et al*. 2006; Romero *et al*. 2008). Moreover, the properties of agar depend on the presence of some chemical groups, such as pyruvate, methyoxyl and sulfate, which all influence gel strength as the main agar quality parameter (Arvizu-Higuera *et al*. 2008; Hurtado *et al*. 2010). The type of treatment prior to the extraction as well as the extraction time and temperature influence the yield and other properties of agar (Oyieke 1993; Kumar & Fotedar 2009).

The sulfate content of agar is one of the main factors by which its high contents could adversely affect the gel properties from *Gracilaria* species (Freile-Pelegrín & Robledo 1997; Freile-Pelegrín & Murano 2005). Application of the alkali hydrolysis treatment with sodium hydroxide, which converts L-galactose-6-sulfate in the agar structure to the 3,6-anhydro-L-galactose form, have allowed *Gracilaria* to compensate for the lack of quantities of *Gelidium* for the agar industry (Duckworth *et al*. 1971; Armisén 1995; Noseda *et al*. 2000; Orduña-Rojas *et al*. 2008). However, alkaline treatment variables, such as temperature, time and concentration of sodium hydroxide, must be adapted for each species of *Gracilaria* to achieve the highest desulfation while inhibiting the yield losses during the treatment (Armisén & Galatas 1987; Andriamananatonio *et al*. 2007; Arvizu-Higuera *et al*. 2008; Orduña-Rojas *et al*. 2008; Li *et al*. 2009).

About 103 species of marine algae are found on the coast of the Persian Gulf (Borgesen 1939) with the highest diversity

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and broadest distribution being in the Rhodophyceae (Sohrabipoor & Rabiei 2008). *Gracilaria corticata* (J. Agardh) J. Agardh is widely distributed on the Iranian coast within the Persian Gulf, lending the potential for commercial harvesting and agar extraction. The present study was conducted to clarify the effect of extraction variables, including soaking time, soaking temperature, alga-to-water ratio, extraction time and extraction temperature on agar yield and other quality properties of *G. corticata* grown along the northeastern coast of the Persian Gulf. An alkali method was also applied to optimise the yield, gel strength and sulfate content of agar gathered through the extraction process.

MATERIAL AND METHODS

Specimens of *G. corticata* were obtained from seashore of the Bushehr province, northeastern Persian Gulf, Iran (27°49′57″N, 51°26′54″E), in July 2012. The live samples were collected by hand from the intertidal mudflat and transported to the laboratory. The seaweeds were washed with tap water to remove external substances (epiphytes, other seaweeds and invertebrates) and then oven dried for 8 h at 60°C to reach a final moisture of 15–20% before agar extraction. Each 5-g sample was considered as an experimental unit. Extraction was done within less than 1 mo to avoid probable degradation of agar during extended seaweed storage (Romero *et al.* 2008). All experiments were carried out with four replications in sterile 500-ml Erlenmeyer flasks each containing an experimental unit of alga by weight.

For soaking time and temperature experiments, replicate flasks were used to soak algal units for 0, 1, 2, and 3 h at 25°C ($n = 4$ per treatment; total of 16 flasks). Extraction was performed by heating all flasks for 1.5 h in 250 ml of deionised water at 80°C in a water bath. In a second experiment, 12 flasks were treated by hydrating the experimental algal units in deionised water for 2 h at three different temperatures of 30, 35 and 40°C. Four experimental algal units were also hydrated at room temperature (25°C) as control ($n = 4$ per treatment; total of 16 flasks). Extraction was carried out using the procedure described above.

To measure the effect of water volume on agar yield, 16 flasks were treated by soaking one part of experimental unit of alga with 50, 100, 150, and 200 parts of deionised water by weight at 40°C. All flasks were then transferred to the water bath and extracted by heating for 1.5 h in 250 ml of deionised water at 80°C.

To determine extraction time and temperature, 16 experimental flasks were pretreated for 1 h at 40°C with an alga-to-water ratio of 1:200 (wt:wt). Agar extraction was carried out by adjusting the temperature of the water bath to 80°C for four different times of 1.0, 1.5, 2.0, and 2.5 h using 250 ml of deionized water ($n = 4$ per treatment). An additional 16 experimental flasks were pretreated with the same procedure described above and then extracted by heating the flasks for 1.5 h in 250 ml of deionised water at four different temperatures of 70, 80, 90, and 100°C ($n = 4$ per treatment).

The alkali treatment was carried out by dissolving 1%, 2%, 3%, and 5% analytical-grade sodium hydroxide (NaOH; Sigma-Aldrich, St Louis, MO, USA) in deionised water. One experimental algal unit was added to each of 16 conical flasks containing 250 ml of alkali solution; the algae were soaked for 1 h at 40°C. After the alkali treatment, algal units were rinsed with running tap water for 1 h to eliminate excess NaOH. Agar extraction was carried out by heating the samples for 1.5 h in 250 ml of deionised water at 80°C, while the pH was adjusted to 7.5.

To determine agar yield, all of the extracts were prefiltered by a cotton tissue and filtered under pressure at 60°C through a glass-fiber filter (Whatman GF/C, Whatman International Ltd, Maidstone, UK). The residues were re-extracted in the same way and poured into plastic containers. The filtrates were gelled at room temperature and then frozen at –20°C overnight. The frozen gels were thawed and oven dried for 24 h at 60°C (Marinho-Soriano *et al.* 1999). Finally, agar yield was determined as the percentage of dry weight by the following equation:

$$\text{Agar yield } = \frac{\text{Agar dry weight (g)}}{\text{Seaweed dry weight (g)}} \times 100$$

To determine the gel strength, agar from each treatment was separately boiled in water and mixed by stirring inside cylindrical containers to obtain 1.5% wet-weight agar solutions. The cylindrical specimens (2.3 cm diameter × 15 cm height) were prepared by decanting the hot gel into clean cylindrical tube and allowed to become cool at ambient temperature for a night after wrapping with aluminum foil. The gel strength was measured as g cm^{-2} according to the procedure of Marinho-Soriano & Bourret (2003) modified by Kumar & Fotedar (2009). Textural properties were evaluated by texture analyzer TA-XT2i (Stable Micro Systems, Surrey, UK) equipped with a cylindrical plunger of 1 cm^2 at a penetration speed of 1 mm s^{-1} to a depth of 5 mm. Sulfate contents were determined turbidimetrically as a percentage of dry weight with the method described by Jackson & McCandless (1978) after hydrolysation of the samples in a sealed tube with 1 N HCl at 105°C for 12 h and subsequent precipitating of liberated sulfate with barium ion.

All statistical analyses were performed using the software SPSS 14.0 for Windows. The data were tested for normality and homogeneity of variances using the Kolmogorov–Smirnov test and the Levene test, respectively. Agar yield and gel characterization were statistically analyzed by a one-way analysis of variance followed by a least significant difference test to discern where differences in means occurred. The results were expressed as mean ± s and a $P < 0.05$ considered statistically significant. To find the correlation between agar properties, Pearson's correlation coefficient was employed.

RESULTS

Soaking time had significant effect on agar yield, gel strength, and sulfate content. Soaking time decreased the yield but increased the gel strength with the strongest gels achieved after 1 h of soaking (Table 1). Sulfate content was significantly affected by soaking time ($P < 0.05$), with the maximum of 4.5% dry weight in 3-h and the minimum of

Table 1. Agar yield and properties (mean ± *s*) of *Gracilaria corticata* at different soaking times and temperatures. Similar lowercase letters within the same column indicate no significant difference between those treatments ($P < 0.05$).

	Agar yield (% dw[1])	Gel strength (g/cm^2)	Sulfate content (% dw)
Soaking time (h)			
0	66.24 ± 2.06 a	144.5 ± 4.0 c	3.1 ± 0.3 c
1	27.76 ± 1.08 b	425.3 ± 13.0 a	3.0 ± 0.3 c
2	20.36 ± 1.02 c	275.4 ± 4.1 b	3.5 ± 0.2 b
3	16.72 ± 1.24 d	267.8 ± 5.3 b	4.5 ± 0.5 a
Soaking temperature (°C)			
25	25.20 ± 1.08 c	144.5 ± 2.5 c	3.0 ± 0.5 c
30	28.26 ± 1.02 b	267.8 ± 4.8 b	4.6 ± 0.9 a
35	29.48 ± 1.02 d	275.6 ± 8.2 b	4.8 ± 0.9 a
40	32.92 ± 2.02 a	425.5 ± 14.1 a	3.5 ± 0.4 b

[1] dw = dry weight.

3.0% dry weight in 1-h treatments. The sulfate content showed significant negative correlation with gel strength ($r = –0.624$; $P < 0.05$) and agar yield ($r = –0.642$; $P < 0.05$) at different soaking times.

Agar yield and gel properties were also significantly affected by soaking temperature ($P < 0.05$). The peak of agar yield was obtained at a soaking temperature of 40°C followed by 30, 25, and 35°C. The gel strength at a soaking temperature of 40°C was significantly higher than other temperatures ($P < 0.05$), while no significant difference was found between 30 and 35°C treatments. There is no significant difference between sulfate contents at soaking temperatures of 30 and 35°C; although, they were significantly higher than 40 and 25°C ($P < 0.05$). With respect to soaking temperature, the gel strength was significantly negatively correlated with sulfate content ($r = –0.624$; $P < 0.05$); although, agar yield illustrated no significant correlation with both gel strength and sulfate content.

The alga-to-water ratio significantly influenced the yield of agar ($P < 0.05$). As shown in Table 2, the highest agar yield was attained in the alga-to-water ratios of 1:100. There was a significant effect of alga-to-water ratio on gel strength, with the maximum of 435.5 ± 11.0 g cm^{-2} in the 1:200 treatment. The lowest sulfate content was also observed in the alga-to-water ratio of 1:100, which was significantly lower than other ratios ($P < 0.05$). Gel strength showed no significant correlation with sulfate content in different alga-to-water ratios ($r = –0.356$; $P < 0.05$).

The effects of extraction times and temperatures on agar yield and properties are given in Table 3. Extraction temperature had a significant effect on agar yield and sulfate content but exhibited no significant impact on gel strength. The peak of agar yield was found in an extraction temperature of 60°C and 2 h, which was significantly higher than the remaining temperatures. An extraction temperature of 80°C also showed the least amount of sulfate content.

There was no significant impact of extraction time on gel strength, except for 1 h that was significantly lower than other times ($P < 0.05$). The maximum amount of sulfate content was observed in an extraction time of 1 h; whereas, the minimum value was identified in an extraction time of 2.5 h. A negative correlation was found between gel strength and extraction time ($r = –0.528$; $P < 0.05$).

Gel strength was not significantly influenced by extraction temperatures; however, gel strength was lower with an extraction temperature of 90°C. The sulfate content at 90°C was significantly higher than other extraction temperatures ($P < 0.05$). The gel strength was significantly negatively correlated with sulfate content ($r = –0.032$; $P < 0.05$).

Agar yield and gel strength were conversely affected by alkali concentrations (Table 4). The maximum agar yield was obtained in 1% concentration of NaOH and continuously declined by increasing of alkali concentration. In contrast, the gel strength was significantly enhanced by increasing alkali concentration ($P < 0.05$) and reached a final strength of 635.2 ± 10.0 g cm^{-2} in concentration of 5% NaOH. The minimum sulfate was also obtained in 5% concentration of NaOH, significantly lower than other concentrations ($P < 0.05$). Gel strength showed a significant negative correlation with agar yield ($r = –0.642$; $P < 0.05$) and sulfate content ($r = –0.719$; $P < 0.05$) at different alkali concentrations.

DISCUSSION

Standardization of agar extraction from each *Gracilaria* species is a serious step for maximization agar yield and quality (Freile-Pelegrín & Robledo 1997; Kumar & Fotedar

Table 2. Agar yield and properties (mean ± *s*) of *Gracilaria corticata* at different alga-to-water ratio. Similar lowercase letters within the same column indicate no significant difference ($P < 0.05$).

Alga-to-water ratios	Agar yield (% dw[1])	Gel strength (g/cm^2)	Sulfate content (% dw)
1:50	16.10 ± 1.02 d	362.2 ± 11.7 c	3.4 ± 0.2 b
1:100	21.20 ± 2.16 a	334.6 ± 10.3 c	3.0 ± 0.5 c
1:150	18.26 ± 1.02 c	398.3 ± 9.1 b	3.4 ± 0.2 b
1_200	19.26 ± 1.08 b	435.5 ± 11.0 a	3.9 ± 0.4 a

[1] dw = dry weight.

Table 3. Agar yield and properties (mean ± *s*) of *Gracilaria corticata* at different extraction time and temperature. Similar lowercase letters within the same column indicate no significant difference between those treatments ($P < 0.05$).

	Agar yield (% dw[1])	Gel strength (g/cm^2)	Sulfate content (% dw)
Extraction time (h)			
1	23.40 ± 1.37 d	397.7 ± 10.9 b	4.0 ± 0.4 a
1.5	27.16 ± 1.52 a	426.6 ± 12.3 a	3.0 ± 0.2 b
2	27.26 ± 1.58 a	459.3 ± 11.5 a	2.7 ± 0.3 b
2.5	24.84 ± 1.71 c	466.6 ± 11.1 a	1.5 ± 0.2 c
3	25.96 ± 1.66 b	438.5 ± 10.0 a	1.7 ± 0.1 c
Extraction temperature (°C)			
60	37.72 ± 1.02 a	360.8 ± 9.3	3.6 ± 0.5 c
70	22.24 ± 1.83 d	358.2 ± 9.1	4.1 ± 0.6 b
80	31.48 ± 1.45 b	364.6 ± 10.7	2.8 ± 0.2 d
90	27.67 ± 1.22 c	330.3 ± 10.2	4.50 ± 0.4 a

[1] dw = dry weight.

2009). Based on the present results, changes in variables extraction could affect agar yield, gel strength and sulfate content from wild *G. corticata* grown along northern coast of the Persian Gulf.

The present study reiterates the findings that soaking time and temperature influence accessibility of agar as the basic soluble polysaccharide of seaweeds, which easily interact with water molecules using hydrogen bonds (Jiménez-Escrig & Sánchez-Muniz 2000). A decrease of agar yield by extending soaking time may be due to its diffusion into the water causing lower agar yields. Besides, a negative correlation between sulfate content and agar yield supports the hypothesis that sulfate plays a notable role on gel strength by the presence of the sulfate ester at C-6 of the L-galactose declining the agar gelling properties (Murano 1995; Falshaw *et al*. 1999; Armisén & Galatas 2000; Orduña-Rojas *et al*. 2008). The period of storage is a serious problem, which has caused agar extraction to be quite expensive (Armisén & Galatas 1987; Vergara-Rodarte *et al*. 2010). The current study shows that agar extraction without soaking pretreatment not only increases agar yield and gel strength but also introduces a more straightforward and commercially practiced method for agar extraction (Selby & Whistler 1993; Armisén & Galatas 2000).

Soaking temperature also affects the agar yield and properties of *G. corticata*. An increase of agar yield and gel strength by increasing soaking temperature observed in the present research could be related to a change of agar structure and its increased discharge through the extraction process. However, the agar yield from other members of *Gracilaria* species, such as *G. cliftonii* A.F. Withell, A.J.K. Millar & G.T. Kraft, was reduced by increment of soaking temperature (Kumar & Fotedar 2009). Differences in response of *Gracilaria* members to soaking temperature before extraction could be related to season of harvest, geographic habitat and specific characters of each species (Whyte *et al*. 1984; Hurtado-Ponce & Umezaki 1988; Murano 1995).

Based on the current results, the alga-to-water ratio influenced the amount of agar yield but had no effect on gel strength. The higher amount of agar yield obtained at a alga-to-water ratio of 1:100 could be associated by swelling of marine algae when exposed to water (Jiménez-Escrig & Sánchez-Muniz 2000). On the other hand, a higher alga-to-water ratio (1:150 and 1:200) had an adverse effect on agar yield by the diffusion of some agar into the water causing lower agar yield at the end of extraction process (Jiménez-Escrig & Sánchez-Muniz 2000; Kumar & Fotedar 2009). Furthermore, it seems that sulfate content more easily solubilizes at a lower alga-to-water ratio (1:100) and therefore has a less negative effect on agar properties. This is contrary to the previous findings that higher amounts of water improve agar properties (Andriamananatonio *et al*. 2007; Arvizu-Higuera *et al*. 2008).

The extraction time has diverse effects on agar properties from agarophytes. Previous studies expressed different extraction times to maximize agar yield of the order Gracilariales, such as 2–4 h for *G. edulis* (Gmelin) P.C. Silva (Thomas & Krishnamurthy 1976), 2.5 h for *G. vermiculophylla* (Ohmi) Papenfuss (Arvizu-Higuera *et al*. 2008), 3 h for *G. cliftonii* (Kumar & Fotedar 2009), 1 h for *G. coronopifolia* J. Agardh, and 2 h for *G. eucheumoides* Harvey (Hurtado-Ponce 1992). The highest agar yield of *G. corticata* in the current research was found at an extraction time of 2 h and declined by elongating of the extraction. It is supposed that a longer time of extraction changes the agar structure of *G. corticata* in comparison to the other members of this genus (Duckworth *et al*. 1971; Freile-Pelegrín & Murano 2005). A

Table 4. Agar yield and properties (mean ± *s*) of *Gracilaria corticata* with different alkali treatment concentrations. Similar lowercase letters within the same column indicate no significant difference between those treatments ($P < 0.05$).

Alkali concentration (%)	Agar yield (% dw[1])	Gel strength (g/cm^2)	Sulfate content (% dw)
1	6.48 ± 2.31 a	479.1 ± 8.9 d	3.12 ± 0.1 b
2	6.40 ± 1.75 ab	532.7 ± 12.4 c	3.45 ± 0.8 a
3	5.38 ± 1.18 bc	568.5 ± 17.3 b	3.47 ± 0.6 a
5	3.54 ± 1.59 c	634.2 ± 10.0 a	2.60 ± 0.2 c

[1] dw = dry weight.

Table 5. Amount of agar yield and gel properties from agar-producing *Gracilaria* species.

Species	Source	Agar yield (%)	Gel strength (g cm^{-2})	Sulfate content (%)	Reference
G. asiatica	China	24.1	465	2.5	Lian (1996)
G. heteroclada	Philippines	20.0	892	—	De la Peña (1996)
G. cornea	Mexico	20.4	930	2.8	Freile-Pelegrín (2000)
G. lemaneiformis	Mexico	29.7	271	3.0	Li *et al.* (2008)
G. cliftonii	Australian	62.3	246	7.5	Kumar & Fotedar (2008)
G. vermiculophylla	Mexico	45.7	84.5	14.3	Orduña-Rojas *et al.* (2008)
G. edulis	India	11	490	1.3	Meena *et al.* (2008)
G. crassa	India	23	250	3.2	Meena *et al.* (2008)
G. foliifera	India	22	100	5.7	Meena *et al.* (2008)
G. gracilis	Argentina	26.7	—	6.8	Rodríguez *et al.* (2009)
G. tenuistipitata	Vietnam	28.7	148	—	Skriptsova & Nabivailo (2009)
G. bailiniae	Vietnam	30.2	155	—	Skriptsova & Nabivailo (2009)
G. corticata	**Iran**	**3.5**	**634**	**2.6**	**Current study**

negative correlation of agar yield with sulfate content also supports this phenomenon.

The response of algae to extraction temperatures in this study was different when compared to those previously reported (Freile-Pelegrín & Murano 2005; Marinho-Soriano & Bourret 2005; Meena *et al.* 2006; Kumar & Fotedar 2009). The highest agar yield for *G. corticata* in the present research was obtained with an extraction temperature of 60°C. This, however, is contrary to the findings for *G. cliftonii* (Kumar & Fotedar 2009) with a maximum agar yield of 60.6% dry weight at an extraction temperature of 100°C. Other studies have also reported temperatures of 85–100°C for agar extraction for other *Gracilaria* species (Santelices & Doty 1989; Marinho-Soriano & Bourret 2003; Meena *et al.* 2006; Arvizu-Higuera *et al.* 2008; Orduña-Rojas *et al.* 2008). These dissimilarities corroborate the hypothesis that extraction properties are species specific and should be separately determined (Li *et al.* 2009).

The present findings confirm that alkali treatments influence agar properties by decreasing agar yield (Armisén 1995; Marinho-Soriano *et al.* 2001; Melo *et al.* 2002; Freile-Pelegrín & Murano 2005; Siddhanta *et al.* 2005). A decline of agar yield in this study appears to be associated with the decomposition of polysaccharides during alkali treatment and their diffusion into the NaOH solution (Freile-Pelegrín & Murano 2005; Li *et al.* 2009), or it may be related to an easier release of agar into the alkaline solution compared to water (Arvizu-Higuera *et al.* 2008; Li *et al.* 2009). Accordingly, instead of decanting the alkali solutions, neutralization of the solution after the treatment using an acid such as HCl prior to the extraction procedure in the same flask may considerably increase agar yield; although, difficulties in maintaining a constant pH during the procedure could lead to the unstable agar quality (Li *et al.* 2008). On the other hand, alkali treatment caused removal of sulfate content, and therefore this increased gel strength. Enhancement of the gel strength after alkali treatment by NaOH could be related to alteration of L-galactose 6-sulfate to 3,6-anhydro-L-galactose (Marinho-Soriano & Bourret 2003).

On the basis of information presented in this study and previous reports, agar yield could be stimulated by manipulating each extraction variable; although, exogenous factors, such as species, location and season of harvest, have critical roles to play in maximizing agar extraction (Hoyle 1978). The gel strength of *G. corticata* in most treatments was more than 350 g cm^{-2}, which has been established as the standard for food-grade agar (Armisén 1995); however, alkali treatment could increase gel strength within or exceeding the range for gel strengths obtained for commercial agarophytes (Table 5).

Different responses of *Gracilaria* species to manipulated parameters do not allow establishing a constant method for agar extraction (Freile-Pelegrín & Robledo 1997; Kumar & Fotedar 2009). On the assumption that gel strength is the main criterion for agar extraction from the order Gracilariales (Arvizu-Higuera *et al.* 2008), a soaking time of 1 h at 40°C with an alga-to-water ratio of 1:200 and then extraction for 2.5 h at 80°C could be recommended for *G. corticata* to achieve agar with better quality. However, a soaking time of 0 h at 40°C with a 1:100 alga-to-water ratio followed by extraction for 2 h at 60°C increases agar yield. Besides, the present study corroborates earlier studies that alkalization is an optional procedure to enhance agar quality, especially for those species with lower agar quality, such as *G. corticata*. A combination of each variable in the factorial experiment may have been designed to test for their possible interactions and find out which combination of factors produce the best agar with higher yield and quality.

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Queries for phya-52-05-11

1. **Author: This article has been edited for grammar, style, and usage. If no change is required in response to a question, please indicate "OK as set." Copy editor**
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