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DEPTH-DEPENDENCY OF THE AGAROPHYTE RED ALGA *Gracilaria corticata* J. AGARDH FOR AGAR YIELD AND QUALITY DURING ITS GROWING SEASON

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Abstract:

The impacts of two depth ranges on the rhodophyte *Gracilaria corticata* for agar yield and quality were determined during its growing season (December to March) at intertidal waters. Average 4-month agar at 2-3 m and 5-6 m were 34 ±0.04% and 42 ±0.05%, respectively. The lowest (30 ±2.89%) and highest (51 ±4.16%) agar yields, respectively, were estimated in December at 2-3 m and in January at 5-6 m. At 2-3 m, the 4-month averages were not significantly different ($P>0.05$). *G. corticata* from a depth of 2-3 m yielded the strongest (30.64 ±1.49 g cm^{-2}) agar gel in December, and the alga sampled at 5-6 m in January revealed the lowest agar strength (7.94 ±0.38 g cm^{-2}). Agar lightness (L) ranged from lowest (59.6 ±3.85) at 2-3 m in December to greatest (86.8 ±3.11) at 5-6 m in February. Agar yellowish factor (a) was highest (11.6 ±3.21) at 2-3 m in December and lowest (4.8 ±1.64) at 5-6 m in February. Agar reddish factor (b) was highest (48 ±2.24) in March and lowest (38.8 ±1.30) in December at a depth of 2-3 m. *G. corticata* growing at relatively deeper intertidal zone yields more quality agar, and December and late winter are the best times to obtain a more rigid and enhanced agar gel.

Keywords: Red algae, Depth dependency, Agar properties, Intertidal

Introduction

Marine algae as a staple food, animal feed, and manure are used in many parts of the world, particularly South-East Asia. Gels and viscous materials extracted from seaweeds especially red and brown macrophytes have been used for centuries in food preparation. These algal extracts are generally called phycocolloids caused by creating colloidal systems in water including agar and carrageenans in red algae. They contribute to four basic applications such as thickness, gel, emulsion, and food stability.

The first phycocolloid used for commercial other than food purposes were the agar as a medium in microbiology (Chapman, 1970) and, more recently, in biotechnology (electrophoresis and chromatography), medicine (anticoagulant), and dental molding material. Agar is a polysaccharide compound in the cell wall of the rhodophytes especially in *Gracilaria* and *Gelidium* species. Agar extracted from *Gelidium* has a better quality whereas *Gracilaria* accounts for the main source for agar extraction because of its large population and intensive growth (McHugh, 2003). Sixty percent of agar production from *Gracilaria* is mainly used for applications concerning food products (Kalesh, 2003). Species of *Gracilaria* are, therefore, some of the most useful algae in the world, combining the production of the valuable polysaccharide agar with fast growth rate, ease of vegetative reproduction, and other attributes favoring their cultivation (Kain and Destombe, 1995).

From 150 species of green, brown, and red macroalgae identified in the coastal province Sistan and Baluchestan (southeast of Iran), eight species belong to genus *Gracilaria* with the highest species diversity in the southeastern region (Chabahar), among which *G. corticata* is the most abundant (33.1%) (Qaranjik, 2010; Sohrabipoor and Rabiee, 2012). Studies on the marine macroalgae of the Iranian southern coastal lines of 1800 km have mostly focused on species identification (e.g. Shoghi, 1995; Sohrabipoor and Rabiee 1998; Ajdari, 2004). Assessment and qualification of ecological characteristics of these valuable macroalgal resources can effectively contribute to their exploitation in aquaculture industry and marine biotechnology. However, the biological properties and compounds of the southern Iranian marine macroalgae including those of rhodophytes have scarcely been investigated. Few applied studies are available on the extraction of industrial materials such as carrageenan from the red alga *Chondrus* spp. (Hassas, 1996), and optimization of agar extraction from *G. corticata* (Karkhane Yousefi et al., 2011).

A variety of environmental factors including irradiation, depth of growing, nutrients, and water temperature can affect both the quality and quantity of agar yield by agarophyte species (Freile-Pelegrin et al., 1997; Eidighaleghazi, 2014) so the depth of growth is a parameter of interest for optimization of agar productivity and gel strength. Hence, considering the lack of sufficient scientific information on the effects of depth on the agar production by *G. corticata* at the study area, the current investigation aimed at assessing agar yield and quality changes in this agarophyte red alga during its growth period (December to March) at two depth ranges. These data will be useful for optimum utilization, and commercial culture of this alga.

Materials and Methods

Preparation of algal biomass

The samples of *G. corticata* were collected once a month from its growing area in the coastal waters of Chabahar, Beris beach, southeastern Iran (25°.8′ N, 61°.11′ E) for 4 months from December to March in shallow (2-3 m) and rather deep (5-6 m) waters. After identification of the collected samples in the Chabahar Research Center for Offshore Coastal Waters (Iran), they were rinsed with tap water to remove extraneous materials and salts. They were then air-dried and transferred to the Department of Fisheries, Sari Agricultural Sciences and Natural Resources, Sari, Iran.

Agar extraction

The dried samples (1.0 g) were cut, sieved, and immersed three times in a water bath containing 150 mL of distilled water and heated at 110 °C for 90 min. The obtained extract was filtered at 70 °C, and the residue re-extracted under the same conditions. The extracts were converted to gel at room temperature and then frozen overnight. Afterward, the samples were oven-dried at 60°C for 24 h. Agar yield as a percentage of dry mass was calculated according to the following equation (Xu and Gao, 2008):

Agar yield (%) = dry agar (g)/ dry alga (g) × 100

Agar color quality

Following drying in the oven and obtaining a homogenized powder, the agar samples were photographed with a digital camera (Nikon, Coolpix Model) located 25 cm above the samples at an angle of 30°C from the camera (according to Hunter L, a, b system). The camera was set to take photos every 10 seconds. The digital images were transferred to a computer to analyze the color differences among the samples by the use of Photoshop 8 software (Yam et al., 2004).

Agar gel strength

To determine agar gel strength, a 1.5% (W/V) agar solution was heated in a water bath (70 °C) for 1 h. Then it was allowed to gel at room temperature for 12 h. The gel strength was measured as g/cm² using a Texture Analyzer (Model CT3, USA) having a piston rod diameter of 7/12 and 35 mm in length (Freile-Pelegrim and Robledo, 1997; Marinho-Sorinha, 2001).

Statistical analysis

Differences among the treatments were tested using two-way analysis of variance (ANOVA) and Duncan's post-hoc test was applied for comparison of means with SPSS software (version 16). A confidence level of 95% was considered in all analyses. Significant differences were determined through Pearson's correlation test.

Results and Discussion

Agar yield

G. corticata displayed elevated agar yield with increasing depth (Fig. 1). Samples from the two depths were different in agar yields ($P<0.05$). Total agar averages at 2-3 m and 5-6 m were 34 ±0.04% and 42 ±0.05%, respectively. The lowest (30 ±2.89%) and highest (51 ±4.16%) agar yields, respectively, were estimated in December at a depth of 2-3 m and in January at a depth of 5-6 m. Moreover, at a depth of 5-6 m, the January measurement revealed a greater yield different from those in the other months ($P<0.05$). At a depth of 2-3 m, no significant differences were observed among the 4-month averages ($P>0.05$).

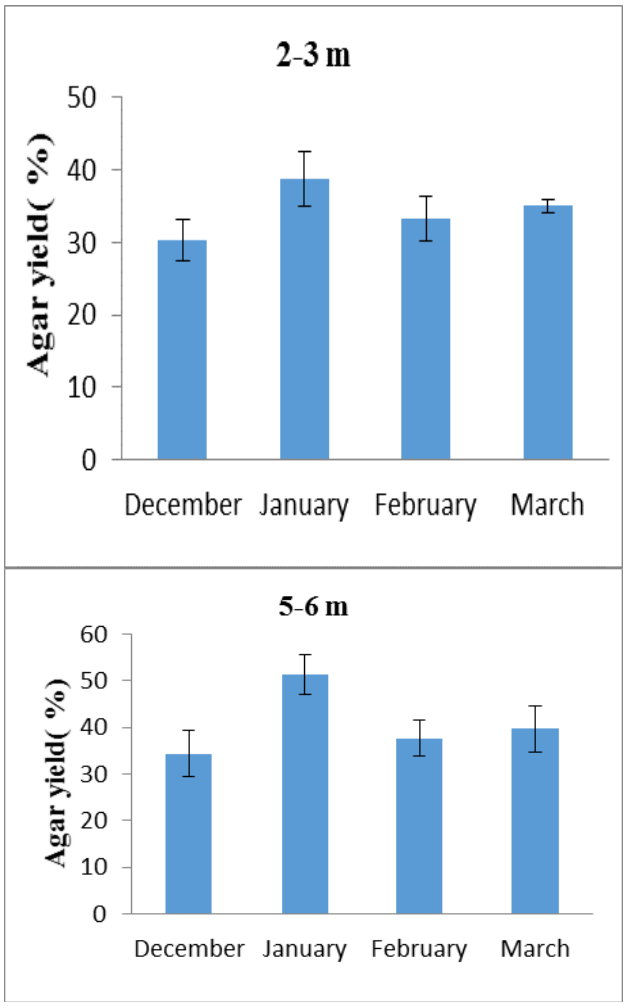

Figure 1. Agar yield fluctuations in *G. corticata* measured at two depths within four months.

Agar gel strength

The gel strenght was significantly different ($P<0.05$) with respect to the depth and monthly changes. *G. corticata* from a depth of 2-3 m yielded the strongest (30.64 ±1.49 g cm^{-2}) agar gel in December, and the alga sampled at 5-6 m displayed the lowest agar strength in January (7.94 ±0.38 g cm^{-2}) (Figure 2). Gel qualities of the agar extracted from samples collected at 2-3m seawater depth were singnificantly different ($P<0.05$) among all four months. At a depth of 5-6 m, December and March algal samples yielded rather the same agar strength ($P>0.05$).

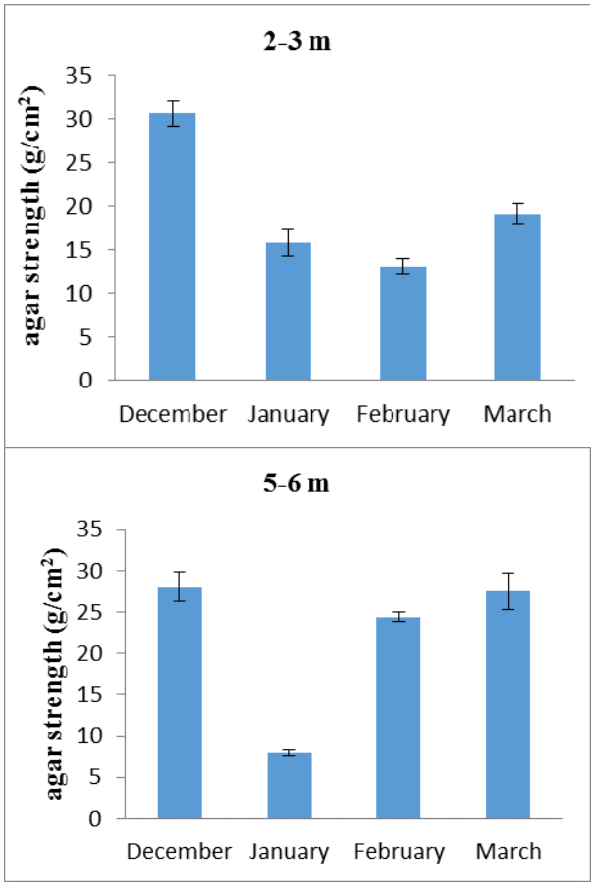

Figure 2.Changes in agar gel strength extracted from *G. corticata* at two depths within four months.

Agar color quality

Agar lightness (L) factor showed significant differences in the algal samples from the examined depths and months (Table 1). This factor ranged from lowest (59.6 ±3.85) at 2-3 m in December to greatest (86.8 ±3.11) at 5-6 m in February. In addition, the color quality displayed marked dissimilarities (P<0.05) at 2-3 m in both December and February compared to the other months, whereas February and March samples revealed no statistical differences (P>0.05) at the same depth. At a depth of 5-6 m, the agar color in February differed (P<0.05) from those obtained in the other months.

The agar yellowish factor (a) changed markedly (P<0.05) with both depth and month (Table 1). Agar yellowish in the samples at a depth of 2-3 m was highest (11.6 ±3.21) in December, and those sampled at 5-6 m exhibited the lowest (4.8 ±1.64) yellow color in February. The 'a' factor at 2-3 m was almost the same (P>0.05) in December, January, February, and March samples; however, this factor was different in February (P<0.05) from those in both December and March. At a depth of 5-6 m, agar yellowish in both January and February was dissimilar (P<0.05) with those in both March and December samples while no significant differences were found (P>0.05) neither between January and February nor between December and March concerning 'a' factors estimated.

The reddish factor (b) of the agar samples did not change with depth (P>0.05); however, it displayed statistical changes (P<0.05) at different months (Table 1). The highest (48 ±2.24) and lowest (38.8 ±1.30) values of 'b' were observed in March and December, respectively, at a depth of 2-3 m. At the same depth, 'b' factor in March differed (P<0.05) from those in the other months but agar samples in January showed comparatively similar (P>0.05) reddish with those in December and February; though, December samples were more reddish (P<0.05) than those in February. At a depth of 5-6 m, 'b' factor in December differed (P<0.05) from those measured in the other months. No considerable alterations were detected (P>0.05) among 'b' factors estimated in the alga sampled in January, February, and March at a depth of 5-6 m.

Table 1. Average values of agar color extracted from *G. corticata* at two depths within four months.

Depth	Month	Lightness (L) factor	Reddish (b) factor	Yellowish (a) factor
2-3 m	December	59.6 ±3.85	38.8 ±1.3	11.6 ±3.21
	January	74.8 ±4.09	40.2 ±2.05	8.8 ±2.28
	February	81.6 ±1.95	41.4 ±1.14	7.0 ±2.74
	March	81.8 ±5.45	48.0 ±2.24	10.8 ±1.64
5-6 m	December	80.2 ±5.45	46.2 ±2.17	10.4 ±1.14
	January	81.0 ±0.71	41.0 ±1.00	6.4 ±1.14
	February	86.8 ±3.11	40.2 ±2.49	4.8 ±1.64
	March	82.8 ±3.42	43.0 ±2.00	9.4 ±1.52

Table 2. Pearson's correlation between agar yield, gel strength, and color factors (L, lightness, a: yellowish, b: reddish) estimated in *G. corticata* at a depth of 2-3 m.

	Agar yield	Gel strength	L	a	b
Agar	1	-0.466	0.433	-0.46	0.234
Gel strength		1	-0.038	0.236	0.265
L			1	-0.565	0.653*
a				1	-0.126
b					1

* Correlation is significant at the 0.05 level

Table 3. Pearson's correlation between agar yield, gel strength and color factors (L: lightness, a: yellowish, b: reddish) estimated in *G. corticata* at a depth of 5-6 m.

	Agar yield	Gel strength	L	a	b
Agar	1	0.085	0.354	-0.465*	0.089
Gel strength		1	-0.289	0.31	0.166
L			1	-0.626**	0.316
A				1	0.161
B					1

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level

According to Tables 2 and 3, agar lightness (L) showed a positive and significant correlation with the reddish factor (b) ($P<0.05$) at a depth of 2-3 m. And at a depth of 5-6 m, agar yield showed a negative correlation with the 'L' factor ($P<0.05$). Furthermore, the 'L' factor showed a negative correlation with the yellowish factor (a) ($P<0.01$).

Agar yield and quality changes have been attributed to a number of determinants including season (Price and Bielig, 1992; Chirapart and Ohno, 1993), environmental parameters (Bird, 1988; Hurtado-Ponce, 1994), growth (Christian et al., 1987), reproductive cycle (Whyte et al., 1981; Marinho-Soriano et al., 1998a), and the algal section examined (Craigie and Wen, 1984). In addition, decreasing effect of high ultraviolet (UV) radiation on agar production was also reported (Eswaran et al., 2002). In this study, the agar yield extracted from the agarophyte *G. corticata* averaged 34% and 42% at depths of 2-3 m and 5-6 m, respectively, that is, the yield elevated as the depth increased. Higher light intensities have been known to promote production of protoplast (affecting rapid growth) rather than wall material (Devlin and Witham, 1983) resulting in lower agar deposition. Accordingly, though solar irradiation was not measured here, agar yields of *G. corticata* at low depth (2-3 m) with naturally higher light levels were lower than those obtained from the alga located at deeper (5-6 m) waters receiving lower light levels. Likewise, *G. lemaneiformis* (Xu & Gao, 2007) afforded uttermost agar at deeper (3.5 m) than at shallower (0.5 m) waters. The same consequence was also observed in *Gracilaria* species grown under high and low light regimes (Araño et al., 2000). In general, the agar yield averages (30-51%) of *G. corticata* in here were higher than those reported for the same species (14.5-22.5%) from Veraval coast of Gujarat (Oza, 1978), and were comparable to those previously found for *Gracilaria* species (Rao et al., 1977, Rao, 1978; Chennubhotla et al., 1979;

Chirapart and Ohno, 1993; Pondevida and Hurtado-Ponce, 1996; Kalesh, 2003).

Monthly variations in agar production found in this investigation were also reported in other agarophyte species. In spite of numerous reports concerning seasonal effects on agar properties of *Gracilaria*, the results are contradictory (Lahaye and Yaphe, 1988), in part because of varying techniques used for agar extraction as well as different geographical nature of seasonal changes. Hence, the seasonal variation of agar yield may or may not display similar trends. The agarophyte *G. bursa-pastoris* from France, for instance, showed an agar peak in summer (Marinho-Soriano, 1999) whereas *G. gracilis* (= *verrucosa*) from Canada performed inversely (Whyte *et al*., 1981). It has recently been reported that no significant seasonal variations in the agar yields were observed for two *Gracilaria* species namely *G. veleroae* and *G. vermiculophylla* (Rodríguez-Montesinos et al., 2013). Our samples of *G. corticata* yielded maximum agar percentage at a depth of 5-6 m in January. At the same depth, Abidizadegan et al. (unpublished data) found marked rises of photosynthetic pigments in winter indicating that the alga maximizes agar production together with increasing growth and biomass since January. Almost in the same way, a rising biomass and agar production near late winter were also observed in *G. corticata* from an adjacent region (Bandar-e-Lengeh, north of the Persian Gulf) (Rafiei et al., 2005). Their estimated agar yield (9.21%), however, was comparatively lower than those averaged in our algal samples; the authors stated that it might be caused by environmental conditions, growth season, and available nutrients. Various environmental stimuli such as temperature, salinity, rainfall, and nutrients as different environmental stimuli affect the physiological responses of algae including agar deposition in the cell walls from season to season (Luhan, 1992). The agar yield was also found to be a function of different life phases of the algae (Whyte et al., 1981).

Gel strength is a particularly important measure of agar quality (Lewis and Hanisak, 1996). In general, the gel strength of agar varies with the species, location, season, and extraction method (Hoyle, 1978). *G. corticata* produced almost equal agar gel quality at both depths studied in December; nonetheless, the agar gel was considerably weaker in winter, particularly at a depth of 2-3 m, which rose again by March especially at a depth of 5-6 m. Taking together, the agar gel determined at 5-6 m in winter was relatively stronger than that at 2-3 m. These observations mostly agree with those found in *G. corticata* from Ettikkulam coast of northern Kerala, India (Kalesh, 2003). Similarly, specific seasonal patterns were shown in the gel strength of other *Gracilaria* species as well (e.g. Hoyle, 1978). Another congener, *G. bursa pastoris*, displayed strongest gel in autumn and winter, while agar gel strength of *G. gracilis* peaked in spring and summer (Marinho-Soriano and Bourret, 2003). Other factors affecting agar gel strength include *in situ* nutrient availability (Chiles et al., 1989), reproductive status (Givernaud et al., 1999), age (Lignell and Pedersén, 1989), and such cation content as methoxyl (McKinnon, 1973). It has also been found that number and position of sulphate groups as well as the amount of 3,6-anhydrogalactose (3,6-AG) fraction of agar (Duckworth and Yaphe, 1971) determine gel properties of agar; high contents of 3,6-AG and low levels of sulphation usually result in strong gels (Nelson et al., 1983). Because the suphated residues lead to weaker and less rigid gels (Rees, 1972) this may also explain the very low agar gel quality of our *G. corticata* samples. The current gel strength was considerably higher than, or within the range of, values reported for agars from some of the Indian *G. corticata*, namely 19 g cm^{-2} (Rao *et al*., 1977), 22 g cm^{-2} (Chennubhotla et al., 1979), 12-67 g cm^{-2} in *G. corticata* var. *cylindrica* (Kaliaperumal et al., 1992), and 17 to 27 g cm^{-2} (Oza, 1978).

Agars with strong brittle gels are used in bacteriological and biomedical applications, and soft elastic gels are consumed in the food industry (Yaphe and Duckworth, 1972). The present agar gel strength (≈8-31 g cm^{-2}) of *G. corticata* is far lower than that of bacteriological grade agar (270 g cm^{-2}), hence, the resultant agar is not suitable for bacteriological purposes. This corroborates previous findings in genus *Gracilaria* (Friedlander et al., 1981; Lobban and Harrison, 1997; Kalesh, 2003). Such an agar is possibly appropriate for food industry usages, probably with subsequent improvement of agar property by methods such as fractionation (Duckworth and Yaphe, 1971; Friedlander *et al*., 1981), alkali manipulation (Nelson et al., 1983), etc.

The color of extracted agar can be influenced by environmental conditions mainly intensity and duration of solar radiation, which affect plant cell wall and the resultant agar color (Rabiei et al., 2004). Accordingly, macroalgae such as *G. corti-*

cata growing at shallow intertidal zones are mostly sun-exposed during low tides leading to yellowish and/or reddish changes in the agar produced. Although agar color variations were observed at both depths, most color changes apparently occurred at low depth (2-3 m) in our algal samples; this may further signify the consequent effect of high solar radiation on the algal products at shallow waters.

Conclusion

The findings from current research indicate that the agar isolated from *G. corticata* is plausibly suitable for food applications following enhancement of agar properties possibly by fractionation technique. Considering the gel strength, the best time of the year to obtain a more rigid and better gel quality is in December and late winter at a depth of 5 m. At the same depth, the agar yield is maximal in winter especially in January. The agar color of this subtidal species is mostly dependent upon the growing depth with more color variations at shallow-water samples. Overall, *G. corticata* growing at relatively deeper intertidal zone has greater potential regarding agar yield and quality. Additionally, the potential values of *G. corticata* render this species a candidate for commercial culture.

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