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Standing Stock, Agar Yield and Properties of *Gracilaria salicornia* Harvested along the Tanzanian Coast

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Abstract—Seasonal biomass variation and agar yield of *G. salicornia* (C. Ag.) Dawson from Oyster Bay, Dar es Salaam, and Chwaka Bay, Zanzibar, were determined, and some properties of the agar examined. Mean biomass and canopy cover values ranged from 21–60 g/m^2 and 7–20 %, respectively. The highest mean biomass and cover values were obtained during the SE monsoon. Agar yield varied from 13.7 to 30.2 % (dry weight) and was highest during the dry NE monsoon period. Alkali treatment generally reduced agar yield by 25–56 %. Gel strength of the agar gels ranged between 118 and 251 g/cm^2 and was significantly higher during the NE monsoon period ($t = 2.2$; $P = 0.04$ and $t = 8.9$; $P << 0.05$) for samples collected from Oyster Bay and Chwaka Bay respectively). Mean gel strengths of native agar samples (205 ± 45 g/cm^2) was about 42 % of that of the standard agar used. The 3,6-anhydrogalactose content was highest during the rainy season (37.4–44.3 %) and tended to coincide with low gel strengths. Sulphate content varied between 0.5 and 2.8 % in both populations. It was concluded that the best period for harvesting *G. salicornia* for agar production in Tanzania is the dry (NE monsoon) period.

INTRODUCTION

Gracilaria is one of the algal genera from which agar is extracted commercially (Lewis et al., 1990). and the main source of agar in the world (Oliveira et al., 2000). The algae are harvested by hand from natural populations in Spain, Portugal, Morocco, the Azores, California, Mexico, New Zealand, South Africa, India, Chile and Japan (Armisen, 1995). Currently, the commercial mariculture of agar-producing seaweeds is led by the production of *Gracilaria chilensis* Bird, McLachlan et Oliveira in Chile (Buschmann & Correa, 2001). The seaweed has also been cultivated in China, Taiwan, Namibia, Venezuela, Mexico (Armisen, 1995), Indonesia and Vietnam (Oliveira et al., 2000).

Seven species of *Gracilaria* including *G. cornea* J. Agardh (previously known as *G. crassa* Harvey, Buriyo et al., in press), *G. corticata* J. Agardh, *G. arcuata* Zanardini, *G. salicornia, G. edulis* (J. Ag.) Silva, *G. fergusoni* J. Agardh and *G. millardetii* J. Agardh have been found in abundance along the Tanzanian coast (Jaasund, 1976). However, with the exception of *G. cornea*, information on biomass productivity of these algae is lacking. Besides, only three of these species have been shown to have potential for agar production. Semesi (1987) reported agar yield in the range of 28–48% (dry weight) for *G. cornea*, *G. corticata* and *G. fergusoni*. More recently, Buriyo (1999) obtained maximum agar yield of 44 % (dry weight) from *G. cornea*. The yield varied with season and the highest yield was obtained during the Northeast (NE) monsoon period.

Agar has many applications depending on its quality. Properties that determine its quality include the melting and gelling temperatures, gel strength, sulphate and the 3,6-anhydrogalactopyranose contents. Agar from *Gracilaria* species is widely used in food preparation.

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We present here the standing stock seasonality of *G. salicornia*, which is the most abundant *Gracilaria* species in Tanzania, and report on investigations on the effects of alkali treatment on the yield and quality of its agar.

MATERIALS AND METHODS

Study areas

Collections were made at Oyster Bay, Dar es Salaam (39°16'–39°17.5'E and 6°46.5'S) and Chwaka Bay (39°40'E , 6°13'S) in Zanzibar. The Oyster Bay shore is characterised by a rocky platform backed by steep and overhanging cliffs. In the platform there are scattered boulders and rocky tidal pools of various sizes both of which form important habitats preferred by different macro- and micro-fauna and flora. Chwaka Bay samples were collected closer to the mangrove thicket, an area permanently submerged with a constantly running tidal stream. The major substrate for the algae is old *Halimeda* segments.

Plant collection

The algae were collected in 2002 during low spring tides in the inter-monsoon wet period (March–April), Southeast monsoon (July–August) and Northeast monsoon (November–December) periods. Sampling for biomass estimation was carried out according to Buriyo (1999). Environmental parameters namely light, water temperature and salinity were recorded during each sampling visit. In the laboratory, samples were sorted and washed thoroughly with tap water to remove rock debris and epiphytes. Plants for biomass determination were then dried in an oven at 105 °C to a constant weight. Plants for agar extraction were rinsed with tap water and dried first in the sun and then at 60 °C in an oven to constant weight.

Agar extraction and quality determination

Agar extraction was done according to the method described by Semesi (1979) and modified by Buriyo (1999). Alkali treatment of plants was carried out according to Oyieke (1993) with modification using a 5% (instead of 2%) NaOH. Agar extraction using an autoclave was done at 121 °C for 1 hour using 10 g dry sample in an Erlenmeyer flask filled with 500 ml distilled water. Agar melting temperature and gel strength at 1.5 % w/v was tested according to Oyieke (1993) and Buriyo (1999). Sulphate content was determined by a turbidimetric method as described in Allen (1989). The 3,6-anhydrogalactose sugar (3,6-AG) was determined by the resorcinol reagent method as described in Craigie & Leigh (1978). The quality of standard agar (Oxoid Bacto Agar) prepared in the same manner was recorded for comparison.

RESULTS

Seasonal variation in biomass and canopy cover

The results for mean dry biomass and percentage canopy cover at Oyster Bay, Dar es Salaam are presented in Table 1. The highest mean biomass and canopy cover values (59.8 g/m^2 and 20.0%, respectively) were obtained in April, and the lowest in December. Generally, biomass and percentage canopy cover were significantly higher during March–July and declined during November–December.

Seasonal variation in agar yield

The results for mean percentage agar yield are presented in Table 2. Agar yield varied from 16.35 % in July to 30.2% in December in the Dar es Salaam samples and 13.7 to 28.0% in the Chwaka Bay samples. Generally agar yield at both stations was lower during April and July and higher

Table 1. Seasonal variation in biomass and canopy cover of *Gracilaria salicornia* at Oyster Bay, Dar es Salaam

Month in 2002	Biomass (g/m^2)	Cover (%)
March	55.6 ± 60.6	18.55 ± 20
April	59.8 ± 66	20.00 ± 22
July	33.1 ± 28	11.06 ± 9
August	26.4 ± 24	8.81 ± 8
November	25.1 ± 34	8.35 ± 11
December	21.0 ± 20	7.00 ± 6.7

in August and December. Statistical analysis (two sample t-test) showed that the agar yield was significantly higher during the NE monsoon period (t = 2.9; P = 0.03) in samples collected from Oyster Bay. Agar yield did not differ significantly between seasons in samples collected from Chwaka Bay (t = 0.8; P = 0.4).

Effect of alkali treatment and extraction method on agar yield

Results of agar yield (% dry wt) of plants treated and not treated with alkali (5 % NaOH) are presented in Table 2. With the exception of seaweeds harvested in August, alkali treatment reduced agar yield by almost 31–56 % for Oyster Bay samples and 25–35 % for Chwaka Bay samples.

Different agar extraction methods appeared to affect the yield of agar. The comparison was arbitrary and was carried out using samples collected in November from Oyster Bay. Agar yield was about 7 % (dry weight) higher for samples extracted by autoclaving at 121 ^{o}C than those for samples extracted using a water bath at 95 ^{o}C. Agar extracted by using the autoclave method was also clearer than that extracted by the water bath method.

Seasonal variation in agar properties

Results of agar gel strength are presented in Fig.1. The native agar extracted from samples collected from Oyster Bay and Chwaka Bay had the highest gel strength during March and April, respectively. Agar from both populations also had generally higher gel strength during November and December than during July to August.

Statistical analysis showed that gel strength in both populations was significantly higher during NE monsoon period (t = 2.2; *P* = 0.04 and t = 8.9; *P* << 0.05 for samples collected from Oyster Bay and Chwaka Bay, respectively). With the exception of July, alkali treatment significantly reduced gel strength of agar in both populations (*t* = 2.7; P = 0.02 and t = 3.8; P = 0.007 for samples collected from Oyster Bay and Chwaka Bay, respectively).

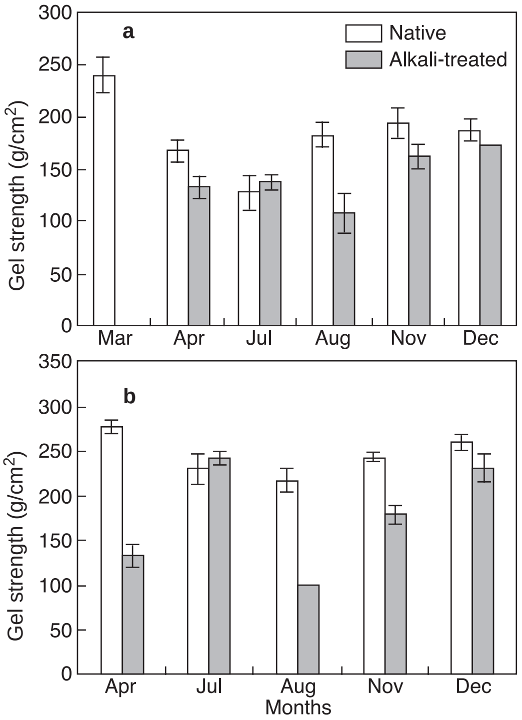

Fig. 1. Gel strength of native and alkali-treated agar extracted from *G. salicornia* harvested from (a) Oyster Bay in Dar es Salaam and (b) Chwaka Bay in Zanzibar in 2002

Table 2. Seasonal variation in agar yield (% mean ± s.d.) from native and alkali-treated *Gracilaria salicornia* (n=3)

	Oyster Bay		Chwaka	
Month in 2002	Native	Alkali-treated	Native	Alkali-treated
March	23.2 ± 1.1	–	–	–
April	22.6 ± 2.0	–	14.1 ± 0.9	–
July	16.3 ± 0.2	7.0 ± 2.2	13.7 ± 1.0	10.4 ± 1.0
August	27.6 ± 2.4	26.1 ± 2.1	28.1 ± 0.3	22.3 ± 3.2
November	26.2 ± 1.1	17.6 ± 0.4	20.3 ± 0.6	13.2 ± 1.0
December	30.2 ± 1.6	15.7 ± 1.0	20.1 ± 1.1	15.7 ± 0.6

Agar extracted from plants collected from Chwaka Bay had significantly higher gel strength than that extracted from plants collected from Oyster Bay, Dar es Salaam (Fig. 1), ($t = 7.2$; $P << 0.05$). There was a general decrease in gel strength during the rainy season, particularly April to August, although agar from samples collected from Chwaka Bay had highest gel strength in April. Overall, the mean gel strength of native agar samples (205 ± 45 g/cm^2) was about 42 % that of the standard agar used. The mean melting temperatures of the agar gels was in the range of 72–94 °C. The highest melting point was measured for the native agar in April and the lowest for the gel from alkali-treated plants in August, both from Chwaka Bay.

The 3,6-anhydrogalactose and sulphate contents of both the native agar and alkali-treated agar are shown in Table 3. The 3,6-anhydrogalactose content was highest during August for both populations and ranged from 16 to 44 %. High 3,6-anhydrogalactose content tended to coincide with low gel strength. Alkali treatment increased the amount of 3,6-anhydrogalactose significantly in agar extracted from Chwaka Bay ($t = 2.5$; $P = 0.02$). On the other hand alkali treatment reduced significantly the amount of 3,6-AG in agar extracted from samples collected from Oyster Bay ($t = 3$; $P = 0.008$).

The sulphate content for native agar varied between 0.8–2.8 % in native agar and 0.52–2.3 % in alkali-modified agar from both populations.

DISCUSSION

Seasonal variation in biomass and canopy cover

The highest biomass recorded in this study was 59.8 g/m^2 (dry weight) during the SE monsoon period (March–July). This value is comparable to the highest biomass value (about 20 g/m^2 dry weight) obtained for the same algal species harvested from Negros Islands in the Philippines (Calumpong et al., 1999). However, these values seem too low for sustainable commercial exploitation, hence the need for cultivation studies.

The higher biomass and canopy cover recorded during the SE monsoon is probably due to improved nutrient availability after the long rains. Other factors which may also promote algal growth during this season are reduced light and temperature; March to July is associated with high cloud cover hence algal thalli are expected to be better protected from damaging light intensities than during September to December. High light intensity accompanied with high temperatures may reduce algal productivity partly due to

Table 3. 3,6 anhydrogalactose (3,6-AG) and sulphate content of native and alkali-treated agar from *Gracilaria salicornia* collected from Oyster Bay in Dar es Salaam and Chwaka Bay in Zanzibar

Collection site	March	April	July	August	November	December
Oyster Bay						
% 3,6-AG						
Native	26.3 ± 2.2	16.3 ± 1.5	26.0 ± 2.2	37.4 ± 0.4	34.1 ± 3.1	23.6 ± 2.8
Alkali-treated	–	18.1 ± 4.1	17.5 ± 1.3	30.0 ± 2.5	34.2 ± 1	19.6 ± 2.7
% Sulphate						
Native	2.21 ± 0.9	2.7 ± 0.44	1.7 ± 0.45	2.33 ± 0.3	2.8 ± 0.83	2.25 ± 0.85
Alkali-treated	–	1.75 ± 0.61	0.9 ± 0.78	1.72 ± 0.28	2.33 ± 0.6	1.7 ± 0.5
Chwaka Bay						
% 3,6-AG						
Native	–	24.9 ± 3.4	28.0 ± 3.4	44.0 ± 3.3	23.0 ± 2.0	19.4 ± 3.0
Alkali-treated	–	24.5 ± 3.5	35.7 ± 1.5	42.6 ± 3.0	25.8 ± 1.7	23.3 ± 3.2
% Sulphate						
Native	–	1.76 ± 0.42	0.8 ± 0.77	2.55 ± 0.3	2.41 ± 0.62	2.11 ± 0.4
Alkali-treated	–	0.97 ± 0.33	0.52 ± 0.5	1.81 ± 1.01	1.51 ± 0.62	0.98 ± 0.5

photoinhibition (Krause & Weis, 1991) and desiccation especially to intertidal algae. Moorjani (1982) found that on reef platforms of southern Kenya higher algal biomass occurred towards the end of the SE monsoon and reached a peak in September; that study emphasised that physical factors were responsible for the observed pattern and suggested that desiccation stress was less severe during the SE monsoon.

A gradual decrease in algal biomass and abundance was observed between August and December (Table 1). This period was associated with higher light intensities, temperatures and salinity. Furthermore, this period normally has relatively longer hours of sunshine (Buriyo, 1999). These conditions affect benthic intertidal organisms especially during the mid-day spring low tides. It was found (Buriyo, 1999) that during this period nitrogen in seawater was low (<2 µg.at.N/l^2). The combined detrimental effects of light stress, desiccation, higher salinity and nutrient limitation probably resulted in the obseved decline of algal biomass.

Seasonal variation in agar yield

Agar yield varied with seasons and strains. Significantly higher mean agar yield in the studied populations was obtained during NE monsoon period. There was an inverse relationship between agar yield and biomass, with the highest agar yield being recorded during August–December and the lowest during April–July (Table 2). The environmental factors which coincided with higher agar yield were higher insolation, salinity, temperature (and low nitrogen availability, Buriyo 1999). The reason for this has not been established. Nevertheless, it is possible that during the period of favourable growth (SE monsoon), plants gradually accumulate resources such as nitrogen in order to survive the adverse period of low nutrients, elevated light intensity, temperature and salinity (i. e. NE monsoon period). In support of this hypothesis, Mshigeni (1974) found that during periods of high levels of nitrate concentration, algal photosynthates were diverted towards protein synthesis instead of polysaccharide synthesis. In a previous study (Buriyo, 1999) agar content in *G. cornea* was inversely correlated with nitrogen levels in seawater and thallus nitrogen and phosphorus. The inverse relationship between water nitrogen and biomass and agar yield has also been reported in *Gracilaria* (Lahaye and Rochas, 1991). Furthermore, Hoyle (1978) found that low agar yields in the winter were associated with higher total thallus nitrogen content in two *Gracilaria* species. Other studies supporting the observations are those of DeBoer (1979); Lahaye & Yaphe (1988); Bird (1988) and Oyieke (1993).

The low agar content observed from April to July may also be related to the onset of the long rains of the SE monsoon. During the rainy season, factors which may contribute to low agar content include low light due to high cloud cover and low salinity. The stress imposed by low salinity might have resulted into the channelling of photosynthetic by-products to sugars responsible for maintaining ionic balance. Moreover lack of adequate CO_2, HCO_3^- and Ca^{2+} in rainwater, may also have a considerable effect on photosynthesis (Lobban & Harrison, 1994; Yarish et al., 1980), and therefore low level of carbohydrate accumulation. Another possibility is the loss of polysaccharides by cellular exudation as a result of stress. It has been reported (Moebus & Johnson, 1974; Moebus et al., 1974; Harlin & Craigie, 1975) that under stress, seaweeds exude more than 1% of their net assimilation. Also exudation of 30–40 % of net assimilation has been reported (Lobban & Harrison, 1994). Other studies corroborating this finding include the work of Santelices & Varela (1993) who analysed the culture media used to culture *Gracilaria chilensis* at low light intensity and temperature (45 µmol photons/m^2/s and 14 ºC) and found a mixture of polysaccharides in which highly sulphated galactans were the most dominant. Furthermore, Ekman & Pedersén (1990) reported that stressful cultivation conditions such as those induced by low light availability may stimulate carbon excretion and increase the cold-water-soluble fraction of the agar.

The agar composition of *Gracilaria* has been reported to be species-dependent (Nelson et al., 1983; Yaphe, 1984) and influenced by seasons (Asare, 1980; Lahaye & Yaphe, 1988).

In this study *G. salicornia* from Oyster Bay yielded more agar than that from Chwaka. The difference in agar yield between these populations may be attributed to the ecology of the two sites. For instance, salinity at Oyster Bay fluctuated between 34–38 ‰ in the water column and 25–30 ‰ in tidal pools exposed to rain during low tides, while at Chwaka Bay it was 33–35 ‰ during the study period. Furthermore, samples collected from Chwaka Bay were permanently submerged hence less exposed to environmental parameter fluctuations. From the previous discussion, the exposure of algae to environmental parameter fluctuations may affect their agar content. The differences in agar yield between the two populations could also be due to the differences in the strains of *G. salicornia* found at Oyster Bay and Chwaka Bay. We observed that populations occurring at the former site were constricted while those at the latter were not.

Alkali treatment significantly affected agar yield as is well known from the literature. The results of the alkali-treated plants suggest that hot-water-soluble polysaccharides that are extracted from alkali-treated plants of *G. salicornia* contain important quantities of alkali-labile sulphates, some of which may have been lost during the processing. These results agree with those reported by Oyieke (1993) on the same species.

The agar yield from plants extracted by autoclaving at 121°C was slightly higher than that from plants extracted at 95 °C in the water bath. The former method was less time-consuming and the resulting agar's clarity was better.

Seasonal variation in agar quality

Higher gel strengths were observed in March for samples collected from Oyster Bay and in April for samples collected from Chwaka Bay compared to July and August. Although March and April are normally rainy months, and July and August are dry along the Tanzanian coast, the situation was the opposite in 2002 when this study was conducted. The occurrence of agar with higher gel strength during the dry period and vice versa has been reported by other workers (Abbott, 1980; Asare, 1980).

The generally low gel strength observed during the rainy season could be related to low salinity, which may result in greater osmotic gradient such that ions like Ca^{2+} and K^+ (Yarish et al., 1980) leave the cell to the medium (Lobban & Harrison, 1994).These ions have been reported (Grant et al., 1973; Semesi, 1979) to increase gel strengths of some algal polysaccharides such as alginates from brown algae and other gels from red algal species such as *Eucheuma*, *Gracilaria*, *Hypnea*, etc. Moreover, Bird (1988) reported low gel strength from agar extracted from *Gracilaria* species cultivated at low salinity (17 ‰).

The 3,6-AG content varied between treatments, sites and months, but was high in all samples during August, which coincided with low agar gel strengths. To the contrary, high 3,6-AG content has been previously reported to increase gel strength. Alkali treatment of either algal plants or agar has been shown to reduce sulphate, increase 3,6-AG content and hence increase gel strength in some *Gracilaria* species (Yenigül, 1993; Chirapart & Ohno, 1993; Givernaud et al., 1999; Montaño et al., 1999). It therefore appears that gel strength is not solely dependent on the 3,6-AG content.

In the present study, alkali treatment slightly reduced 3,6-AG content but had little effect on the sulphate content. These results are in conformity with those obtained for *G. caudata* from Brazil and *G. tenuistipitata* from China (Macchiavello et al., 1999). The presence of high sulphate content in tropical agarophytes has been reported to affect greatly its agar quality. It has also been reported (Lignell & Pedersén, 1989) that the content of 3,6-AG decreased while that of sulphate and 4-O-methyl-L-galactose increased in *Gracilaria tikvahiae* cultivated at higher temperatures. The more sulphated chains have the greater tendency to enter into solution (Rees, 1972; Tsuneaki & Suzuki, 1975). As such, the association of agar chains during gelation is inhibited by increasing the sulphate content.

Lower gel strengths and higher sulphation levels have also been reported for samples grown during summer or at high temperatures (Hoyle, 1978; Cote & Hanisak, 1986). However, Wang & Yang (1980), Abbott (1980) and Asare (1980) all reported high gel strengths for agar extracted from samples collected in summer with temperatures higher than 30 ºC. In the present study there was no clear relationship between the variations in sulphate and 3,6-AG contents and gel strength. These observations from the literature and the present study suggest that none of the single factors tested significantly affects gel properties.

Other factors which influence agar quality include nutrient status of the media in which algae grow (Bird, 1988; Chiles et al., 1989), age (Lignell & Pedersén, 1989) and reproductive status of the plants (Givernaud et al., 1999), molecular weight of the polymer (McKinnon et al., 1969), concentration of cations such as K^+ (McKinnon, 1973), methoxyl content (Arnott et al., 1974a), pyruvic acid (Arnott et al., 1974b), the concentration of alkali for seaweed treatment prior to extraction.

Since all of the factors enumerated above were not investigated in this study, their effects on our agar quality results cannot be discussed. Furthermore, different concentrations of alkali have been employed to treat seaweeds before extractions; hence it is difficult to compare its effect. There are also seasonal differences between locations and years in the magnitude and timing of the environmental parameters affecting algal growth.

CONCLUSIONS

The best period for harvesting wild populations of *G. salicornia* for agar production in Tanzania is during the dry NE monsoon. However, the low biomass measured during this period might be a constraint to the exploitation of this agarophyte unless it is cultivated. Besides, as its agar quality is below the standard for commercial bacteriological agar, it can be only used in applications that require soft gels unless its quality is improved.

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