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Seasonal changes in carrageenan yield and gel properties in three commercial eucheumoids grown in southern Kenya

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Abstract

Three eucheumoid strains (brown *Eucheuma denticulatum*, green and brown *Kappaphycus alvarezii* growing in fixed, off-bottom seaweed farms) were examined for carrageenan properties at three sites (Gazi, Kibuyuni and Mkwiro) in Kenya, monthly for one year; these properties were related to a suite of environmental factors and seaweed growth condition. The mean carrageenan yield was significantly higher for green *K. alvarezii* (59.1% dry wt) than both brown *E. denticulatum* (56.6% dry wt) and brown *K. alvarezii* (56.5% dry wt). Thalli at Gazi had a higher carrageenan yield (58.0% dry wt) than both those at Kibuyuni (57.1% dry wt) and Mkwiro (57.3% dry wt), although this small difference does not appear meaningful commercially. Both green and brown *K. alvarezii* exhibited much higher gel strengths (1042.1 g cm^{-2} and 1053.7 g cm^{-2}, respectively) than brown *E. denticulatum* (100.8 g cm^{-2}). Thalli at Kibuyuni had higher gel strengths (783.0 g cm^{-2}) than those at Gazi (690.1 g cm^{-2}), while those at Mkwiro had intermediate values (747.8 g cm^{-2}). In both green and brown *K. alvarezii*, the gel strengths were positively correlated with photon fluence rate, while the gel strengths of brown *K. alvarezii* showed an inverse correlation with both relative growth rate and percentage “ice-ice” syndrome. Brown *E. denticulatum* had carrageenan with higher viscosity (81.7 mPa s) and sulphate content (29.1% dry wt) than both green and brown *K. alvarezii*. The gel viscosities of all the strains were higher during the southeast monsoon (April–September: 67.3 mPa s) than during the northeast monsoon (October–March: 46.3 mPa s), and were positively correlated with gel strengths. The results show that the three strains produced carrageenans of commercial quality.

Keywords: carrageenan; *Eucheuma*; *Kappaphycus*; Kenya; yield.

Introduction

Red seaweeds of the genera *Kappaphycus* and *Eucheuma* (commercial eucheumoids) are cultivated in tropical countries for the commercially important phycocolloid, carrageenan. Carrageenans are sulphated polysaccharides with a common structural framework of alternately 4-linked α-D-galactopyranosyl and 3-linked β-D-galactopyranosyl units (Anderson et al. 1968). There are several carrageenan types, but only the iota (ι-), kappa (κ-) and lambda (λ-) carrageenans are available commercially (Craigie 1990). *Kappaphycus* produces kappa carrageenan while *Eucheuma* produces iota carrageenan. The naturally occurring κ- and ι-carrageenans contain μ- and ν-precursor residues, respectively, with 4-linked α-D-galactopyranosyl-6-sulphate or 2,6-disulphate units, and alkali extraction is used to increase their gel strengths (Stanley 1990).

Today, most of the world’s supplies of κ- and ι-carrageenans are from various strains of *Kappaphycus alvarezii* (Doty) Doty *ex* P.C. Silva and *Eucheuma denticulatum* (Burman) Collins *et* Hervey, respectively. In the following discussion, the term “eucheumoid” will be used to include the different commercial strains of *E. denticulatum* and *K. alvarezii*, which were formerly recognized as species of *Eucheuma* (Doty 1987).

The κ- and ι-carrageenans are different in their chemical structures and properties, and have different uses in industry related to their ability to form gels. Kappa gels are firm, brittle and syneretic (exude water), while iota gels, by contrast, are elastic, resilient, and dry (Stanley 1990). The gel properties and viscosities of carrageenans from eucheumoids have made them commercially important as water binders, gellants, stabilizers, and thickeners in food, cosmetic, pharmaceutical, and textile industries (McHugh 2003).

The carrageenan industry has a total market value of about US$ 300 million with an estimated increase in demand for carrageenan growing at 5% annually (McHugh 2003). This high demand for carrageenans in the world market has encouraged the introduction of commercial eucheumoid farming in several tropical countries (Doty 1987, Ask et al. 2003). Although the carrageenan industry’s concern for eucheumoids is based on their hydrocolloids, the information on carrageenan yield and quality from these seaweeds is limited (Doty 1987, Ohno et al. 1996, Muñoz et al. 2004). The lack of information on carrageenan properties in the introduced commercial eucheumoids is even more marked. There are some published data on carrageenan characteristics of introduced eucheumoids in the subtropical waters of Japan (Ohno et al. 1994) and Brazil (Paula et al. 1999), and tropical waters of Vietnam (Ohno et al. 1996) and Mexico (Muñoz et al. 2004). However, the reports do not provide year-round data on carrageenan properties in these regions.

Commercial cultivation of introduced eucheumoids is now well established in the Western Indian Ocean and Central Pacific regions in areas such as the Fiji Islands, Kiribati, Madagascar and Tanzania (Ask et al. 2003). However, to date, little has been published on carrageenan properties of introduced eucheumoids in the Western Indian Ocean region; indeed, no such studies have been published on *Kappaphycus alvarezii*. Except for the work of Braud and Perez (1978) on introduced *Eucheuma denticulatum* [as *Eucheuma spinosum* (Linnaeus) J. Agardh] in Djibouti waters, most of the studies on carrageenan properties of seaweeds in the region are on indigenous materials of *E. denticulatum* and *Kappaphycus striatus* (F. Schmitz) Doty *ex* P.C. Silva [as *Kappaphycus striatum* (F. Schmitz) Doty *ex* P.C. Silva] from Tanzania (Buriyo et al. 2001) and Madagascar (Mollion and Braud 1993). The influence of local environmental factors on carrageenan yield and quality from introduced eucheumoids is not known or inadequately documented. Such information is useful in siting eucheumoid farms to yield the best quality carrageenan (Ohno et al. 1996). Chemical and physical properties of carrageenans from eucheumoids vary with genus, species and morphotypes (Trono and Lluisma 1992, Hurtado-Ponce 1995) and season (Trono and Lluisma 1992, Buriyo et al. 2001), among other factors.

As there is no commercial exploitation or farming of marine algae in Kenya, there is, in the southern part of the country, an ongoing research project investigating the feasibility of eucheumoid cultivation using the fixed off-bottom method (Wakibia et al. 2006). Attention should be given to the commercial quality of the cultivated material, so this study was conducted to determine carrageenan yield and quality of three commercial eucheumoids grown at three sites with different environmental conditions. The carrageenan properties were also related to seaweed growth condition (relative growth rate and percentage “ice-ice”). Gel quality factors measured were gel strength, viscosity and sulphate content, since these parameters are used as indicators of carrageenan of commercial quality.

Materials and methods

Collection and drying of seaweed materials

Samples of three commercial eucheumoids (brown strain of *Eucheuma denticulatum* and brown and green strains of *Kappaphycus alvarezii*), each weighing about 1 kg (wet wt) were collected monthly for one year from pilot demonstration seaweed farms at three study sites in southern Kenya: Gazi (shallow mangrove system; 4°25′ S, 39°30′ E), Kibuyuni (intertidal reef flat; 4°38′ S, 39°20′ E), and Mkwiro (lagoon; 4°40′ S, 39°23′ E). These sites were chosen to represent different available habitats that may be suitable for growth of the seaweeds. The eucheumoid stocking material was imported from Zanzibar, originally having come from Bohol, Philippines (see Wakibia et al. 2006, for quarantine procedures).

Various environmental factors, viz., water temperature (°C), water motion (diffusion factor), and photon fluence rate (μmol photons m^{-2} s^{-1}), and seaweed parameters: relative growth rate (% day^{-1}), thallus N (% of dry wt) and % “ice-ice” syndrome [presence of white and transparent spots (resembling ice) found within eucheumoid thalli] were also measured at each study site when the eucheumoid samples were collected. A detailed description of the measurements of environmental factors and seaweed parameters is published elsewhere (Wakibia 2005, Wakibia et al. 2006). The relative growth rate (RGR), expressed as percent increase in wet weight per day, was determined for each strain according to the formula

$$RGR = [(Wt/Wo)^{1/t} - 1] \times 100,$$

where Wo and Wt were initial biomass and final biomass at day t, respectively (Evans 1972). The number of plants with “ice-ice” syndrome were recorded and expressed as a percentage. Water temperature and photon fluence rate were determined using a mercury thermometer and a LI-193SA Spherical Quantum Sensor and LI-1400 Datalogger (Li-Cor, Lincoln, USA), respectively. Water motion was determined by the dissolution of spherical plaster of Paris balls according to the clod card method from Doty (1971). The balls were mounted on stakes for up to 24 h, and amounts of dissolution of plaster of Paris were evaluated by comparing their initial and final dry weights. The diffusion factor was calculated by the ratio of weight lost compared to plaster of Paris balls in still water.

All harvested materials were sun-dried for three days, oven dried at 45°C to constant weight and stored in sealed polyethylene bags in a dry place for carrageenan analysis. All dried materials were stored in a desiccator at room temperature (25°C) in a dry place for two to three months prior to carrageenan extraction. The stored algal samples were thoroughly washed and cleaned with tap water to remove salt, sand and epiphytes, and oven dried at 60°C for 24 h.

Carrageenan extraction

Prior to carrageenan analyses, the seaweeds were redried to a constant weight at 60°C and cooled in a desiccator over silica gel. Carrageenan extraction followed the procedures of Dawes et al. (1977), with some modifications. According to Wakibia (2005), the best extraction conditions for *Eucheuma denticulatum* and *Kappaphycus alvarezii* were 8 and 18 h, respectively, and hence these extraction times were used in this study. To evaluate the commercial value of the plant material, the extraction was performed using alkaline pretreatment.

Carrageenan extraction was done by soaking 20.0 g of the chopped seaweeds in 700 ml of hot distilled water in a 2-l stainless steel beaker and adding 4.0 g of calcium hydroxide. The mixture was heated with mild agitation in a water bath (95±2°C) for 30 min, blended to a paste, and 300 ml of hot water were added to the mixture. The paste was then thoroughly mixed with a magnetic stirrer. The steel beaker was capped with aluminium foil (to reduce evaporation) and placed in a water bath (95±2°C) for 8 h and 18 h for *E. denticulatum* and *K. alvarezii* samples, respectively. To each of the hot mixtures, 20.0 g Celite 545 was added and stirred for 30 min, and the hot slurry pressure filtered through Whatman filter paper # 2 in a preheated stainless steel pressure filter holder (Sartorius GmbH, Goettingen, Germany). The residue was then removed from the filter paper, placed in 200 ml boiling distilled water and pressure filtered as above. The resulting hot filtrate was adjusted to pH 8.5±0.5 with diluted reagent grade HCL (w/v, 10%). The filtrate was poured into two litres of 99% reagent grade isopropanol in a steady stream while stirring the alcohol gently until the carrageenan coagulum had completely formed and hardened as much as possible. The coagulum was drained on a 100-mesh sieve, squeezed and kneaded to remove as much liquid as possible. The coagulum was spread out in a glass Petri dish to allow alcohol to evaporate for an hour in a ventilated fume chamber. The coagulum was oven-dried at 60°C to constant weight, cooled in a desiccator over silica gel and weighed to calculate the carrageenan yield (% algal dry wt). The dried carrageenan was then ground to a powder using a Wiley (Philadelphia, USA) mill (40-mesh size), placed in a bottle with a lid and stored in a desiccator for 2 weeks prior to carrageenan quality analysis. Each sample was extracted in duplicate for carrageenan yield determinations.

Carrageenan quality analysis

The physical properties (gel strength and viscosity) were determined in duplicate using a 1.5% carrageenan gel in 0.2% potassium chloride (KCL) according to procedures outlined in Wakibia (2005). Water gel of the carrageenan was made up using 1.5 g of extract, 0.2 g KCL, and 100 ml of deionised water. The mixture was weighed and heated in a boiling water bath with constant stirring until the carrageenan was dissolved (30 min). The solution was maintained at 80°C for 10 min. The volume was adjusted to initial weight using hot deionised water followed by stirring to incorporate the added water. The hot gel was poured into a crystallising dish (70×50 mm) and allowed to stand for 20 min at room temperature (25°C). The crystallising dish was covered with a plastic film and the carrageenan was allowed to gel overnight in a 10°C incubator. The resulting gel was inverted in the crystallising dish to expose the fresh surface of the gel to the gel testing plunger. Gel strength (g cm^{-2}) was determined in duplicate, with a laboratory-made apparatus that measures the force required to break the gel surface. The apparatus consists of 1 cm^{-2} stainless steel cylindrical probe connected to a motor drive, and a Sartorius balance (BP 4100) attached to a PC with Winwedge® version 1.2 software. Viscosity was determined by soaking 9.0 g of the extract in 600 ml of 0.2% KCL. The solution was allowed to cool and at 75°C, viscosity (in mPa s) was measured with a Brookfield DV-II viscometer (Brookfield Engineering Labs. Inc., Middleboro, USA) using spindle no. 1 at 30 rpm. The gel strength and viscosity values of the samples were compared to standard commercial carrageenan gels (κ-carrageenan: Lot no. 41K1413 and ι-carrageenan: Lot no. 41K1424) from Sigma Chemicals Co. (St. Louis, USA) prepared and measured in the same way.

The sulphate content of the carrageenan extracts was determined by hydrolysing 0.5 g of each sample (previously dried to a constant weight at 60°C) in concentrated nitric acid at 105°C for 2 h. After the hydrolysis, the sample was diluted to 50 ml and the sulphur concentration was determined with a Varian Vista-MPX ICP spectrophotometer (Cambridge, UK). Percentage sulphur of carrageenan was then converted into percentage sulphate. The sulphur analysis was carried out by Bemlab (Somerset West, South Africa).

The study was performed from October 2001 to October 2002, covering both the northeast monsoon (NEM) prevailing from October to March and the southeast monsoon (SEM) from April to September. The two seasons are characterised by distinct differences in physical and chemical conditions of the coastal waters (McClanahan 1988). The SEM is associated with strong winds, low air and water temperatures, low solar radiation and heavy rains, with the lowest tides occurring during the night. During the NEM, these conditions are reversed with the lowest tide occurring during the day. The tides are mixed semi-diurnal, with maximum tidal ranges of about 4.0 m (McClanahan 1988). However, due to logistical problems it was not possible to collect samples at Mkwiro from May to July, and in July at Kibuyuni.

Statistical analysis

The carrageenan yield and gel properties (gel strength, viscosity and sulphate content) of the carrageenan measured in the present study were compared with environmental factors and seaweed parameters that were determined in a previous study (Wakibia 2005). Data were tested for normality (Shapiro-Wilk's test) and homogeneity of variance (Bartlett's test). Statistically significant differences in carrageenan properties between strains, months, sites and seasons were tested by the general linear model (GLM) procedures followed by determination of pairwise differences among individual mean values by the least significant difference (LSD) test at $p<0.05$. Pearson's product moment correlation test was used to determine the linear relationship between treatments. In all these analyses, the SAS Program was used (SAS 1999).

Results

Carrageenan yield

The monthly variations in carrageenan yields of three eucheumoid strains at three sites are presented in Figure 1. The monthly average carrageenan yield varied between 49% and 63% dry wt. The carrageenan yield varied significantly among strains ($F_{2,174}=22.23$, $p<0.05$) and was higher in the green strain of *Kappaphycus alvarezii* (59.1% dry wt) than in both brown *Eucheuma denticulatum* (56.6% dry wt) and brown *K. alvarezii* (56.5% dry wt). The carrageenan contents were very similar at the three sites. However, the small differences were significant ($F_{2,174}=3.58$, $p<0.05$); thalli at Gazi (58.0%) had a higher carrageenan yield than both those at Kibuyuni (57.1%) and Mkwiro (57.3%). Carrageenan yield of the strains varied significantly among months ($F_{12,96}=24.98$, $p<0.05$). The carrageenan yields were higher from plants harvested at all sites during the month of September

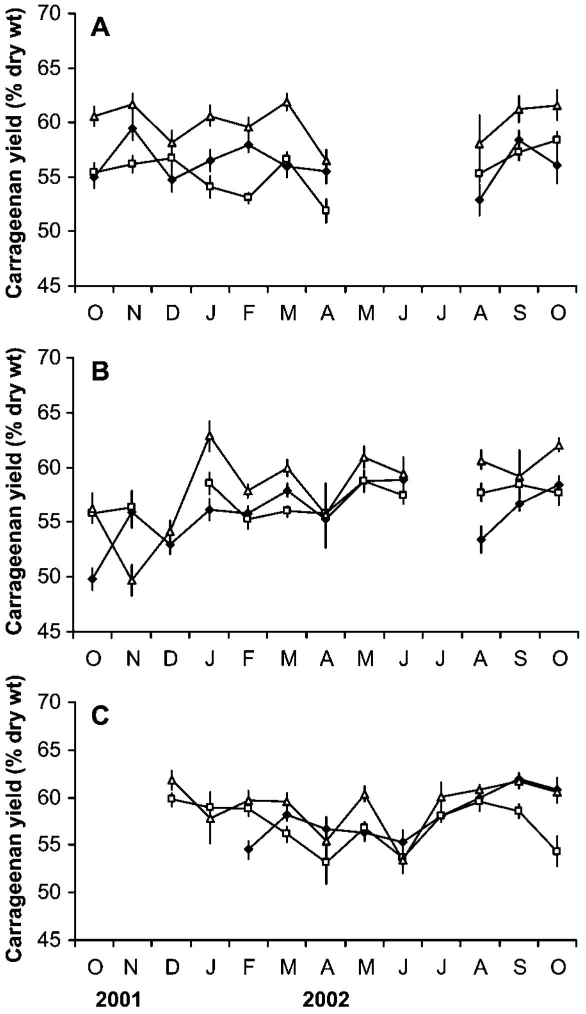

Figure 1 Mean monthly carrageenan yields (% dry wt) of brown *Eucheuma denticulatum* (prism), green (triangle) and brown (square) *Kappaphycus alvarezii* at three sites (A=Mkwiro; B=Kibuyuni; C=Gazi) in southern Kenya from October 2001 to October 2002. Mean and range (vertical line), n=2.

2002 (59.3%) while minimal values were obtained in both October 2001 (55.5%) and April 2002 (55.1%) (Figure 1). The three strains showed no significant difference ($F_{1,174}=0.20$, $p=0.65$) in carrageenan yields between the northeast monsoon (57.3% of dry wt) and the southeast monsoon (57.2% of dry wt).

Carrageenan quality properties

Significant monthly variations ($F_{2,174}=339.73$, $p<0.05$) in carrageenan gel strengths occurred in the three eucheumoid strains at all the sites (Figure 2). Higher gel strengths were obtained for both green (1042.1 g cm^{-2}) and brown (1053.7 g cm^{-2}) strains of *Kappaphycus alvarezii* than for the brown (100.8 g cm^{-2}) strain of *Eucheuma denticulatum*. However, the gel strengths of the two *K. alvarezii* strains were similar. The 1.5% gel solution under the laboratory conditions exhibited gel strengths of 1600.1 and 82.8 g cm^{-2}, respectively, for commercial κ-and ι-carrageenans (Sigma).

Gel strength varied significantly among sites ($F_{2,174}=3.17$, $p<0.05$). Thalli at Kibuyuni had carrageenans with higher gel strengths (783.0 g cm^{-2}) than those at Gazi and Mkwiro (with gel strength values of 690.1 and 747.8 g cm^{-2}, respectively). The gel strengths of carrageenans from the three strains varied with the month of sampling ($F_{12,96}=208.91$, $p<0.05$). A higher gel strength was obtained in January (900.0 g cm^{-2}), while lower values were recorded in November 2001 (362.9 g cm^{-2}) and December 2001 (458.7 g cm^{-2}). The three strains showed similar ($F_{1,174}=2.39$, $p=0.12$) gel strengths of carrageenans in the SEM (764 g cm^{-2}) and NEM (724 g cm^{-2}).

Gel viscosities of carrageenans varied significantly among the eucheumoid strains but not between sites. The brown strain of *Eucheuma denticulatum* had carrageenan with a significantly higher viscosity (81.7 mPa s) than both green (46.0 mPa s) and brown (40.0 mPa s) strains of *Kappaphycus alvarezii* (LSD test, $p<0.05$). The carrageenan viscosities of the three strains varied significantly between the seasons, with higher values (LSD test, $p<0.05$) during the SEM (67.3 mPa s) than during the NEM (46.3 mPa s). The gel viscosities for the commercial κ- and ι-carrageenans (Sigma) were 61.4 and 54.3 mPa s, respectively.

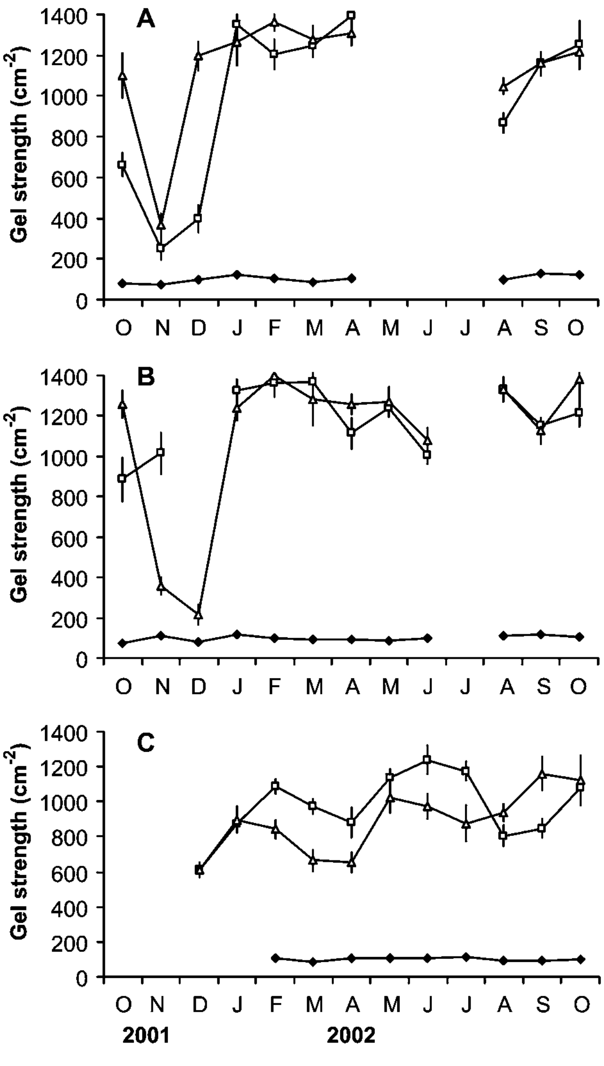

Figure 2 Mean monthly gel strength (g cm^{-2}) of brown *Eucheuma denticulatum* (prism), green (triangle) and brown (square) *Kappaphycus alvarezii* at three sites (A=Mkwiro; B=Kibuyuni; C=Gazi) in southern Kenya from October 2001 to October 2002. Mean and range (vertical line), n=2.

Table 1 Correlation coefficients of carrageenan yield (% algal dry wt) of three eucheumoid strains (brown *Eucheuma denticulatum*, brown and green *Kappaphycus alvarezii*) with environmental factors, seaweed parameters, and carrageenan quality factors in southern Kenya.

Factor	*E. denticulatum* (brown)	*K. alvarezii* (brown)	*K. alvarezii* (green)
Environmental factor			
Water temperature (°C)	0.078	0.038	0.055
Water motion (diffusion factor)	0.300	0.016	0.408*
Photon fluence rate (μmol photons m^{-2} s^{-1})	-0.270	-0.451	-0.242
Seaweed condition			
Relative growth rate (% d^{-1})	0.361*	0.203	0.067
% "Ice-ice" syndrome	-0.206	-0.251	0.239
Carrageenan quality factor			
Gel strength (g cm^{-2})	0.066	-0.207	-0.400*
Viscosity (mPa s)	0.107	-0.096	0.392*
Sulphate (% gel dry wt)	-0.173	0.047	-0.020

* Significant at $p<0.05$ (Pearson's product moment correlation).

Significant differences in monthly average sulphate contents of carrageenans were found in the three eucheumoid strains. Among the three strains, brown *Eucheuma denticulatum* had the highest sulphate content (29.1% dry wt), and the lowest mean value occurred in green *Kappaphycus alvarezii* (20.9% dry wt), with intermediate values in brown *K. alvarezii* (26.5% dry wt). However, the three strains showed similar sulphate contents among the three sites and between the two seasons.

Correlations

Table 1 shows the correlation coefficients of carrageenan yield (% algal dry wt) of three eucheumoids with environmental factors, seaweed parameters, and carrageenan quality parameters. Among the environmental factors, water motion showed a positive correlation with carrageenan yield from green *Kappaphycus alvarezii*. In brown *Eucheuma denticulatum*, the carrageenan yield was positively correlated with relative growth rate ($p<0.05$, Table 1). In green *K. alvarezii*, the carrageenan yields were inversely correlated with gel strengths, and positively correlated with viscosities ($p<0.05$, Table 1).

Table 2 presents the correlation coefficients between gel strengths of carrageenans of three eucheumoid strains and environmental factors, seaweed parameters, and carrageenan quality factors. In both the green and brown strains of *Kappaphycus alvarezii*, the gel strengths were positively correlated with photon fluence rate. The gel strengths of brown *K. alvarezii* showed an inverse correlation with both the relative growth rates and percent "ice-ice" syndrome ($p<0.05$, Table 2). In the three strains, the viscosities were positively correlated with gel strengths ($p<0.01$, Table 2). The gel viscosity values of both brown *Eucheuma denticulatum* and green *K. alvarezii* were inversely correlated with water temperatures ($r=-0.365$ and $r=-0.441$, $p<0.05$, respectively). However, the carrageenan gel strengths from the three eucheumoids showed no significant correlations with the sulphate contents (Table 2).

Discussion

Carrageenan yield

The production of carrageenans by eucheumoids varies with species. A wide range of carrageenan yields from *Eucheuma* and *Kappaphycus* species is reported by several authors (Dawes et al. 1977, Doty 1987, Azanza-Corrales and Sa-a 1990, Muñoz et al. 2004). In the present study, the average monthly carrageenan yields from the three eucheumoids varied from about 49 to 63% dry wt. These yields were higher than those obtained for *Kap-*

Table 2 Correlation coefficients of gel strength (g cm^{-2}) of carrageenans of three eucheumoid strains (brown *Eucheuma denticulatum*, brown and green *Kappaphycus alvarezii*) with environmental factors, seaweed parameters, and carrageenan quality factors in southern Kenya.

Factor	*E. denticulatum* (brown)	*K. alvarezii* (brown)	*K. alvarezii* (green)
Environmental factor			
Water temperature (°C)	-0.196	-0.195	-0.284
Water motion (diffusion factor)	0.107	0.041	0.282
Photon fluence rate (μmol photons m^{-2} s^{-1})	-0.142	0.606*	0.518*
Seaweed condition			
Relative growth rate (% d^{-1})	-0.145	-0.440*	-0.221
% "Ice-ice" syndrome	0.076	-0.368*	0.141
Carrageenan quality factor			
Yield (% algal dry wt)	0.066	-0.207	-0.400*
Viscosity (mPa s)	0.873**	0.673**	0.626**
Sulphate (% gel dry wt)	-0.092	-0.199	-0.031

* Significant at $p<0.05$. ** Significant at $p<0.01$ (Pearson's product moment correlation).

paphycus alvarezii (8 to 12%) in the Philippines (Hurtado-Ponce 1995), but lower than 47–80% in Floridian *Eucheuma* species (Dawes et al. 1977). Muñoz et al. (2004) obtained 30–41% carrageenan yield for *K. alvarezii* in Mexico. The carrageenan yields obtained in the present work were similar to gel values for eucheumoids grown in the Philippines (Trono and Lluisma 1992), Vietnam (Ohno et al. 1996), China (Li et al. 1990) and Brazil (Paula et al. 1999). Buriyo et al. (2001) reported a range of 44–59% of carrageenan yields from natural stocks of *Eucheuma denticulatum* in Tanzania.

The production of carrageenans by eucheumoids varies by strain as well as with other factors. In the current study, the green strain of *Kappaphycus alvarezii* had a higher carrageenan yield than the brown *K. alvarezii*, as observed similarly by Trono and Lluisma (1992). Conversely, Hurtado-Ponce (1995) obtained higher carrageenan yields for brown morphotypes of *K. alvarezii* than the green morphotypes in the Philippines, whereas Muñoz et al. (2004) found no significant differences among red, green and brown morphotypes of *K. alvarezii* in Mexico.

Here, we show significant differences in carrageenan yields among the sites, with the highest gel yields observed in Gazi thalli. The phycocolloid yields in seaweeds have been reported to vary by site (Wang and Yang 1980, Ohno et al. 1996). Wang and Yang (1980) reported that the day length in various locations was considered to affect the agar yields extracted from *Gracilaria* species cultivated in Taiwanese ponds. In Vietnam, Ohno et al. (1996) obtained higher carrageenan yield for *Kappaphycus alvarezii* thalli grown in offshore waters (53.2%) than those in lagoons (51.9%) and ponds (47.1%). Although no data on water motion were provided, these authors reported that the offshore cultivation area had a strong water current. In our study, water motion (diffusion factors) measured for Gazi (6.16) were higher than those for Mkwiro (5.54) and Kibuyuni (4.72) (Wakibia 2005) and consequently, the thalli at Gazi could have adapted to the strong water environment by synthesising additional structural cell wall polysaccharides. Phycocolloids are structural cell wall polysaccharides that provide flexible structural support in response to water currents and wave action in seaweeds (Kloareg and Quatrano 1988). However, strong water currents at Gazi could also have provided sufficient carbon and nitrogen to support high relative growth rates and high carrageenan yields.

In the present investigation, high carrageenan yield from the three eucheumoids occurred towards the end of the southeast monsoon (September), corresponding to the period of high relative growth rate (6.0% day^{-1}) (Wakibia et al. 2006). Similarly, increased phycocolloid content with increasing growth rates has been obtained in eucheumoids elsewhere (Li et al. 1990, Trono and Lluisma 1992). Conversely, some authors have reported an inverse relationship between phycocolloid yields and growth rates in carrageenophytes (Dawes et al. 1974, Guist et al. 1982). It has been suggested that during the active growth of algae, sufficient nitrogen levels promote the synthesis of proteins and protoplasmic constituents at the expense of deposition of cell wall materials, including phycocolloids (Fogg 1964, Dawes et al. 1974). However, reduced phycocolloid content may also be due to carbon limitation, as thalli in most culture systems grow under suboptimal inorganic carbon availability (Moseley 1990). Thus, if both nitrogen and carbon are in sufficient supply, it may be possible to produce seaweeds with both high growth and phycocolloid levels, as was observed in the field culture of *Kappaphycus alvarezii* by Li et al. (1990) and in this study at the Gazi site. In Tanzania, Buriyo et al. (2001) obtained high carrageenan yield and high biomass from *Eucheuma denticulatum* during the southeast monsoon.

Carrageenan quality

Kappaphycus produces kappa carrageenan while *Eucheuma* produces iota carrageenan. Gel strength is one of the important indices of carrageenan quality. *Kappaphycus* and *Eucheuma* species generally produce carrageenans with gel strengths of about 1000 g cm^{-2} and 100 g cm^{-2}, respectively (Santos 1989). The gel strengths obtained in the present study are within these ranges but a little lower than those reported for *Kappaphycus alvarezii* grown in Japanese (Ohno et al. 1994) and Vietnamese waters (Ohno et al. 1996). Higher gel strengths were obtained in the present study than those reported for eucheumoids in China (Li et al. 1990), Tanzania (Buriyo et al. 2001) and the Philippines (Azanza-Corrales and Sa-a 1990, Hurtado-Ponce 1995). The higher gel strengths obtained in the present study may be attributed to the strong alkali used (20% of the weight of seaweed) and the long duration of extraction.

The gel strengths for the eucheumoid strains showed variation among the sites, with the highest values observed in plants at Kibuyuni and those at Gazi having the lowest values. This difference may be due to the site characteristics such as the photon fluence rate. The photon fluence rate was higher at Kibuyuni (1254 μmol photons m^{-2} s^{-1}) than at Gazi (1042 μmol photons m^{-2} s^{-1}) (Wakibia et al. 2006). In this study, in both brown and green strains of *Kappaphycus alvarezii*, the gel strengths were positively correlated to photon fluence rate. Wang and Yang (1980) also observed that location and sunlight affected gel strengths of agars from *Gracilaria* species cultivated in Taiwan, with areas experiencing more sunlight producing gels with high gel strengths.

High contents of sulphate are usually associated with weak gels. The formation of gels is reported to involve a coil-helix transition of the gel molecules, followed by aggregation and network formation (Morris et al. 1980). According to Rees (1969), sulphates in the cell wall polysaccharide cause kinks in the helical structure responsible for gel formation resulting in phycocolloids of lower gel strength. An inverse relationship was observed between carrageenan sulphate content and gel strength for morphotypes of *Kappaphycus alvarezii* (Hurtado-Ponce 1995). On the contrary, no significant relationship was observed between sulphate content and gel strength in our investigation, as was also observed by Azanza-Corrales and Sa-a (1990) for *Eucheuma* and *Kappaphycus* strains. Similarly, Mouradi-Givernaud et al. (1992) did not find any significant correlation between sulphate and gel strength in agar of *Gelidium spinosum* (S.G. Gmeli) P.C. Silva [as *Gelidium latifolium* (Greville) Bornet *et* Thuret].

It appears rather that factors other than sulphate content probably play a role in gel formation, as was evident in this study where a significant difference in sulphate contents between the brown and green *K. alvarezii* did not result in different gel strengths. The lack of correlation between sulphate content and gel strength is probably due to the complex nature of the cell wall polysaccharides. It has been suggested that the length of phycocolloid molecules may be related to the gel strength, with longer chains interacting with each other to form gels of higher strengths, and the reverse for shorter chains (Mouradi-Givernaud et al. 1992, Mendoza et al. 2002). A high content of 3,6-anhydrogalactose (not determined in this study) is also associated with strong gels (Anderson et al. 1968).

In this work, positive correlations were observed between gel strengths and viscosities in the three eucheumoids. Both the carrageenan from *Gymnogongrus griffithsiae* (Turner) Martius (Breden and Bird 1994) and the agar from *Gelidium spinosum* (Mouradi-Givernaud et al. 1992) showed similar patterns. In contrast to these observations, an inverse relationship between gel strength and viscosity was observed for three members of the family Cystocloniaceae (Cosson et al. 1990) and *Gigartina teedei* (Roth) Lamouroux (Zinoun et al. 1993). The difference in the relationships may be due to the type of carrageenans and their molecular weights, among other factors (Stanley 1990). Mendoza et al. (2002) reported decreased gel strength and viscosity of "ice-ice" infected carrageenans and attributed the low values to a decrease in the molecular weight of the gel (from about 700 kDa to 32 kDa). In this investigation, similar low gel strengths were observed at Kibuyuni during the November–December period when 15% of the plants were infected with "ice-ice" syndrome. A significant negative relationship ($r=-0.611$, $p<0.05$) was obtained between viscosity and the "ice-ice" syndrome (Wakibia et al. 2006).

Gel viscosities were also inversely correlated with water temperature, and were higher during the southeast monsoon than during the northeast monsoon. These results corroborated findings of Braud and Perez (1978), which indicated higher viscosity of carrageenan of *Eucheuma denticulatum* during the cooler season in Djibouti waters.

The gel strength and viscosity of phycocolloids depend on the method of extraction, measurement device, and concentration of the gels, as well as growth conditions and other factors (Levy et al. 1990, Stanley 1990). For reference purposes, the quality of gel extract should be compared to a standard commercial gel (Levy et al. 1990). The gel strengths and viscosities of carrageenans extracted from the three strains in the present study were comparable to the Sigma commercial carrageenans. All three carrageenan extracts had sulphate levels which fit the US Food and Drug Administration purity standard of 20–40% (dry wt) and meet the industrial requirements of minimum carrageenan yield and viscosity value of 39% (dry wt) and 5 mPa s, respectively (Bixler 1996).

From the carrageenan data presented here, eucheumoid cultivation for producing good quality material appears feasible in Kenya, but attention should be given to site selection particularly if high quality carrageenans are required. However, information on the ecological impacts of eucheumoid cultivation, including the introduction of non-indigenous species, conflicts over water space, and marketing is needed before commercial farms are established in Kenya.

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References

Anderson, N.S., T.C.S. Dolan, A. Penman, D.A. Rees, G.P. Mueller, D.J. Stancioff and N.F. Stanley. 1968. Carrageenans. Part IV. Variations in the structure and gel properties of κ-carrageenan, and the characterisation of sulphate esters by infrared spectroscopy. *J. Chem. Soc. (C)*: 602–606.

Ask, E.I., A. Batibasaga, J.A. Zertuche-González and M. de San. 2003. Three decades of *Kappaphycus alvarezii* (Rhodophyta) introduction to non-endemic locations. *Proc. Int. Seaweed Symp. 17*: 49–57.

Azanza-Corrales, R. and P. Sa-a. 1990. The farmed *Eucheuma* species (Gigartinales, Rhodophyta) in Danajon Reef, Philippines: carrageenan properties. *Hydrobiologia 204/205*: 521–525.

Bixler, H.J. 1996. Recent developments in manufacturing and marketing carrageenan. *Hydrobiologia 326/327*: 35–57.

Braud, J.P. and R. Perez. 1978. Farming on pilot scale of *Eucheuma spinosum* (Florideophyceae) in Djibouti waters. *Proc. Int. Seaweed Symp. 9*: 533–539.

Breden, P.C. and K.T. Bird. 1994. Effects of environmental factors on carrageenan from *Gymnogongrus griffithsiae* (Gigartinales, Rhodophyta). *J. Appl. Phycol. 6*: 371–380.

Buriyo, A.S., A.K. Semesi and M.S.P. Mtolera. 2001. The effect of seasons on yield and quality of carrageenan from Tanzanian red alga *Eucheuma denticulatum* (Gigartinales, Rhodophyta). *S. Afr. J. Bot. 67*: 488–491.

Cosson, J., E. Deslandes and J.P. Braud. 1990. Preliminary approach to the characterization and seasonal variation of carrageenans from four Rhodophyceae on the Normandy coast (France). *Hydrobiologia 204/205*: 539–544.

Craigie, J.S. 1990. Cell walls. *In*: (K.M. Cole and R.G. Sheath, eds) *Biology of the red algae*. Cambridge University Press, Cambridge. pp. 221–257.

Dawes, C.J., J.M. Lawrence, D.P. Cheney and A.C. Mathieson. 1974. Ecological studies of Floridian *Eucheuma* (Rhodophyta, Gigartinales). III. Seasonal variation of carrageenan, total carbohydrate, protein and lipid. *Bull. Mar. Sci. 24*: 286–299.

Dawes, C.J., N.F. Stanley and D.J. Stancioff. 1977. Seasonal and reproductive aspects of plant chemistry and ι-carrageenan from Floridian *Eucheuma* (Rhodophyta, Gigartinales). *Bot. Mar. 20*: 137–147.

Doty, M.S. 1971. Measurement of water movement in reference to benthic algal growth. *Bot. Mar. 14*: 32–35.

Doty, M.S. 1987. The production and use of *Eucheuma*. FAO Fisheries Technical Paper 28. pp. 123–164.

Evans, G.C. 1972. *The quantitative analysis of plant growth*. Blackwell, Oxford. pp. 734.

Fogg, G.E. 1964. Environmental conditions and the pattern of metabolism in algae. *In*: (D. Jackson, ed.) *Algae and man*. Plenum Press, New York. pp. 77–85.

Guist, G.C., C.J. Dawes and J.R. Castle. 1982. Mariculture of the red seaweed *Hypnea musciformis*. *Aquaculture 28*: 375–384.

Hurtado-Ponce, A.Q. 1995. Carrageenan properties and proximate composition of three morphotypes of *Kappaphycus alvarezii* Doty (Gigartinales, Rhodophyta) grown at two depths. *Bot. Mar. 38*: 215–219.

Kloareg, B. and R.S. Quatrano. 1988. Structure of the cell walls of marine algae and ecophysiological functions of the matrix polysaccharides. *Oceanogr. Mar. Biol. Annu. Rev. 26*: 259–315.

Levy, I., S. Beer and M. Friedlander. 1990. Growth, photosynthesis and agar in wild-type strains of *Gracilaria verrucosa* and *G. conferta* (Gracilariales, Rhodophyta), as a strain selection experiment. *Hydrobiologia 204/205*: 381–387.

Li, R., J.J. Li and C.Y. Wu. 1990. Effect of ammonium on growth and carrageenan content in *Kappaphycus alvarezii* (Gigartinales, Rhodophyta). *Hydrobiologia 204/205*: 499–503.

McClanahan, T.R. 1988. Seasonality in East Africa's coastal waters. *Mar. Ecol. Prog. Ser. 44*: 191–199.

McHugh, D.J. 2003. *A guide to the seaweed industry*. FAO Fisheries Technical Paper 441. pp. 105.

Mendoza, W.G., N.E. Montaño, E.T. Ganzon-Fortes and R.D. Villanueva. 2002. Chemical and gelling profile of *ice-ice* infected carrageenan from *Kappaphycus striatum* (Schmitz) Doty "sacol" strain (Solieriaceae, Gigartinaceae, Rhodophyta). *J. Appl. Phycol. 14*: 409–418.

Mollion, J. and J.P. Braud. 1993. A *Eucheuma* (Solieriaceae, Rhodophyta) cultivation test on the south-west coast of Madagascar. *Hydrobiologia 260/261*: 373–378.

Morris, E.R., D.A. Rees and G. Robinson. 1980. Cation-specific aggregation of carrageenan helices: domain model of polymer gel structure. *J. Mol. Biol. 138*: 349–362.

Moseley, C.M. 1990. The effect of cultivation conditions on the yield and quality of carrageenans in *Chondrus crispus*. *In*: (I. Akatsuka, ed.) *Introduction to applied phycology*. SPB Academic Publishing, The Hague. pp. 565–574.

Mouradi-Givernaud, A., T. Givernaud, H. Morvan and J. Cosson. 1992. Agar from *Gelidium latifolium* (Rhodophyceae, Gelidiales): Biochemical composition and seasonal variations. *Bot. Mar. 35*: 153–159.

Muñoz, J., Y. Freile-Pelegrín and D. Robledo. 2004. Mariculture of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) color strains in tropical waters of Yucatán, México. *Aquaculture 239*: 161–177.

Ohno, M., D.B. Largo and T. Ikumoto. 1994. Growth rate, carrageenan yield and gel properties of cultured kappa-carrageenan producing red alga *Kappaphycus alvarezii* (Doty) Doty in the subtropical waters of Shikoku, Japan. *J. Appl. Phycol. 6*: 1–5.

Ohno, M., H.Q. Nang and S. Hirase. 1996. Cultivation and carrageenan yield and quality of *Kappaphycus alvarezii* in the waters of Vietnam. *J. Appl. Phycol. 8*: 431–437.

Paula, E.J., R.T.L. Pereira and M. Ohno. 1999. Strain selection in *Kappaphycus alvarezii* var. *alvarezii* (Solieriaceae, Rhodophyta) using tetraspore progeny. *J. Appl. Phycol. 11*: 111–121.

Rees, D.A. 1969. Structure, conformation, and mechanism in the formation of polysaccharide gels and networks. *Adv. in Carbohyd. Chem. Biochem. 24*: 267–332.

Santos, G.A. 1989. Carrageenans of species of *Eucheuma* J. Agardh and *Kappaphycus* Doty (Solieriaceae, Rhodophyta). *Aquat. Bot. 36*: 55–67.

SAS. 1999. SAS/STAT user's guide, version 8.2, 1st printing, volume 2. SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513.

Stanley, N.F. 1990. Carrageenans. *In*: (P. Harris, ed.) *Food gels*. Elsevier Applied Science, London. pp. 79–119.

Trono, G.C. and A.O. Lluisma. 1992. Differences in biomass production and carrageenan yields among four strains of farmed carrageenophytes in Northern Bohol, Philippines. *Hydrobiologia 247*: 223–227.

Wakibia, J.G. 2005. *Linking biology and sustainable livelihoods to the proposed establishment of community-based eucheumoid farming in southern Kenya.* Ph.D. thesis, University of the Western Cape, South Africa. pp. 196.

Wakibia, J.G., J.J. Bolton, D.W. Keats and L.M. Raitt. 2006. Factors influencing the growth rates of three commercial eucheumoids at coastal sites in southern Kenya. *J. Appl. Phycol.*: in press.

Wang, C.Y and S.S. Yang. 1980. Seasonal variation of the quality of *Gracilaria* cultivated in Taiwan. *Proc. Natl. Sci. Counc. ROC 4*: 78–86.

Zinoun, M., J. Cosson and E. Deslandes. 1993. Influence of culture conditions on growth and physicochemical properties of carrageenans in *Gigartina teedii* (Rhodophyceae-Gigartinales). *Bot. Mar. 36*: 131–136.

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