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A comparative analysis of phycocolloids produced by underutilized versus industrially utilized carrageenophytes (Gigartinales, Rhodophyta)

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Abstract Carrageenan (E-407) and semi-refined carrageenan (E-407a) are some of the main additives used by the food industry for their gelling, emulsifying, thickening, and stabilizing properties. These are natural ingredients, which have been used for decades in food applications and are generally regarded as safe. Internationally, sub-tropical carrageenophytes (e.g., *Kappaphycus alvarezii*) are cultivated extensively as a source of raw materials for industrial extraction, and their use as potential candidates in integrated multitrophic aquaculture is tentative. We analyzed carrageenan yield (as a percentage of dry weight) and chemical composition (by Fourier transform infrared attenuated total reflection and Fourier transform-Raman) of extracts produced by several carrageenophytes (Gigartinales, Rhodophyta), from different origins, e.g., *K. alvarezii* (Tanzania, Indonesia, the Philippines, Panama, and Mexico), *Kappaphycus striatum* (Madagascar), *Eucheuma denticulatum* (Tanzania, the Philippines, and Madagascar), *Betaphycus gelatinum* (the Philippines), *Chondracanthus chamissoi*, and *Sarcothalia crispata* (Chile). For comparison, some underutilized carrageenophytes were also analyzed, e.g., *Chondrus crispus*, *Mastocarpus stellatus, Gigartina pistillata*, *Chondracanthus teedei* var. *lusitanicus*, *Chondracanthus acicularis*, *Calliblepharis jubata*, *Gymnogongrus crenulatus*, and *Ahnfeltiopsis devoniensis* (Portuguese carrageenophytes). The main findings were that the highest carrageenan yield was obtained from *K. striatum* (Madagascar) with 75.6 (percent dry weight (% DW)); *B. gelatinum* and *K. alvarezii* (both from the Philippines) had yields of 71.0% and 68.0% (% DW), respectively; and *G. pistillata* (Portugal) 65.4% (% DW). Spectroscopic analysis of the phycocolloids allowed determination of a wide range of carrageenan types, e.g., pure iota carrageenan, several kappa–iota hybrid carrageenans with different iota/kappa ratios, and kappa–beta, xi–theta, and xi–lambda hybrid carrageenans.

Keywords Seaweed · Cultivation · Phycocolloids · Carrageenan · FTIR-ATR · FT-Raman

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Introduction

Carrageenans are the third most important hydrocolloid in the food industry, after gelatin (animal origin) and starch (van de Velde and de Ruiter 2002). The main commonly used, commercial carrageenans are extracted from *Kappaphycus alvarezii* and *Eucheuma denticulatum* (McHugh 2003).

Carrageenan processors have fuelled the development of *K. alvarezii* ("cottonii" to the trade) and *E. denticulatum* ("spinosum" to the trade) farming in several countries including the Philippines, Indonesia, Malaysia, Tanzania, Kiribati, Fiji, Kenya, and Madagascar; total market volume now exceeds 140,000 tonnes per annum of commercially dried biomass with a value of over $70 million (Guiry 2008). Primarily, wild-harvested genera such as *Chondrus*, *Furcellaria*, *Gigartina*, *Chondracanthus*, *Sarcothalia*, *Mazzaella*, *Iridaea*, *Mastocarpus*, and *Tichocarpus* are also mainly cultivated as carrageenan raw materials and producing countries include Argentina, Canada, Chile, Denmark, France, Japan, Mexico, Morocco, Portugal, North Korea, South Korea, Spain, Russia, and the USA. According to Guiry (2008), carrageenan production exceeded 50,000 tonnes in 2007/2008 with a value of over US$600 million (not including China).

Shortages of carrageenan-producing seaweeds suddenly appeared in mid-2007, resulting in doubling of the price of carrageenan; some of this price increase was due to increased fuel costs and a weak US dollar (most seaweed polysaccharides are traded in US dollars). The reason for weed shortages are less certain: perhaps it is a combination of environmental factors, sudden increases in demand, particularly from China, and some market manipulation by farmers and traders (Guiry 2008).

In the last months of 2008, the tight supply of kappa carrageenan persists. Prices have reached an all-time high and long delivery delays are frequent. Most hydrocolloids are experiencing severe price movements. Turmoil in global financial markets may even have an impact on some pending hydrocolloid corporate transactions (IMR 2008).

The original source of carrageenans was from the red seaweed *Chondrus crispus*, which continues to be used, but in limited quantities. *Betaphycus gelatinum* is used for the extraction of beta (β) carrageenan. Some South American red algae used previously only in minor quantities have, more recently, received attention from carrageenan producers, as they seek to increase diversification of raw materials in order to provide for the extraction of new carrageenan types with different physical functionalities and therefore increased product development, which in turn stimulates demand (McHugh 2003). *Gigartina skottsbergii*, *Sarcothalia crispata*, and *Mazzaella laminaroides* are currently the most valuable species and all are harvested from natural populations in Chile and Peru. Small quantities of *Gigartina canaliculata* are harvested in Mexico and *Hypnea musciformis* has been used in Brazil (Furtado 1999). The use of high value carrageenophytes as a dissolved organic nutrient sink to boost economic viability of integrated multitrophic aquaculture (IMTA) operations has been considered (Pereira 2004; Chopin and Robinson 2006).

Materials and methods

Algal material

All of the algae studied belong to the order Gigartinales, a taxonomic group widely represented on the North Atlantic shores. In addition to the carrageenophyte species with current industrial interest, we also analyzed representative species of the Iberian Peninsula marine flora. The origin of each underutilized and commercially used carrageenophyte sample and information on the family, species, life cycle, and harvest season are respectively presented in Tables 1 and 2. Commercially used carrageenophyte samples were provided by the Raw Material Division, Cargill Texturing Solution, based in Baupte, France.

For the representative species from the Iberian Peninsula, 100 plants larger than 3 cm in length were collected in Buarcos (Portugal). The algal material was divided into three lots following examination under a stereomicroscope: tetrasporophytes, female gametophytes bearing cystocarps, and non-fructified thalli.

Preparation of ground seaweed samples for FTIR-ATR and FT-Raman

Seaweed samples were rinsed in distilled freshwater to remove salt and debris and oven-dried to a constant weight at 60°C. The dried seaweeds were finely ground (50 mesh) to render the samples uniform. For Fourier transform infrared (FTIR) analysis, the samples required no further treatment. FT-Raman required depigmentation which was achieved by sun drying (a similar process used by collectors/producers of commercial seaweeds) or by pigment removal in the laboratory by the addition of a calcium hypochlorite solution (4%, 30 to 60 s, 4°C), according to Pereira (2006) and Pereira et al. (2009).

Phycocolloid extraction

Prior to phycocolloid extraction, the ground, dry material was rehydrated and pre-treated with methanol 100% and acetone 100% to eliminate the organosoluble fraction (Zinoun and Cosson 1996).

For the extraction of the "native" phycocolloid, the seaweed samples were placed in distilled water (50 mL g^{-1}), pH 7 at 85°C for 3 h. For an alkaline extraction (closely resembling the industrial extraction), the samples were placed in a solution (150 mL g^{-1}) of NaOH (1 M) at 80–85°C for 3–4 h and neutralized to pH 6–8 with HCl (0.3 M), according to Pereira (2006).

The solutions were hot filtered twice under vacuum by using a cloth and glass fiber filter. The extract was evaporated under vacuum to one-third of the initial volume.

Table 1 Underutilized carrageenophytes: carrageenan composition determined by vibrational spectroscopy (FTIR-ATR and FT-Raman)

Family	Species	Life cycle phase	Harvest season	Origin	Carrageenan			
					Yield	Alkali-extracted	Iota/kappa ratio	Native[a]
Cystocloniaceae	*Calliblepharis jubata*	NF	Late winter	Buarcos (Portugal)	10.8	Iota–kappa	0.80	Iota–kappa (nu)
	C. jubata	T	Late spring	Buarcos (Portugal)	28.4	Iota–kappa	0.84	Iota–kappa (nu)
	C. jubata	FG	Spring	Buarcos (Portugal)	24.2	Iota	0.90	Iota (nu)
Gigartinaceae	*Chondracanthus acicularis*	T	Summer	Buarcos (Portugal)	36.6	Xi–theta	–	Xi–theta
	Chondracanthus teedei var. *lusitanicus*	NF	Late spring	Buarcos (Portugal)	35.0	Kappa–iota	0.72	Kappa–iota (mu/nu)
	C. teedei var. *lusitanicus*	T	Late spring	Buarcos (Portugal)	36.6	Xi–theta	–	Xi–theta
	C. teedei var. *lusitanicus*	FG	Late spring	Buarcos (Portugal)	–	–	0.69	Kappa–iota (mu/nu)
	C. teedei var. *lusitanicus*	FG	Late spring	Buarcos (Portugal)	43.6	Kappa–iota	0.83	–
	Chondrus crispus	NF	Winter	Buarcos (Portugal)	14.0	Kappa–iota	0.59	Kappa–iota (mu)
	C. crispus	NF	Spring	Buarcos (Portugal)	29.1	–	–	Kappa–iota (mu)
	C. crispus	T	Spring	Buarcos (Portugal)	36.6	Lambda	–	Lambda
	C. crispus	FG	Late winter	Buarcos (Portugal)	23.2	Kappa–iota	0.53	Kappa–iota (mu)
	C. crispus	FG	Late spring	Buarcos (Portugal)	35.0	–	–	Kappa–iota (mu)
	Gigartina pistillata	NF	Late spring	Viana do Castelo (Portugal)	65.4	Kappa–iota	0.69	Kappa–iota (mu/nu)
	G. pistillata	T	Spring	Buarcos (Portugal)	55.6	Xi–lambda	–	–
	G. pistillata	H (⊕)	Late winter	Buarcos (Portugal)	58.5	–	–	Xi–lambda
	G. pistillata	FG	Spring	Buarcos (Portugal)	49.8	Kappa–iota	0.69	Kappa–iota (mu/nu)
	G. pistillata	H (♀)	Summer	Buarcos (Portugal)	58.5	–	–	Kappa–iota (mu/nu)
Petrocelidaceae	*Mastocarpus stellatus*	G	Winter	Buarcos (Portugal)	19.4	–	–	kappa–iota (mu/nu)
	M. stellatus	G	Summer	Buarcos (Portugal)	20.3	Kappa–iota	0.64	Kappa–iota (mu/nu)
Phyllophoraceae	*Ahnfeltiopsis devoniensis*	G	Summer	Buarcos(Portugal)	13.6	Iota–kappa	0.71	Iota–kappa (nu)
	A. devoniensis	G	Winter	Buarcos (Portugal)	11.5	Iota–kappa	0.73	–
	A. devoniensis	NF	Summer	Buarcos (Portugal)	11.5	Iota–kappa	0.76	Iota–kappa (nu)
	Gymnogongrus crenulatus	TB	Spring	Buarcos (Portugal)	9.7	Kappa–iota	0.73	–
	G. crenulatus	TB	Autumn	Buarcos (Portugal)	11.0	Iota–kappa	0.88	–

Yield expressed as percentage of dry weight

T tetrasporophyte, *FG* female gametophyte, *G* gametophyte, *NF* non-fructified thalli, *TB* tetrasporoblastic thalli, *H* heterosporic thalli: (♀ branch; ⊕ branch)

[a] Composition determined by FTIR-ATR and FT-Raman analysis of ground seaweed samples; the carrageenans are identified according to the Greek lettering system; the letters between parentheses ( ) correspond to the biological precursors of the carrageenans, present in native samples (or ground seaweed)

The carrageenan was precipitated by adding the warm solution to twice its volume of ethanol (96%). The coagula were dried in an oven for 48 h at 60°C, and then weighed to determine the carrageenan content (percentage of dry weight) (Pereira and Mesquita 2004). Each sample was extracted in quadruplicate for carrageenan yield determinations.

FTIR-ATR and FT-Raman analysis

The FTIR spectra from the samples (ground dried seaweed, native, and alkali-modified carrageenan) were measured on an IFS 55 spectrometer, using a Specac Golden Gate MKII single reflection diamond ATR system. All spectra presented are the average of two repeated measurements on the same sample with 128 scans each at a resolution of 2 cm^{-1}.

The corresponding FT-Raman spectra were recorded on a RFS-100 Bruker FT-spectrometer using an Nd/YAG laser with an excitation wavelength of 1,064 nm. Each spectrum was the average of two repeated measurements on the same sample with 150 scans at a resolution of 2 cm^{-1} (Pereira et al. 2003).

The ratio between 805 and 845 cm^{-1} absorption bands in Fourier transform infrared attenuated total reflection (FTIR-ATR) spectra was calculated (Correa-Díaz et al. 1990) and used as a parameter to determine the degree of the iota/kappa hybridization (Fig. 1). The ratio between 845 and

Table 2 Carrageenophytes used industrially: carrageenan composition determined by vibrational spectroscopy (FTIR-ATR and FT-Raman)

Family	Species	Life cycle phase	Harvest season	Origin	Carrageenan			
					Yield	Alkali-extracted	Iota/kappa ratio	Native[a]
Gigartinaceae	*Chondracanthus chamissoi*	NF	Summer	Chile (W)	13.5	Kappa/iota	0.77	Kappa/iota (mu/nu)
	C. chamissoi	T	Late spring	Chile (W)	24.6	Xi/theta	–	Xi/theta
	C. chamissoi	FG	Summer	Chile (W)	14.2	Kappa/iota	0.79	Kappa/iota (mu/nu)
	Chondrus crispus	G + T	Late spring	Canada (W)	33.8	Kappa/iota	–	Kappa/iota (mu/nu)
						Lambda		Lambda/alpha
	Sarcothalia crispata	NF	Late winter	Chile (W)	14.6	Kappa/iota	0.81	Kappa/iota (mu/nu)
	S. crispata	NF	Spring	Chile (W)	16.7	Kappa/iota	0.79	Kappa/iota (mu/nu)
	S. crispata	FG	Late winter	Chile (W)	5.4	Kappa/iota	0.81	Kappa/iota (mu/nu)
Petrocelidaceae	*Mastocarpus papillatus*	G	Winter	Chile (W)	5.4	Kappa/iota	0.83	Kappa/iota (mu/nu)
Solieriaceae	*Betaphycus gelatinum*	–	June–October	Philippines (W)	71.0	Kappa/beta	1.004[b]	Kappa/beta (mu/gamma)
	Eucheuma denticulatum	–	October–February	Philippines (F)	39.7	Iota	0.92	Iota (nu)
	E. denticulatum	–	Late spring	Madagascar (F)	35.3	Iota	0.93	Iota (nu)
	E. denticulatum	–	Spring	Tanzania (F)	31.5	Iota/kappa	0.88	Iota/kappa (nu)
	Eucheuma isiforme	–	Late summer	Colombia (F)	20.4	Kappa/iota	0.71	Kappa/iota (mu)
	Kappaphycus alvarezii	–	October–February	Indonesia (F)	20.0	Kappa/iota	0.64	Kappa/iota (mu)
	K. alvarezii (Ph)	–	October–February	Philippines (F)	30.4	Kappa/iota	0.72	Kappa/iota (mu)
	K. alvarezii	–	June–October	Philippines (F)	68.0	Kappa/iota	0.70	Kappa/iota (mu)
	K. alvarezii (Tz)	–	Winter	Tanzania (F)	18.7	Kappa/iota	0.69	Kappa/iota (mu)
	K. alvarezii (M1)	–	2 weeks[c]	Mexico (C)	58.1	Kappa/iota	0.80	Kappa/iota (mu/nu)
	K. alvarezii (M2)	–	4 weeks[c]	Mexico (C)	60.2	Kappa/iota	0.75	Kappa/iota (mu)
	K. alvarezii (M3)	–	6 weeks[c]	Mexico (C)	62.4	Kappa/iota	0.76	Kappa/iota (mu)
	K. alvarezii (M4)	–	8 weeks[c]	Mexico (C)	48.0	Kappa/iota	0.80	Kappa/iota (mu/nu)
	K. alvarezii (P1)	–	2 weeks[c]	Panama (C)	–	–	0.59	Kappa/iota (mu)
	K. alvarezii (P2)	–	3 weeks[c]	Panama (C)	–	–	0.66	Kappa/iota (mu)
	K. alvarezii (P3)	–	4 weeks[c]	Panama (C)	–	–	0.65	Kappa/iota (mu)
	K. alvarezii (P4)	–	5 weeks[c]	Panama (C)	–	–	0.70	Kappa/iota (mu)
	K. alvarezii (P5)	–	6 weeks[c]	Panama (C)	–	–	0.67	Kappa/iota (mu)
	K. alvarezii (P6)	–	7 weeks[c]	Panama (C)	–	–	0.71	Kappa/iota (mu)
	K. alvarezii (P7)	–	8 weeks[c]	Panama (C)	–	–	0.60	Kappa/iota(mu)
	Kappaphycus striatum	–	Late spring	Madagascar (F)	75.6	Kappa/iota	0.66	Kappa/iota (mu)

Yield expressed as percentage of dry weight

Ph the Philippines, *Tz* Tanzania, *M* Mexico, *P* Panama, *C* experimental cultivation, *F* farmed, *W* wild, *T* tetrasporophyte, *FG* female gametophyte, *G* gametophyte, *NF* non-fructified thalli

[a] Composition determined by FTIR-ATR and FT-Raman analysis of native carrageenan or ground seaweed samples; the carrageenans are identified according to the Greek lettering system; the letters between parentheses ( ) correspond to the biological precursors of the carrageenans, present in native samples (or ground seaweed)

[b] The ratio between 845 and 890 cm^{-1} absorption bands in FTIR spectra was calculated and used as a parameter to determine the degree of the kappa/beta hybridization

[c] Carrageenophytes subjected to increasing duration of culture

890 cm^{-1} absorption bands in the FTIR spectra was calculated (Pereira et al. 2009) and used as a parameter to determine the degree of the kappa/beta hybridization (Table 2).

Results and discussion

Yield, type (alkali-extracted and native), and iota/kappa ratio of carrageenan produced are presented in Tables 1 and 2.

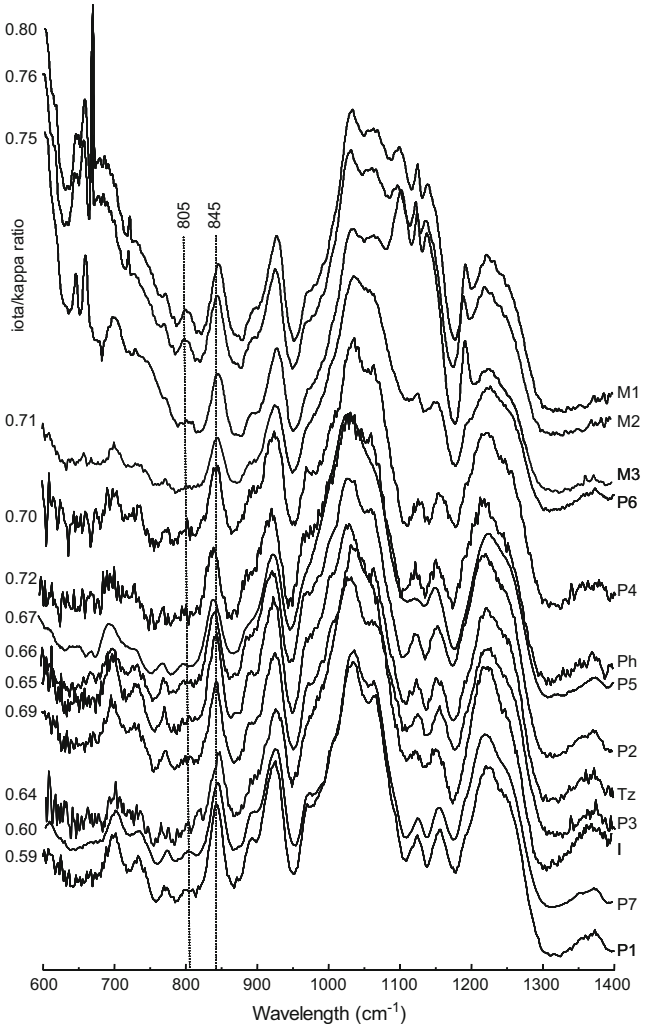

Fig. 1 FTIR-ATR spectra of *Kappaphycus alvarezii* samples: *M1–M3* ground seaweed samples (seaweed cultivated in Mexico, see Table 2), *P1–P7* native carrageenan extracted after increasing duration of seaweed culture in Panama (see Table 2), *I* ground seaweed samples from Indonesia, *Tz* ground seaweed sample from Tanzania, *Ph* ground seaweed sample from the Philippines

Spectroscopic analysis of the phycocolloids

The ATR-FTIR spectra (Fig. 1) of ground *K. alvarezii* (Solieriaceae), the main industrial source of kappa carrageenan, have strong absorption bands in the region of 930 cm^{-1} (CO of 3,6-anhydrogalactose) and in the 845 cm^{-1} region (CO-SO_4 in C4 of galactose), typical of the presence of kappa carrageenan. These spectra have reduced absorbance in the region 805 cm^{-1} (CO-SO_4 in C2 of anhydrogalactose), which indicates the presence of small quantities of iota carrageenan. The ratio iota/kappa ranged from 0.59 (P1) to 0.80 (M1), and these ratio variations seem related to the duration of the seaweed cultivation and the amount of precursor (mu) present at the time of harvest of *K. alvarezii* (Pereira 2004). The ratio iota/kappa ranged from 0.59 (P1) to 0.80 (M1), and these variations appeared to be related to the duration of the seaweed cultivation and the amount of precursor (mu) present at the time of harvest of *K. alvarezii* (Pereira 2004); however, a more detailed study of cultivation duration and presence of carrageenan forms is required to determine the nature of the correlation if one exists.

In relation to the nature of the phycocolloid, our spectroscopic analysis showed that the carrageenophytes studied seem to present a variation similar to that existing in other species of Cystocloniaceae, Gigartinaceae, Petrocelidaceae, Phyllophoraceae, and Solieriaceae (Chopin et al. 1999; Pereira 2004). The gametophyte and non-fructified stages of *Chondracanthus teedei* var. *lusitanicus*, *C. crispus*, *Gigartina pistillata*, *Ahnfeltiopsis devoniensis*, *Gymnogongrus crenulatus* (Table 1), *Chondracanthus chamissoi*, and *S. crispata* (Table 2) produce carrageenans of the kappa family (hybrid kappa/iota/mu/nu carrageenan). The tetrasporophyte stages produce carrageenans of the lambda family (hybrid xi/theta or xi/lambda carrageenan).

The gametophytes of the genus *Mastocarpus* produce hybrid kappa/iota/mu/nu carrageenan (Tables 1 and 2) and all stages of *Calliblepharis jubata* (Table 1) produce iota or iota–kappa hybrid carrageenan.

Those species of the family Solieriacae which were examined (Table 2) produce hybrid kappa/iota/mu/nu carrageenan in *Eucheuma isiforme*, *K. alvarezii*, and *K. striatum*; hybrid kappa/beta carrageenan in *B. gelatinum*; and predominantly iota carrageenan in *E. denticulatum*.

The alkali-extracted carrageenan from female gametophytes showed a lower sulfate content and a decrease in galactose to the benefit of 3,6-anhydrogalactose. This corresponds to the conversion of the 4-linked galactose-6-sulfate in native samples to anhydro-galactose in the alkali-extracted carrageenans. Thus, the biological precursor mu and nu carrageenan were converted into kappa and iota carrageenan, respectively (Pereira and Mesquita 2004). The increment in the ratio 805/845 in the alkali-extracted carrageenan samples, as compared to ground whole seaweed samples (or native carrageenans), corresponded to an increment of the iota fraction relative to kappa fraction: see *C. teedei* var. *lusitanicus* FG late spring in Table 1.

The combination of a high content of carrageenan (see Table 1) and its widespread presence on the north coast of Portugal and Galicia (Spain; Barbara and Cremades 1996) allows us to consider that *C. teedei* var. *lusitanicus* is a potential source of industrial co-polymers of kappa/iota and xi/theta carrageenan (see Table 1) in addition to the traditionally harvested carrageenophytes in the North West of the Iberian Peninsula (northern coast of Portugal and Galicia; Santos and Duarte 1991; Sousa-Pinto and Araújo 2006; Pereira and Mesquita 2003). Examples such as *G. pistillata* and *Chondracanthus acicularis* have a high

content of carrageenan (found not only as kappa/iota, but also as lambda, theta, and xi; see Table 1) and are sustainably harvested together with *C. teedei* in Morocco (Thierry Givernaud, pers comm. 2003).

At the present time, the commercial carrageenan industry is largely based on cultivated sub-tropical species (approximately 88.3%) from the major world producers in Indonesia and the Philippines. This may be viewed as being detrimental to cold water, wild species. However, commercial demand for carrageenophytes producing co-polymers of kappa/iota carrageenan has renewed the interest of collectors of raw materials and major companies of food additives for cold water carrageenophyte species (Bixler et al. 2001; Falshaw et al. 2003; Nunes et al. 2003). There is also a renewed interest in the cultivation (both intensive tank and extensive open-water operations) for cold water carrageenophytes, e.g., *C. crispus* (Chopin and Yarish 1998); however, the economics of scale and real operational costs of these endeavors need to be carefully scrutinized.

One of the most important factors responsible for the success of industrially used carrageenophytes is related to the mono-specificity of the colloids they produce, a feature that allows substantial savings for the extraction industry, e.g., *Kappaphycus* spp. (predominantly kappa carrageenan), *Eucheuma* spp. (predominantly iota carrageenan), and *Betaphycus* spp. (predominantly beta carrageenan). Unfortunately, responsible harvesting is not always the norm and non-sustainable harvesting can have severe economic and environmental impacts including total loss of valuable biomass (Pereira 2004; Pereira and Mesquita 2004).

With the increase in the number of published studies regarding both laboratory and industrial small-scale experiments for the culture of carrageenophytes, it is now possible to develop projects of co-culture of algae, shellfish, fish, and seafood (e.g., IMTA; Chopin et al. 2001; Neori et al. 2004; Neori 2007). The integrated culture of algae is an alternative option to the decline of traditional carrageenophyte harvest (Chopin 1998; Sousa-Pinto 1998), and it could provide sources of pure kappa and lambda-type carrageenans with high quality, instead of mixtures of kappa/lambda carrageenans, which is a result of collections from natural populations (Chopin et al. 1990). The IMTA concept would also provide for simultaneous and natural bioremediation of eutrophication resulting from intensive fish aquaculture. It may be necessary to educate end users on their potential negative attitudes to growing food grade products in "fish waste" (Pereira 2004; Chopin and Robinson 2006).

The comparative characteristics of carrageenophytes from a wide range of both used and potential sources (see Tables 1 and 2) indicate several candidates with potential for development of sustainable utilization of resources on the coast of northern Portugal for industrial utilization.

In conclusion, we noted that the *B. gelatinum* and *K. alvarezii* (both from the Philippines) and *K. striatum* (from Madagascar) were carrageenophytes with the highest yields which could even exceed 70% dry weight as carrageenan. Other species have values close to 30% carrageenan on a dry weight basis, i.e., *E. denticulatum* (samples from Madagascar, the Philippines, and Tanzania—producing predominantly iota carrageenan), *K. alvarezii* (from Indonesia—producing predominantly kappa carrageenan with an elevated iota content), and *C. crispus* (from Canada). The remaining carrageenophytes have relatively low yields; the values vary between 5.4% DW of *S. crispata* FG and *M. papillatus* FG (from Chile) and 24.6% DW for *C. chamissoi* (from Chile). Note that all alternative "competitors" form the presently "underutilized" carrageenophytes and have a similar or a lower yield in carrageenan, e.g., 33.8% DW for C. *crispus* (from Canada) and 35% DW for samples from Buarcos in spring, e.g., 24.6% DW for *C. chamissoi* NF in late spring and 16.7% DW for *S. crispata* NF in spring (from Chile) versus 43.6% DW from *C. teedei* var. *lusitanicus* in late spring (Buarcos).

Our analyses show variability in both carrageenan yield and ratio of kappa/iota fractions in different geographic commercial sources of sub-tropical carrageenophyte raw materials. Such differences may be related to the duration of culture and also site-specific, environmental conditions (see Fig. 1) as outlined by previous workers with *K. alvarezii* (Ohno et al. 1996; de Paula et al. 2002; Muñoz et al. 2004; Wakibia et al. 2006; Hayashi et al. 2007a, b; Hung et al. 2008; Hurtado et al. 2008), *K. striatum* (Hurtado et al. 2008), *E. denticulatum* (Wakibia et al. 2006), and *E. isiforme* (Freile-Pelegrín and Robledo 2006). The present study further supports the need to undertake more detailed investigations of the availability of cold water carrageenophytes and their sustainable harvesting and/or cultivation for industrial hydrocolloids.

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