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Rheological properties of agar and carrageenan from Ghanaian red seaweeds

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ABSTRACT

Red seaweeds contain unique galactose-rich hydrocolloids, carrageenans and agar, which find use as gelling agents in high value applications. This study examined the chemical and rheological properties of hydrocolloids from selected wild red seaweed species collected in Ghana: *Hypnea musciformis* and *Cryptonemia crenulata*, expected to hold carrageenan, contained 21–26% by weight of galactose. A commercial *Kappaphycus alvarezii* carrageenan sample had 30% galactose residues by weight. *Hydropuntia dentata*, expected to contain agar, contained 15% by weight of galactose-monomers. Fourier transform infrared spectroscopy (FTIR) analysis on the hydrocolloids extracted from *H. musciformis* (and *K. alvarezii*) indicated κ-carrageenan, *C. crenulata* hydrocolloids were mainly ι-carrageenan, and the *H. dentata* hydrocolloids were agar. Gelling temperatures ranged from 32 to 36 °C for the κ-carrageenan hydrocolloid samples. The ι-carrageenan and agar samples had gelling temperatures of 70–74 °C and 38–52 °C, respectively. Gel strengths, G' at 25 °C, of carrageenan samples extracted via alkali-treatment were 4000–6500 Pa. The agar gel strength was 287 Pa. The rheological properties of the *H. musciformis* κ-carrageenans were comparable with κ-carrageenan from *K. alvarezii*, whereas the *H. dentata* agar properties were different from those of a commercial agar sample. This work shows that certain red seaweed species in Ghana contain hydrocolloids with desirable properties for high value applications.

1. Introduction

Seaweed hydrocolloids currently have a global value of approximately US$ 1.1 billion with products from the Asia-Pacific region dominating the market (Bixler & Porse, 2011; Hurtado, Neish, & Critchley, 2015; Rhein-Knudsen, Ale, & Meyer, 2015). Red seaweed species contain particularly valuable hydrocolloids such as agar and carrageenan that are used in food, pharmaceutical, and biotechnological applications due to their unique physico-chemical properties and gelling characteristics (Mchugh, 2003). Extraction of such high value hydrocolloids from native red seaweed species in Ghana could enable a new type of green growth in the West African coastal region.

Carrageenans are principally made up of repeating disaccharides of D-galactopyranose units bound together with alternating α-1,3 and β-1,4 linkages. Some of the galactose moieties are present in a 3,6-anhydro-α-D-galactopyranose form and a significant portion of the galactose-moieties may also be sulfated at C-2, C-4 or at C-6 (De Ruiter & Rudolph, 1997). The presence of 3,6-anhydro-galactopyranose and the amount and position of the sulfate substitutions form the basis for categorizing carrageenans into three chemically distinct types of structures, that also designate the main commercially used types, namely κ-, ι-, and λ-carrageenan. In general, κ-carrageenan has one sulfate ester per galactose dimer, whereas ι-and λ-carrageenan have two and three sulfates per dimer, respectively (De Ruiter & Rudolph, 1997). Agar is chemically similar to carrageenan, and is made up of galacto-pyranose dimers, i.e. alternating galactose and 3,6-anhydro-α-galactopyranose units connected by alternating α-1,3 and β-1,4 linkages, but with the

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important difference that in agar the 3,6-anhydro-α-galactopyranose units are in the L-configuration and not in the D-configuration as is the case for carrageenans (Usov, 2011).

Both carrageenans and agar form gels in aqueous environments via helix formation and aggregation of the polysaccharide chains through inter-molecular hydrogen bonds (Morris, 1986; Schafer & Stevens, 1995) - for carrageenans the gelation is supported by the presence of cations that induce the formation of a stable three-dimensional gel-network (usually with potassium for κ-carrageenan and with calcium for ι-carrageenan) (Montero & Pe, 2002; Rhein-Knudsen et al., 2015). However, being biologically synthesized in nature, natural carrageenans and agar are inherently heterogeneous. Extracted κ- and ι-carrageenans may thus contain traces of their biosynthetic precursors, i.e. μ- and ν-carrageenan, respectively, whereas agar may hold porphyran structures, i.e. the precursor for agar, having α-L-galactose-6-sulfate instead of 3,6-anhydro-α-L-galactopyranose, along with other hybrid structures (Rhein-Knudsen et al., 2015). The level of these different precursors and the extent of structural differences vary in different red seaweed species, and this variation obviously affects the rheological properties of the hydrocolloids.

Tanzania has long been a producer of carrageenan from seaweed farming (Hurtado et al., 2015), but neither seaweed collection nor farming are currently practiced in Ghana, even though the 540 km long Atlantic coastline in the south is a habitat for different seaweed species with potential for local hydrocolloid production. Some of the wild red seaweed species such as *Hypnea* spp., *Cryptonemia crenulata* and *Hydropuntia* spp. found along the coast of Ghana are known, however, to contain hydrocolloids, notably carrageenan and agar (Mtolera & Buriyo, 2004; Pereira-Pacheco, Robledo, Rodríguez-Carvajal, & Freile-Pelegrín, 2007; Saito & de Oliveira, 1990). Cultivation of e.g. *Hypnea musciformis* for extraction of κ-carrageenan has been studied in India and Brazil, but has not reached a commercial stage (Berchez, Pereira, & Kamiya, 1993; Ganesan, Thiruppathi, & Jha, 2006). Only few studies have been conducted on Ghanaian seaweeds, and these have mainly focused on elemental analysis and assessments of iodine levels (e.g. Serfor-Armah, Nyarko, Osae, Carboo, & Seku, 1999; Serfor-Armah et al., 2000). In 1975, John and Asare (John & Asare, 1975) published a preliminary assessment study of the yields and properties of hydrocolloids extracted from certain Ghanaian red seaweeds, but to our knowledge, no recent data are available on the rheological characteristics or hydrocolloid levels in wild red seaweed species from Ghana. The hypothesis of the present work was that red seaweed species native to Ghana hold galactose-rich hydrocolloids of the carrageenan or agar type, and that the rheological properties of hydrocolloids extracted from these seaweed species could be on par with commercially used carrageenans and agar. The present study was conducted to assess the carbohydrate composition of local red seaweed species found along the coast in Ghana, and to characterize the rheological properties of the hydrocolloids extracted as a base for considering local carrageenan and agar production from red seaweed resources in the region.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) unless stated otherwise. Guluronic acid was purchased from Chemos GmbH (Regenstauf, Germany).

2.2. Seaweed sampling and sample preparation

Wild red seaweed samples were collected in the coastal areas of Ghana, except the *Kappaphycus alvarezii* (Table 1). All samples obtained from Ghana were immediately frozen in aliquout portions at −20 °C after collection. Before use, the seaweed samples were gently thawn, and then rinsed to remove epiphytes, entangled materials, and sand. The washed seaweed samples were then freeze-dried and milled, then passed through a 1 mm mesh sieve (MF 10 basic Microfine grinder drive, IKA) to obtain uniform particle sizes. The milled seaweed samples were stored in sealed plastic bags at −20 °C. Cultured *Kappaphycus alvarezii* was received in dried form from Vietnam (Nhatrang Institute of Technology Research and Application) and used as a benchmark for carrageenan extraction and rheological properties of carrageenan.

2.3. Composition analysis

The amount of dry matter and ash in the seaweed samples were determined according to the National Renewable Energy Laboratory (NREL) procedure and the weight of biomass used in the experiments was mathematically corrected for the amount of moisture present in the samples (Sluiter et al., 2004, 2008). For carbohydrate composition analysis, the collected seaweed samples were hydrolyzed according to a modification of the NREL two-step sulfuric acid hydrolysis procedure, as described by Manns et al. (Manns, Deutschle, Saake, & Meyer, 2014). In brief, 100 mg dry matter seaweed material per mL was mixed with 72% H_2SO_4 (weight/volume, w/v) and left to react at 30 °C for 1 h. The reaction mixture was then diluted to 4% w/v H_2SO_4 and hydrolyzed in an autoclave at 120 °C for 40 min (Manns et al., 2014). The acid hydrolysate and seaweed residuals were then separated by vacuum filtration and the supernatants filtered through a 0.22 μm nylon syringe tip filter (Frisenette Aps, Knepel, DK) and diluted in 500 mM NaOH prior to injection for high performance anion exchange chromatography (HPAEC) analysis. HPAEC separation of the seaweed polysaccharides was performed using a HPAEC-PAD, ICS3000 system (Dionex Corp. Sunnyvale, CA) equipped with a CarboPac™ PA20 column by a method principally as described by Arnous and Meyer (2008). L-fucose, L-arabinose, L-rhamnose, D-galactose, D-glucose, D-xylose, D-mannose, D-galacturonic acid, D-guluronic acid, and D-glucuronic acid were used as monosaccharide standards for quantification; quantification was done using Chromeleon software (Dionex Corp. Sunnyvale, CA). Recovery values for the monosaccharides were estimated from parallel runs of monosaccharide standards (Arnous & Meyer, 2008).

2.4. Hydrocolloid extraction and characterization

2.4.1. Carrageenan extraction

Water extraction of carrageenan was performed using 1.5 g (dry matter) samples from *H. musciformis*, *C. crenulata* and *K. alvarezii* that were hydrated overnight in 30 mL milli-Q water before direct extraction at 99 °C for 1.5 h. The pH following overnight soaking was between 7.5 and 8.5 and based on previous results, this pH was high enough to prevent depolymerization of the seaweed galactans (Capron, Yvon, & Muller, 1996; John & Asare, 1975). Alkali-treatment was carried out using a slightly modified procedure of the method described by Istinii, Ohno, and Kusunose (1994). Briefly, 60 mL 6% w/v KOH was added to 1.5 g of the seaweed samples and reaction was carried out at 80 °C for 3 h. KOH was removed by washing the seaweed samples and soaking in water overnight. Carrageenan extraction was then performed on the alkali-treated, washed, seaweed samples (1.5 g dry matter) in 30 mL milli-Q water at 99 °C for 1.5 h. The extracts were pressure filtered (filter paper, PP filter cloth, Sigma-Aldrich) after being mixed with diatomaceous earth (Celite, Sigma-Aldrich), precipitated in 80% isopropanol, filtrated, and recovered by freeze-drying. Yields were