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Carrageenan and biochemical composition of three species of *Halymenia* (*Halymenia durvillaea* Bory de Saint-Vincent, *Halymenia maculata* J. Agardh and *Halymenia dilatata* Zanardini) in Initao, Misamis Oriental, Mindanao, Philippines

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Abstract

Laboratory experiments were conducted to determine the biochemical composition and carrageenan content of three species of *Halymenia* (*Halymenia durvillaea*, *Halymenia maculata* and *Halymenia dilatata*) that occurred along the coast of Iligan Bay. Powdered samples of *Halymenia* were analyzed for carrageenan content using extraction method while analysis of crude protein was done using Kjeldahl method, total fat by hydrolysis and solvent extraction method, ash content by gravimetric method, moisture content by air oven method, and total carbohydrate content by computational method. Result showed significant variation in the levels of carrageenan and biochemical composition among species. *Halymenia durvillaea* ranked highest in carrageenan yield, protein, fat, carbohydrate, ash and moisture content. This species can be a potential source for carrageenan production.

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Introduction

Carrageenan is a generic name for a family of gel-forming and viscosifying polysaccharides, which is obtained by extraction from certain species of red seaweeds (Necas and Bartosikova, 2013). It is derived from a number of seaweeds of the class Rhodophyceae. Carrageenan has no nutritional value and is used in food preparation for its gelling, thickening, and emulsifying properties (Van de Velde *et al.*, 2002) and in pharmaceutical applications (Takamatsu and Tosa, 1993) and experimental medicine where this substance is often used for the testing of anti-inflammatory agents (Zacharopoulos and Phillips, 1997). According to Freile-Pelegrin and Robledo (2006), there is an increasing worldwide demand for an additional raw materials source for carrageenan, particularly given the processing industry's quality, price and volume flow requirements (Ask *et al.,* 2003). Since the Philippines is the major source of carrageenophytes in the world market (James, 1990), there is a great help that the country can provide for the world production of carrageenan. The genus of *Halymenia* C. Agardh is a promising carrageenan source that grows along Iligan Bay (Kho, 2014). The vegetative and reproductive morphology and anatomy of *Halymenia* specimens from the Philippines are well documented (De Smedt *et al.,* 2001). Determining the biochemical composition and carrageenan content may help in providing data for its use as raw material for carrageenan industry. In an effort to develop the different species of *Halymenia* for industrial use, the carrageenan content and biochemical composition were studied.

Materials and Methods

Collection of red seaweed

Samples of *Halymenia durvillaea*, *Halymenia dilatata* and *Halymenia maculata* were collected randomly in the coastal area of Barangay Tubigan, Initao, Misamis Oriental (08° 32.0' North Latitude 124° 18.7' East Longitude) during low tide in December 2013. Barangay Tubigan was chosen as the sampling area because of its high diversity in red seaweeds. Collected samples were placed in an ice bucket and brought to the laboratory where they were sorted and cleaned with water.

Carrageenan analysis

Carrageenan extraction was done following the method of Mtolera and Buriyo (2004) and Hayashi *et al.* (2007). About 3 g of powdered seaweeds was hydrated in 105 mL distilled water for 12 h at room temperature under agitation (to remove internal salts). The hydrated material was boiled using 105 mL distilled water at 60°C for 4 h with constant stirring (to dissolve algal particles and evaporate insoluble materials). The digested product was precipitated in three volumes of 95% ethanol (210 mL). The precipitate was then filtered through a nylon cloth, dried in oven at 60°C for 24 h and at 105°C for 2 h to constant weight, and then weighed. The final carrageenan was grounded into powder for storage. The carrageenan yield (% dry weight) was determined according to the formula of Hung *et al.* (2009):

$$\text{Yield} = (W_c / W_m) \times 100$$

Where:

W_c = dry carrageenan weight

W_m= dry algal weight

Protein, fat, carbohydrate, ash and moisture analysis

The collected samples of *Halymenia durvillaea*, *Halymenia dilatata* and *Halymenia maculata* were oven dried at 60°C for 24 hours. After drying, the samples were ground into powder and were brought to the Chemical Testing Laboratory of the Department of Science and Technology, Cagayan de Oro City, Mindanao, Philippines for analysis. Analysis of crude protein was done using Kjeldahl method, total fat by hydrolysis and solvent extraction method, ash content by gravimetric method, moisture content by air oven method, and total carbohydrate content by computational method [Carbohydrate = 100% - (% protein + % fat + % ash + % moisture)]. The method used for the analysis of crude protein, total fat, ash content and moisture content was based on OMA AOAC (2008) and the values were expressed as percentage on dry weight basis.

Statistical analysis

The difference in carrageenan, protein, fat, carbohydrate, ash and moisture contents among the three species of *Halymenia* was determined by Analysis of Variance (One-Way ANOVA, level of significance, P of 0.05) in SPSS (version 8.0). Tukey's Pairwise Comparison of Means was used to determine which variables were significantly different.

Results and discussion

Carrageenan yield

The carrageenan yield obtained in this study ranged from 9.74-28.41% and comprised about 74% of the carrageenan content in *Kappaphycus alvarezii* (Muñoz *et al.,* 2004). The highest carrageenan yield was found in *H. durvillaea* (28.41±2.77%, Table 1 and 2) and the value was comparable to the carrageenan yield of *Hypnea musciformis* (20-35%) in Oyster Bay, Tanzania (Mtolera and Buriyo, 2004).

Table 1. Statistical analysis (One-Way ANOVA) on the effects of species on protein, fat, carbohydrates, ash, moisture and carrageenan content (mean ± SD).

Dependent variable	Independent variable	d.f.	*F*-statistics	*p*	Analysis
Protein	Species	2	153.18	0.000	Significant
Fat	Species	2	327.27	0.000	Significant
Carbohydrate	Species	2	651.48	0.000	Significant
Ash	Species	2	1639.00	0.000	Significant
Moisture	Species	2	3604.00	0.000	Significant
Carrageenan	Species	2	51.17	0.000	Significant

Several species of carrageenophytes are present in the Philippines: *Kappaphycus alvarezii*, *K. cottonii*, *K. striatum*, *K. procrusteanum*, *Eucheuma denticulatum*, *E. gelatinae* and *E. arnoldii*. Likewise, several species of *Hypnea* and *Acathophora* have also been reported to contain carrageenan. The genus *Kappahycus* produces kappa-carrageenan while *Eucheuma* produces iota-carrageenan. At present, only *K. alvarezii* and *E. denticulatum* are produced commercially through farming. The result of this study suggests that *H. durvillaea* could potentially be used as raw material for carrageenan production in the country.

Protein, fat, carbohydrate, ash and moisture contents

Significant variation in protein, fat, carbohydrate, ash and moisture contents among the three species of *Halymenia* was visible (Table 1).

The range of protein in this study was from 9.99-13.02% and was within the range of 10-47% for green and red seaweeds (Fleurence, 1999). *H. durvillaea* had the highest protein content (13.02±0.33, Table 3) followed by *H. maculata* (11.07±0.09) and *H. dilatata* (9.99±0.05).

Table 2. Carrageenan yield (%) in *H. durvillaea, H. maculata* and *H. dilatata* (mean ± SD).

Yield	Species		
	H. durvillaea	*H. maculata*	*H. dilatata*
Carrageenan (%)	28.41±2.77[a]	11.50±3.17[b]	9.74±0.93[c]

Letters represent differences in group means as determined by Tukey's multiple comparison test.

The protein content (11.77±1.37% DW) recorded from the three species of *Halymenia* is higher than the concentrations found in higher plants (Norziah and Ching, 2000). Seaweeds are considered to be a good source of nutritional protein and bioactive peptides with health promoting properties, and also show potential for development as functional food ingredients (Harnedy and FitzGerald, 2011). The

protein in seaweeds is called complete protein with all the essential amino acids at levels close to that recommended by FAO/WHO (Wong and Cheung, 2000; Matanjun *et al .,* 2009). Importantly, red seaweeds have the highest amount of protein compared to brown and green seaweeds (Kolb *et al.,* 1999).

The concentrations of fat were within the range of 0.29-1.29%. According to the following authors (Morrissey *et al.,* 2001; Dawczynski *et al.,* 2007; MacArtain *et al.,* 2007; Taboada *et al.,* 2011, 2013), the fat content in seaweed is generally low (<5% dry weight). In fact, in comparison to other chemical constituents, fat contents were the smallest component observed for the three species studied. However, as reported by Arasaki and Arasaki (1983), Foster and Hodgson (1998) and Ahmad *et al.* (2012), the fat content obtained from this study was higher compared to other edible red seaweeds such as *Porphyra tenera* (0.70%), *Gelidium pristoides* (0.90%) and *Kappaphycus alvarezii* (green variety; 0.18%).

Table 3. Levels of protein, fat, carbohydrate, ash and moisture in *H. durvillaea, H. maculata* and *H. dilatata* (mean ± SD).

Biochemical constituents	Species		
	H. durvillaea	*H. maculata*	*H. dilatata*
Protein (%)	13.02±0.33[a]	11.07±0.09[b]	9.99±0.05[c]
Fat (%)	1.29±0.06[a]	1.09±0.03[b]	0.29±0.01[c]
Carbohydrate (%)	53.65±0.07[a]	51.18±0.54[b]	40.53±0.55[c]
Ash (%)	39.54±0.52[a]	23.61±0.18[b]	21.92±0.49[c]
Moisture	12.40±0.05[a]	11.39±0.06[b]	8.85±0.08[c]

Letters represent differences in group means as determined by Tukey's multiple comparison test.

The amount of carbohydrates varied from 40.53-53.65% dry weight and was within the range of 40-75% specified for seaweed (MacArtain *et al.,* 2007; Holdt and Kraan, 2011). Likewise, the values were comparable to other species of seaweeds found in different biotopes of the world (El-Tawil and Khalil, 1983; Arasaki and Arasaki, 1983; Banerjee *et al.,* 2009; Kumar *et al.,* 2011; Ahmad *et al.,* 2012; Chakraborty and Bhattacharya, 2012; Nor Salmi *et al.,* 2012; Khairy and El-Shafay, 2013; Omar *et al.,* 2013; Siddique *et al.,* 2013). *H. durvillaea* had the highest amount of carbohydrates (53.65%) and it differed significantly with *H. maculata* and *H. dilatata*. This variation might be due to different season and growth stage experienced per individual (Fleurence, 1999).

The ash content in all three species varied from 21.92-39.54% DW and was within the range of 8-40% for seaweed (Mabeau and Fleurence, 1993). On the other hand, these results were comparatively higher than those of terrestrial counterparts with only 5-10% DW (USDA, 2001). The high ash content is a general feature of seaweeds, and these values are generally much higher than those of terrestrial vegetables other than spinach (Ruperez, 2002). High ash content invariably indicates the presence of appreciable amounts of diverse mineral components (Mantanjun *et al.,* 2008). Seenivasan *et al.* (2012) reported that seaweeds contain higher concentration of more than sixty trace elements. Among the three species being studied, *H. durvillaea* had the highest level of ash (39.54±0.52) followed by *H. maculata* (23.61±0.18) and *H. dilatata* (21.92±0.49). The level of ash obtained from the three species was higher than those reported in other species of red seaweeds; i.e. *Hypnea pannosa* (18.65% DW), *Hypnea musciformis* (21.57% DW), *Hypnea japonica* (22.10% DW), *Hypnea charoides* (22.80% DW), *Porphyra tenera* (8.70% DW), *Gelidium pristoides* (14.00% DW), *Gracilaria cervicornis* (7.72% DW) and *Gracilaria changgi*

(22.70% DW), respectively (Arasaki and Arasaki, 1983; Foster and Hodgson, 1998; Wong and Cheung, 2000; Marinho-Soriano *et al.,* 2006; Siddique *et al.,* 2013a; Siddique *et al.,* 2013b;).

Moisture content is an important criterion in determining the shelf-life and quality of processed seaweed meals as high moisture may hasten the growth of microorganisms (Rohani-Ghadikolaei *et al.,* 2012). It is preferably not more than 40% (McHugh, 2006) however the recommended value is not more than 35% (Pelinggon and Tito, 2009). In this study, the moisture content of *H. durvillaea* (12.40%), *H. maculata* (11.39%) and *H. dilatata* (8.85%) are within the recommended level of not more than 35% (Pelinggon and Tito, 2009).

Conclusion

The biochemical composition of the three species of *Halymenia* (*H. durvillaea, H. maculata* and *H. dilatata*) were within the values specified for seaweeds hence there is a potential for these species to be used as raw material or ingredients in human diet and animal feed. Likewise, *Halymenia durvillaea* obtained the highest carrageenan yield and the values were within the commercial requirement needed for commercial crops, thus can be a potential source for carrageenan production.

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