RUHUNA JOURNAL OF SCIENCE
Vol 13 (1): 1-13, June 2022
eISSN: 2536-8400
http://doi.org/10.4038/rjs.v13i1.111

Faculty of Science
University of Ruhuna

Gel properties of agar from two *Gracilaria* species in Sri Lanka and development of food jellies

Piyumi S. De Alwis and Isuru Wijesekara*

Department of Food Science & Technology, Faculty of Applied Sciences,
University of Sri Jayewardenepura, Nugegoda, Sri Lanka

*Correspondence: *isuruw@sci.sjp.ac.lk*, ORCID: https://orcid.org/0000-0003-1688-8801

Received: 1st May 2021, Revised: 21st December 2021, Accepted: 19th April 2022

Abstract *Gracilaria* species are found to be a rich source of natural hydrocolloid agar-agar but are currently under-exploited in Sri Lanka. The gel characteristics of agar extracted from *Gracilaria corticata* and *Gracilaria edulis* from Sri Lankan waters were investigated in this study. The agar yields (%, dry weight basis) obtained from *G. corticata* and *G. edulis* by hot-water extraction at 90°C for 1 h, were 39.91 ± 0.17 and 67.52 ± 1.43, respectively. The gel properties of both agar gels (1.5%, w/v) such as pH, viscosity, gelling and melting temperatures, and syneresis were examined. The viscosity of agar gels (cP) at 80°C was lower than that at 70°C for both species, while *G. edulis* has shown higher viscosities at both temperatures (1.96 ± 0.06 and 2.76 ± 0.39, respectively). Moreover, the textural analysis revealed that *G. corticata* has a higher gel hardness (316.7 ± 20.8 g) than *G. edulis* (113.33 ± 12.58 g). Strawberry-flavored food jellies were developed from agar gels of two *Gracilaria* species with either artificial (Carmoisine, E122) or natural food color (Annato). Collectively, this study revealed that agar from both red seaweed species has the potential to be developed as plant-based food jellies, and *G. edulis* can be suggested as the best source to extract agar with higher yield compared to *G. corticata*.

Keywords: Agar, food jellies, *Gracilaria* spp., red seaweeds, value-addition.

1 Introduction

Seaweeds are an important plant-based food source that contains reduced levels of calories and lipids but are abundant with polysaccharides, proteins, steroids, dietary fibres, vitamins, and minerals (Gupta and Abu-Ghannam 2011, Rioux and Turgeon 2015). Primary and secondary bioactive metabolites of seaweeds are increasingly recognized as valuable sources of dietary nutrition (Almeida *et al*. 2011). Previous studies have reported the use of seaweed not only for developing different types of processed foods for human consumption but also for formulating animal feeds and

non-food applications such as bio-fertilizers, biofuels and cosmetics (Buschmann *et al.* 2017).

Red algae are generally found in the coastal environments, while they are relatively rare in freshwaters (Dodds and Whiles 2010). Red seaweeds are the major sources of hydrocolloids including agar and carrageenan. Among red seaweeds, the genus *Gracilaria* serves as a major agar source worldwide and *Gelidiella* and *Gelidium* contain minute quantities. *Gracilaria* species are called agaroides since it includes elevated amounts of sulfates which leads to low-quality agars (Freile-Pelegrin and Robledo 1997). Although agar has a higher sulfur content, its unique physical gel structure, high hysteresis, and extensive gelling power with ideal gel reversibility lead to distinctive properties which give an advantage in diverse applications (Rioux and Turgeon 2015, Chaudhary *et al.* 2015). As one of the applications, hydrocolloid confectionery uses agar gels as a gelling agent with sugar, for instance, table jelly which is also known as water gels.

As an island, Sri Lanka is a rich source of nutritious but underutilized seaweeds, with potential value-additions. According to previous reports, the agar from *G. verrucosa* from Trincomalee, Sri Lanka was incorporated into yoghurts as an alternative to animal-derived gelatin (Paththuwe Arachchi *et al.* 2018). Considerable quantities of *Gracilaria* species have been discovered in the coastal area of Sri Lanka and *G. edulis* has been one of the most abundant *Gracilaria* species (Sivapalan and Theivendirarajah 1985, Vinobaba *et al.* 2016). The seaweed polysaccharides of *Gracilaria* species were found to be present with a higher nutritional profile other than natural hydrocolloids (Jayasinghe *et al.* 2016, Rosemary *et al.* 2019). The aim of the study was to extract and determine the gel properties of agar from two *Gracilaria* species, *G. corticata* and *G. edulis* collected from Sri Lankan waters. Moreover, attempts were taken to formulate edible food jellies using extracted agar from those *Gracilaria* species with either natural or artificial food colour as a commercial formulation for the Sri Lankan jelly market. Thus, this study demonstrates the first scientific study of the food application of agar gel from Sri Lankan red seaweed, *G. corticata*.

2 Material and Methods

2.1 Seaweed collection and preparation

Red seaweed species *Gracilaria corticata* was manually collected during late August 2019 from Moruwala and Pitipana Beaches, Negombo, Sri Lanka. *Gracilaria edulis* was collected during early September 2019 from Kalpitiya lagoon, Kalpitiya, Sri Lanka. Species identification was done in reference to Coppejans *et al*. (2009) and Vinobaba *et al.* (2016) and under the guidance of The National Aquatic Resources Research and Development Agency (NARA). The fresh samples were thoroughly cleaned with running tap water and were dried at 60°C for 10 h in a drying cabinet.

Dried samples were pulverized by using a food-grade mechanical grinder and sieved through the 355-micron (BS 410 – No. 44) sieve mesh. Finally, they were kept in sealed polypropylene bags and stored at room temperature (27°C) till further analysis.

2.2 Agar extraction

Agar was extracted from dried seaweed powders (*G. corticata* and *G. edulis*) by the hot water extraction method. Briefly, 50 g of each dried seaweed powder sample was added to a beaker that contains 1000 ml distilled water and the beaker was kept in a hot water bath at 90°C for 1 h. The extracted agar solution was kept at room temperature (27°C) until gel formation and that gel was dried at 60°C for 12 h until getting a constant weight. The dried agar was subsequently ground, and the agar powders were stored in sealed HDPE bags at 4°C till further analysis.

2.3 Fourier transform infrared (FT-IR) spectroscopy

The FT-IR measurements of the yielded agar powder samples of *G. corticata* and *G. edulis* were performed using KBr pellets to identify the functional compounds in the dried agar powders. The FT-IR spectrums were recorded in the wavelength between 4000 and 500 cm^{-1}.

2.4 Agar gel preparation and determination of gel properties

Agar gels (1.5 % w/v) from yielded agar powders from two *Gracilaria* spp. were prepared according to a previously published method (Kumar and Fotedar 2009). All analyses were triplicated for accuracy. The pH was determined according to the Association of Official Analytical Chemists (AOAC 943.02) method using mV meter-(SPER SCIENTIFIC pH probe, 860031 Bench-top pH). Viscosity profiles of 1.5 % agar gels from two *Gracilaria* spp. were assessed using a viscometer by using No. 1 spindle, 60 rpm at 90°C and 80°C (BDV- 1S/ 5S/ 8S/9S type). The gelling and melting temperatures of agar gels were determined according to Rodríguez *et al.* (2009). For the syneresis of agar gels, hot dispersions (1.5% w/v, 20 ml) of two species were prepared according to a previously published method with modifications (Yarnpakdee *et al*. 2015).

2.5 Development of agar-based food jellies

Agar-based food jellies were developed using the extracted dried agar powder from *G. corticata* and *G. edulis*. Either artificial food color (Carmoisine) or natural pigment (Annato seed extract) was used as the coloring agent for the agar-based (agar 1.5%

w/v) food jellies with strawberry flavor (Table 1). Finally, the solutions were poured into the transparent plastic containers and allowed to cool at room temperature (27°C) followed by storing in the refrigerator at 4°C.

Table 1. Ingredients for food jelly formulation.

Ingredients	g/100 ml
Agar powder	1.50
Sugar	20.00
Water	100.00
Color [a]	0.50
Flavor [b]	1.00
Citric acid [c]	0.25

[a] Red Color (Delmege®, Carmoisine – INS. No. 122 or natural pigment Annatto seed)
[b] Strawberry Flavor (Delmege®, nature identical strawberry ester)
[c] Citric acid (E 330)

2.6 Analysis of moisture, Brix, pH and texture of jelly samples

The moisture content was determined using the san pan method by Tray drier demonstrator B 35355 for both jelly samples *G. corticata* (GC 1) and *G. edulis* (GE 1) with artificial food colour according to Riedel *et al*. (2015). The amount of dry solid dissolved in the solutions of GC 1 and GE 1 with artificial food colour was determined by using a refractometer/ brix meter (OPTIKA HR-150, Hand refractometer, Italy) and pH of the jelly samples was determined by using a pH meter (860031 Bench-top pH / mV meter –SPER SCIENTIFIC pH probe) before solidification of the samples. From stored agar food jelly samples of GC 1 and GE 1, acidity was analyzed using a piercing electrode attached to a probe, pH meter with 0 days, 5 days, 10 days and 15 days' time intervals. Texture profile analyses (TPA) for agar jelly samples, GC 1 and GE 1 was determined using a computer-controlled CT3 texture analyzer (50 kg, Brookfield, USA) with a load cell of 25000 g and cross-head speed of 1 mm/s, equipped with a Flat-faced cylindrical TA25/1000 Probe.

2.7 Statistical analysis

All experiments were carried out in triplicate and data were presented as mean ± SD (standard deviation). The statistical analysis of data was carried out using a one-way analysis of variance (ANOVA) test and the significance of each variable was at $p=0.05$ and followed by comparisons performed using the Tukey test by the statistical software MINITAB 17 (Pennsylvania, USA).

3 Results and Discussion

3.1 Agar yield

Gracilaria corticata and *Gracilaria edulis* are two underutilized red seaweeds that were used to extract agar in the present study. *G. edulis* (67.52 ± 1.43) yielded a significantly higher percentage of agar than *G. corticata* (39.91 ± 0.17) by hot-water extraction at 90°C for 1 h. Lower yield extraction of agar from *G. corticata* than *G. edulis* may be due to leaching out of the water insoluble agar polymers during the extraction process (Montaño *et al*. 1999, Praiboon *et al*. 2006). It has been stated that alkali pre-treatment may also have an impact on the yielded agar amount of *Gracilaria* species (Freile-Pelegrin and Murano 2005, Meena *et al.* 2008).

3.2 FT-IR spectrums

FT-IR spectrums from the dried agar powders from *G. corticata* and *G. edulis* provide peaks with different wavelength ranges which are unique to each functional group and present informative details on its chemical nature (Gómez-Ordóñez and Rupérez 2011).

For *G. corticata* (Figure 1), major FT-IR peaks were observed at 3434.99, 1640.64, 1113.21, and 617.52 cm^{-1}. A dominant absorption peak at 3434.99 cm^{-1} indicated the presence of phenols and alcohols with free OH group. Reference data further explicated that O–H bonds occur around 3650-3200 cm^{-1} wavelength range. Carbonyl stretch in proteins is shown by the resulting peak at 1640.64 cm^{-1} and may specify the presence of amide I groups in line with previous data (Kumar *et al*. 2012). Most of *Gracilaria* spp. have been reported of containing amino acids, vitamins, carbohydrates, fatty acids, and phenolic constituents and this may be attributed to the peak of 617.52 cm^{-1}. This arisen peak may be due to the stretching vibration of an aromatic ring that is connected to the free OH group (Kumar *et al*. 2013). The observed absorption peak of a weak band at 2360.12-2341.90 cm^{-1} in the sample may be correlated to the C-O stretching band. The weak band peak of 889 and 931 cm^{-1} can be assigned to Vinyl C-H out-of-plane bend vibrations. The weak absorption band of 657.26 cm^{-1} observed near 600 - 670 cm^{-1} from the sample may be due to C-S and C=S stretching vibrations and a Weak band of 470.88 cm^{-1} near 415-480 cm^{-1} may be attributable to the disulfides (Kannan 2014).

For *G. edulis* (Figure 1), the FT-IR spectrum of agar was similar to the spectrum of *G. corticata.* For instance, the absorption peak of weak band 2359.41 cm^{-1} observed in *G. edulis* sample may correspond with the C-O stretching band which is similar to the agar sample of *G. corticata*. The weak absorption bands, 656.81, 667.51, 617.52 cm^{-1} were detected near 600 - 670 cm^{-1} due to C-S and C=S stretching and this may be an indication of sulfated polysaccharides (Kannan 2014). Previous findings also have justified the existence of 3,6-anhydro-a-L-galactopyranose unit and risen levels of

sulfate amount characteristically sulfate group on galactose residues through the FT-IR analysis of *G. edulis* polysaccharides (Coates 2000, Sakthivel and Devi 2014). The FT-IR spectra of agar powder of two samples showed band values of 889.81-931 cm^{-1} for *G. corticata* and 751 cm^{-1} for *G. edulis* may be characterized as diagnostic bands for agarans (Kim and Shin 2017).

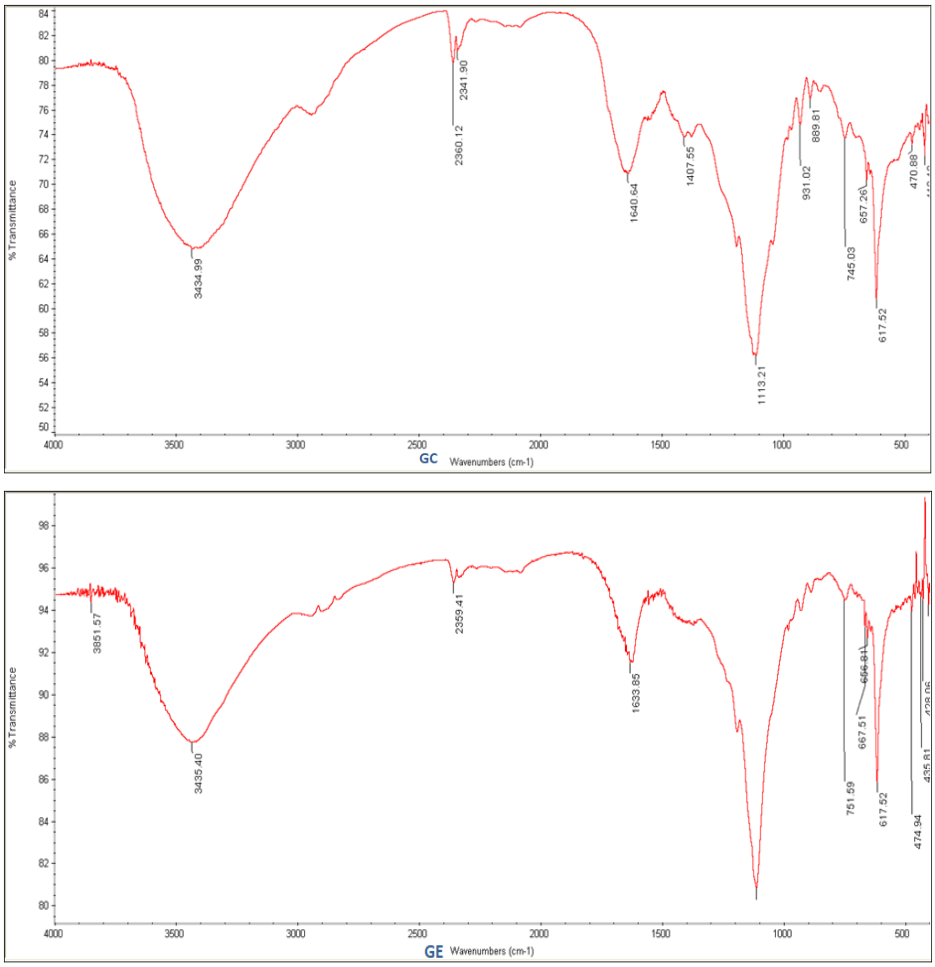

Fig 1. FT-IR analysis of agar from *G. corticata* (GC, Top) and *G. edulis* (GE, Bottom)

3.3 Agar gel properties of *G. corticata* and *G. edulis*

In the present study, gel properties of agar gels from *G. corticata* and *G. edulis* were compared and results were given in Table 2. *G. corticata* (6.7 ± 0.03) showed a significantly higher pH value than *G. edulis* (6.38 ± 0.02) at a 5% significance level.

The pH of a substance is the degree of acidity or alkalinity and it has a significant impact on the ultimate quality of the food product. Thus, these lower pH values of the agar gels from both species may affect the viscosity of the agar gel solutions (Andrés-Bello *et al*. 2013, Yu *et al*. 2020). Previous research have shown that the higher viscosity of agar gel has a correlation to its higher melting temperatures and the molecular chain length or weight, and this can be used to describe the viscosity of agar solution (Murano 1995, Yarnpakdee *et al*. 2015).

Table 2. Agar gels properties of *G. corticata* and *G. edulis*.

Parameter	*G. corticata*	*G. edulis*
pH	6.70 ± 0.03^{a}	6.38 ± 0.02^{b}
Viscosity at 80°C (cP)	1.68 ± 0.08^{b}	1.96 ± 0.06^{a}
Viscosity at 70°C (cP)	1.89 ± 0.05^{b}	2.72 ± 0.39^{a}
Gelling Temperature (°C)	38.10 ± 0.40^{a}	36.37 ± 0.51^{b}
Melting Temperature (°C)	54.27 ± 0.40^{b}	60.57 ± 1.00^{a}
Syneresis (%)	2.75 ± 0.05^{b}	29.69 ± 1.95^{a}

Values are Mean ± SD, n=3.
Different superscripts within the same row are significantly different ($p < 0.05$).

In this study, viscosity has experimented with two temperatures in order to determine the changes in the viscosity of agar gel with respect to the different temperatures. The viscosity at 80°C was lower than at 70°C for both species and *G. edulis* has shown the highest value for both temperatures. The reason for this difference might be due to the thermal movement of the molecular structure slowing down with a temperature decrement, which leads to the gradual formation of the double helix structure. Hence, this makes for the arrangement of macromolecular aggregates which increases the viscosity (Yu *et al*. 2020). *G. edulis* has shown viscosity (cP) of 1.96 ± 0.06 and 2.72 ± 0.39 at 80°C and at 70°C respectively. *G. corticata* has shown significantly lower values ($p<0.05$) than G. edulis, which were 1.68 ± 0.08 and 1.89 ± 0.05 at 80°C and at 70°C, respectively.

Gelling and melting temperatures were examined according to Rodríguez *et al.* (2009). The gelling temperatures (°C) of *G. corticata* and *G. edulis* were 38.10 ± 0.40 and 36.37 ± 0.51, respectively, and values are significantly different ($p<0.05$). It has been stated that the content of 6-O-methyl-D-galactose and 3,6-anhydro-2-O-methyl-L-galactose in *Gracilaria* agar have a direct association with its gel formation temperature (Murano *et al.* 1992, Tako *et al.* 1999, Villanueva *et al.* 1999, Melo *et al.* 2002, Rodríguez *et al.* 2009). Armisen and Galatas (2009) explained that the identification of agarophyte can also be done through the gel formation temperature. Gelling temperature of agar in *Gracilaria* spp. has been reported to be in the range of 40-42°C (Rodríguez *et al.* 2009). Villanueva *et al.* (1999) stated that gelling and melting temperatures of *G. vermiculophylla* agar were the lowest which is 21.6-26.4°C and 62.7-70.0°C, respectively.

The melting temperatures (°C) of agar of *G. corticata* and *G. edulis* were 54.27 ± 0.40 and 60.57 ± 1.00, respectively and the values were significantly different ($p<0.05$). The higher melting temperature is attributed to the higher energy required to break down the network structure, indicating that agar gel from *G. edulis* was more stable than the gel from *G. corticata* (Villanueva *et al.* 2010, Yarnpakdee *et al.* 2015). Furthermore, differences in melting or gelling temperature may be associated with the molecular weight patterns in agar, which strongly emphasized the gel characteristics (Freile-Pelegrin and Murano 2005). Hence, the increment of molecular weight increases the likelihood of stable associations within gelling sequences in the polymer. However, sulphate content and gelling temperature have a negative relation with each other (Andriamanantoanina *et al.* 2007, Yarnpakdee *et al.* 2015).

The term syneresis is characterized as the liquid loss of the gels with time, showing the instability of the gel network (Yarnpakdee *et al*. 2015). Previous studies have concluded that the quantity of water lost from the agar gel has an inverse relation to the amount of total sulfate concentration (Matsuhashi 1990, Mizrahi 2010). The lowest syneresis (%) has resulted in agar gel of *G. corticata* (2.75 ± 0.05) and the highest syneresis (%) was observed in *G. edulis* (29.69 ± 1.95) ($p < 0.05$). The lowest syneresis in *G. corticata* might be related to a strong gel network which could retain more water with ageing (Yarnpakdee *et al*. 2015). Thus, the dried agar powder of *G. corticata* can be incorporated as a gelling agent for food products which is needed to be stored for relatively longer time period.

3.4 Developed agar-based food jellies

In the development process of agar-based food jellies, the dried agar powders were added to a known amount of cold water and soaked for 5 min because agar is needed to be properly hydrated for a satisfactory jelly product (Riedel *et al*. 2015). Sugar plays multiple roles in jellies as a sweetening agent and contributes to the gel texture and microbial stability. Moreover, the sugar level in the product also gives a noticeable impact on agar gel by physically reinforcing the junctions of polysaccharide helixes. However, increment sugar levels may give a harder and firmer but less cohesive texture to the jelly (Meschter 1990). Food acid is added at reduced temperatures to minimize hydrolysis risks. Following the addition of acid, the gelling process starts irreversibly (Prakash and Priya 2016). The artificial red colour was used as the food colouring agent to mask the greenish yellow colour given by the dried agar powder to the food jellies. Additionally, the strawberry flavour was chosen as the flavour since it matches the colour and also aids in concealing the seaweed smell of food jellies. The sensory and shelf-life studies of the developed food jellies are in progress. Moreover, *Gracilaria* species have been reported with Pb contaminations, thus it is crucial to ensure good environmental conditions and maintain water quality parameters in cultivating areas (Jayakody *et al*. 2021) and further studies are required for the determination of the

heavy metal composition of these dried agar powders in order to be utilized in the food industry.

3.5 Analysis of moisture, Brix, pH and texture of jelly samples

Statistical analysis of data showed that moisture content in GE 1 (agar food jelly from *G. edulis* with artificial food colour) and GC 1 (agar food jelly from *G. corticata* with artificial food colour) jelly types are significantly different (p<0.05) (Table 3). The moisture content of the jelly samples was determined by the san pan method because jellies contained approximately a higher liquid (water) percentage. In confectionery gels, water aids in the formation of gel and has an impact on the gel properties. Thus, the variations in moisture content may lead to quality variations such as differences in texture and product alterations (Burey *et al.* 2009).

Table 3. Results of the moisture content, Brix value and instrumental texture analysis in jelly samples.

Parameter	Product type	
	GE 1	GC 1
Moisture content	77.48±0.95^{b}	79.18±0.24^{a}
Brix value (°Brix)	24.25±0.35^{a}	24.17±0.29^{a}
Hardness cycle 1 (g)	113.33±2.89^{b}	196.7±17.6^{a}
Hardness cycle 2 (g)	86.67±7.64^{b}	145.00±15.00^{a}

Values are Mean ± SD, n=3.
Different superscripts within the same row are significantly different (p < 0.05).
Gracilaria edulis - 1.5% Agar (GE 1), *Gracilaria corticata* - 1.5% Agar (GC 1)

Soluble solids content is expressed as °Brix and is used to express the sugar content of an aqueous solution. One °Brix is equal to 1g of sucrose in 100 g of solution (Priya and Prakash 2017). Results showed that the Brix values of both GE 1 and GC 1 jelly samples are not significantly different (p>0.05) from each other (Table 3). For the preparation of jellies, 20% of granulate sucrose sugar was added to each jelly sample as a sweetener (Burey *et al.* 2009, Riedel *et al*. 2015). Furthermore, according to Kasapis and Al-Marhoobi (2003), the added sugar significantly contributes to gel texture and strength.

Texture Profile Analysis (TPA) is a technique for characterizing the many aspects of a jelly sample's texture (Table 3). TPA hardness is a measure of firmness and jelly hardness (N) refers to the maximum force obtained in the first penetration to attain a given deformation (Zahn *et al*. 2010, Intarasirisawat *et al*. 2014, Riedel *et al*. 2015, Yarnpakdee *et al*. 2015). During consumption, the texture of the product may have a major influence on the customer preference for the product. Besides flavour release, chewing characteristics are a crucial factor in determining consumer acceptance and this is affected by the texture of the product. The product's texture is affected by the gelling agent's unique structural network, which is built as a pillar to bind and provide

certain firmness. For instance, products with agar gel might have a dry and brittle texture (Endress and Mattes 2003). Therefore, agar has a major impact on the texture profile of hydrocolloid confectionery like food jellies (Ong *et al.* 2003). According to the statistical results, hardness in cycle 1 and 2 of TPA for both jelly samples are significantly different (p<0.05) and GC 1 jelly sample have shown a higher value of hardness than the GE 1 jelly sample. However, it has been reported a higher value for the gel strength (gcm^{-1}) of agar extracted from *G. edulis* with the pretreatment (Meena *et al.* 2008).

Table 4. Results of the pH changes of the jelly samples of *Gracilaria edulis* - 1.5% Agar (GE 1) and *Gracilaria corticata* - 1.5% Agar (GC 1) during storage at 4°C.

Product type	0 Day	5 Days	10 Days	15 Days
GE 1	3.49 ± 0.03^{b}	3.42 ± 0.01^{b}	3.12 ± 0.03^{b}	3.17 ± 0.02^{b}
GC 1	3.88 ± 0.01^{a}	3.77 ± 0.03^{a}	3.83 ± 0.03^{a}	3.83 ± 0.03^{a}

Values are Mean ± SD, n=3.
Different superscripts within the same column indicate significant differences ($p < 0.05$).
Gracilaria edulis - 1.5% Agar (GE 1), *Gracilaria corticata* - 1.5% Agar (GC 1)

As stated in the results (Table 4), the pH of agar jelly samples is significantly different ($p < 0.05$) between agar jellies from the two *Gracilaria* species. Moreover, the pH did not significantly change among 0 days, 5 days, 10 days and 15 days' time intervals throughout the storage at 4°C for both jelly samples of GE 1 (3.49 ± 0.03, 3.42 ± 0.01, 3.12 ± 0.03, 3.17 ± 0.02) and GC 1 (3.88 ± 0.01, 3.77 ± 0.03, 3.83 ± 0.03, 3.83 ± 0.03) respectively. The pH of the confectionery gels can be altered by food acids and lower pH values may indicate the preservation action which promotes the gel stability and shelf life (Burey *et al.* 2009).

5 Conclusions

This study demonstrates that *Gracilaria edulis* and *Gracilaria corticata* possess good amounts of agar as phycocolloides and are successfully utilized to develop food jellies as a vegan food product and as a substitute for gelatin. In conclusion, these two *Gracilaria* spp. can be recommended as potential agar sources for the local confectionery food industry.

Acknowledgements

This research was partially supported by a research grant (ASP/01/RE/SCI/2021/30) from the Research Council, University of Sri Jayewardenepura, Sri Lanka. The authors wish to thank Mr. Chathura Medagedara, Scientist at Regional Research Center, National Aquatic Resources Research and Development Agency, Kalpitiya for providing the raw materials. Furthermore,

The Custom Headquarters, Department of Customs, Colombo, Dr. Heshani Rathnayake, and Mr. Mohammed Atheeq are gratefully acknowledged for their technical assistance. The authors are grateful to two anonymous reviewers for their valuable comments to improve the quality of the manuscript.

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