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Physicochemical properties of edible alginate film from Malaysian *Sargassum polycystum* C. Agardh

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ABSTRACT

The edible brown seaweed, *Sargassum polycystum* was harvested from the coastal region of Malaysia. This study introduces the preparation of *S. polycystum* alginate through the external (protocol A) and internal (protocol B) gelation methods with 1% and 3% calcium chloride solutions. The physicochemical characteristics of the films such as transparency, internal viscosity, solubility, swelling index and chemical structure were studied. Results indicated that the films cross-linked with Ca^{2+} through Protocol A resulted in uneven films with rough surface compared to Protocol B that produced film with a uniform surface. Additionally, Protocol B with 0.5% glycerol produced films that was flexible and slightly soluble (7.11%) with the shape and integrity maintained. The ^{1}H-NMR analysis estimated the mannuronic: guluronic (M: G) ratio of the film as 0.733 and thus, confirming the characteristics of the alginate gel as less viscous but rigid. Comparatively, the commercial alginate of the *Laminarian* species showed a lower M: G ratio of 0.351 that resulted in a highly viscous gel. Besides that, the FTIR analysis showed that glycerol at increasing concentrations reduces the intensity of the absorption band at 3451.0 cm^{-1} (stretching vibrations of O–H). This indicated that the strong intermolecular bonds between the alginate polymer were reduced and thus, improved the flexibility of the films.

1. Introduction

Over the years, the reliance of consumers on products derived from fossil fuels such as plastics has caused damage to the Earth with 91% of them remained in wasteland and not recycled (Geyer et al., 2017). The United States alone discard about 33.6 million tons each year with only 6.5% recycled and 7.7% combusted in waste-to-energy facilities (Sharuddin et al., 2016). Therefore, there is an urgency to find an alternative material that is cheap, safe and biodegradable to accommodate the current demand of plastic for various applications.

There are over 400 species of *Sargassum* species distributed in the warm and temperate waters of Indo-West Pacific region, which include Malaysia, China, Japan, Indonesia and Australia (Noiraksar and Ajisaka, 2008). The sodium alginate (NaAlg) is a type of polysaccharide that is found abundantly in the cell wall of brown seaweed and consists of homo-polymeric blocks of (1 − 4) -linked β-D-mannuronate (M) and α-L-guluronate (G) that are covalently linked (Venkatesan et al., 2014). Their arrangements may differ across the seaweed species and the ratios of monomer affect the physicochemical properties of alginate (Fertah et al., 2017). Due to the non-toxic property of NaAlg, they are widely reported for various industrial applications, mainly in food, pharmaceutical and chemical industries as thickening, gelling or stabilizing agents (Choi et al., 2009). Currently, there is an increasing demand for safe food package that are eco-friendly and thus, the NaAlg films meet those requirements because they effectively bio-degrade over time with a 90% loss in weight after 35 days (Deepa et al., 2016) and 92% at the end of 80-days (Solak and Dyankova, 2014).

The alginate is compatible to form films with di- and tri-valent elements such as calcium, magnesium and ferrous ion (Cazón et al., 2017). The complex formed between the association of ions and the M and G residues, results in a stable and a three-dimensional network that resembles an “egg-box” model (Tavassoli-Kafrani et al., 2016). Thus, these cross-linked structures have shown improvements in the water barrier, mechanical resistance, cohesiveness and stiffness properties (Cazón et al., 2017). Even though, external gelation is the most common method employed for fast cross-linking of the polymer, however, this results in a localized gelling area that unfortunately compromises the uniformity and quality of films (Al-Remawi, 2012).

Plasticizer is a group of low molecular weight compound that can be added to polymers to provide plasticity to otherwise rigid and fragile polymers (Vieira et al., 2011). For plasticizing hydrophilic biopolymer-based films, especially for the food and pharmaceutical industry, the type of plasticizers used are polyols such as glycerol, sorbitol, mannitol and xylitol (Siepmann et al., 1998). Plasticizer has been commonly used

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to reduce the brittleness and increase the flexibility of the films which normally take place during handling and storage (Antoniou et al., 2014). However, it was reported that films incorporated with glycerol or sorbitol as plasticizers has shown to reduce their mechanical properties such as tensile strength and elongation at break (Sanyang et al., 2015). This study aims to investigate the effects of different concentrations of glycerol and gelation methods with calcium chloride on the structure and the chemical properties of the *S. polycystum* alginate edible films. In addition to the antioxidant properties of the edible films (Sellimi et al., 2015), this could serve as an alternative material that could provide additional health benefits when consumed. Thus, the NaAlg from *S. polycystum* harvested from Malaysia would add economic value to marine seaweeds of this part of the world.

2. Materials and methods

2.1. Sample collection and preparation

The S. polycystum samples were collected from Teluk Kemang, Port Dickson, Malaysia. The samples were rinsed with 0.1% NaCl solution to remove dirt and epiphytes. Then, the samples were air dried and powdered prior to analysis.

2.2. Extraction of sodium alginate and phytochemical analysis

The dried and powdered seaweed was soaked in 0.2 M hydrochloric acid at room temperature for 24 h. Subsequently, the residues were rinsed with distilled water and agitation for 5 h with 2% sodium carbonate. The extract was filtered, precipitated with ethanol (99%) to obtain a final concentration of 70%. The sample was rinsed with ethanol to a final concentration of 50% and this step was repeated twice, followed by methanol (99%) and acetone (99%). To access the purity of the crude polysaccharide samples, the presence of phytochemicals; terpenoids, cardiac glycosides, phenolics, flavonoids, saponins, alkaloids, and tannins were analysed based on the methods of Harbone (1973).

2.3. pH

The pH of sample was measured with a pH meter (Eutech Instruments P700, USA).

2.4. Antioxidant assays

Briefly, 600 μL of 0.16 mM DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) solution was added to 400 μL of 1% alginate sample and incubated in the dark at 37 °C for 30 min and the absorbance of the mixture was read at 540 nm. The DPPH value was expressed as μM Trolox equivalent (TE) per gram extract.

2.5. Preparation of sodium alginate films

1% sodium alginate solution was prepared by dissolving 5 g of powdered alginate in 500 mL of distilled water and stirred overnight to ensure homogeneity. Then, 100 mL of NaAlg solution was separated into 5 different Falcon tubes and glycerol was added at concentrations of 0.1%, 0.5%, 1.0%, 1.5% and control (without glycerol), respectively. The NaAlg was then subjected to 2 different gelation methods (protocol A and protocol B) with both, 1% and 3% $CaCl_2$ solutions.

2.6. Intrinsic viscosity

The viscosity of 1% NaAlg samples at different glycerol concentrations prior to treatments with $CaCl_2$ solutions were measured using DV2TLVTJ0 viscometer (Brookfield). The viscometer was operated with spindle no. 62 with mixing speed of 100 rpm and the results were recorded every 30 s. The intrinsic viscosity values were expressed as Pa s^{-1}.

2.7. External gelation method

Ten milliliters of alginate solutions with different glycerol concentrations (0.1%, 0.5%, 1.0%, 1.5%) and control were poured separately into petri-dishes with a diameter of approximately 8 cm and left to dry for 12–16 h in the oven at 50 °C. After drying, the films were transferred into a desiccator to remove moisture and keep them dry until further testing. Then, the dried films were soaked separately in 1% (w/v) and 3% (w/v) $CaCl_2$ solutions for 3 min and rinsed with distilled water before drying them in the oven at 50 °C for 2–3 h.

2.8. Internal gelation method

The 10 mL NaAlg solutions containing glycerol were heated to 70 °C and 1 mL of 1% and 3% $CaCl_2$ solutions were added separately into 100 mL NaAlg solution in a drop-like manner with constant stirring. To ensure equal size of drops of $CaCl_2$ at a consistent rate, a Biuret was used in the dripping process. Then, 10 mL of all the NaAlg samples were transferred to petri-dishes and dried in the oven at 50 °C for 12–16 h.

2.9. Transparency test

The films were cut into small rectangular strips (10 × 20 mm) and placed in a clear cuvette and absorbance readings were taken at 600 nm. The cuvette without the film was used as blank.

2.10. Swelling test

The film pieces (10 × 20 mm) were immersed in distilled water for 30 min. The films were removed and blotted with filter paper to remove excess water. The films were then weighed immediately and the swelling index was calculated as follows:

$$\text{Swelling index (\%)} = (\text{weight after immersion} - \text{weight before immersion}) / (\text{weight before immersion}) \times 100$$

2.11. Solubility test

Pieces of NaAlg films (20 × 30 mm) were cut and weighed to the nearest 1.0×10^{-4} g and placed in Falcon tubes with 50 mL deionized water. The samples were maintained under constant agitation for 30 mins at room temperature (approximately 25 °C). The remaining pieces of film after soaking were filtered through filter paper (Whatman no. 1), followed by oven drying at 50 °C to constant weight. Samples were measured in 3 replicates and the percentage of total soluble matter (% solubility) was calculated as follows:

$$\text{\% of solubility} = (\text{initial dry weight} - \text{final dry weight}) / (\text{initial dry weight}) \times 100$$

2.12. Transparency test

The films were cut into small rectangular strips (10 × 20 mm) and placed in a clear cuvette and absorbance readings were taken at 600 nm. The cuvette without the film was used as blank.

2.13. Fourier transform infrared (FTIR) spectroscopy measurement

Analysis of sodium alginate samples was performed by ATR-FTIR spectrophotometer with absorption region of 650–4000 cm^{-1}. The

dried alginate film was placed onto universal diamond ATR top-plate and the IR spectra were obtained based on the results of over 16 scans in the spectrophotometer resolution of 4 cm^{-1}.

2.14. Proton nuclear magnetic resonance (^1H NMR) spectrometry

The pH of 0.1% NaAlg solution in Milli-Q water was adjusted to pH 5.6 using HCl (1.0 and 0.1 M) and reflux at 100 °C for 1 h. After cooling at room temperature, the pH was adjusted to pH 3.8 and reflux for an additional 30 min. Then, the sample was cooled in ice to stop the hydrolysis, neutralized (~pH 7) using NaOH (1 and 0.1 M) and freeze-dried. The spectra were acquired on 0.1% w/v solutions of NaAlg in D_2O with a Fourier-transform Bruker 250 BioSpin supplied with an inverse multi-nuclear gradient probe-head with z-shielded gradient coils, and with a Silicon Graphics Workstation. The composition of the sugar and the block structure of alginate can be derived from the following relationships:

$$F_G = AA/(AB + AC); \quad F_{GG} = AC/(AB + AC)$$

The mole fraction of M (F_M) was derived from the normalization condition:

$$F_G + F_M = 1.0$$

The relations between the mole fractions and the doublet frequencies are given by

$$F_{GG} + F_{GM} = F_G; \quad F_{MM} + F_{MG} = F_M$$

2.15. Statistical analysis

All data are presented as the mean and standard deviation (SD). Statistical analyses were done using the IBM® SPSS 21.0 statistical package (Chicago, IL, USA).

3. Results and discussion

3.1. Yield of extract

The yield of NaAlg extracted from *Sargassum polycystum* was 15.85% of DW. Similar yield of NaAlg (20.00%) from this species was reported by Mushollaeni (2011) and from *S. wightii* (21.71%) and *S. myriocystum* (20.10%) (Subramanian et al., 2015). However, the yield of alginate varies depending on factors such as species, seasonal growth, pretreatment and extraction methods. The *S. polycystum* alginate was pH 7.42 and the commercial was pH 7.20.

3.2. DPPH˙ scavenging activity and phytochemical analysis

The *S. polycystum* alginate showed promising DPPH scavenging activity at 61 ± 0.27 μM TE/g extract with commercial sample exhibiting a lower activity at 0.57 ± 0.22 μM TE/g extract. Sellimi and colleagues, 2015 have also reported high radical scavenging activity of 0.5 mg mL^{-1} NaAlg extracts from *Cystoseira barbata* with 75% radical scavenging activity. The phytochemical analysis of *S. polycystum* identified saponins and flavonoids in the extract. However, tannin, terpenoid, cardiac glycoside, phenolics and alkaloid were absent. The commercial NaAlg samples of *Laminaria* species reported negative for all phytochemicals.

Even though, these compounds compromised the purity of the *S. polycystum* alginate, nevertheless, they could be an added value to the food preservative as they possess several biological activities such as antimicrobial, antiviral, antioxidant and anticoagulant (Jeyaraman et al., 2013). A study by Cox et al. (2011) reported that phytochemical compounds are incorporated into various industrial products due to their high tolerance level towards various factors such as heat and UV rays. Thus, it is worth exploring the synergistic effects of the phytochemicals and NaAlg as a powerful antioxidant tool.

Table 1
Transparency and viscosity of sodium alginate samples.

Glycerol	Viscosity (Pa s^{-1})		Transparency (OD_{600} nm)	
	Commercial	*S. polycystum*	Commercial	*S. polycystum*
0	0.149 ± 0.002	0.047 ± 0.009	Abs: 0.03 ± 0.001 %T: 92.9 ± 1.56	Abs: 0.09 ± 0.004% T: 81.00 ± 0.72
0.1	0.130 ± 0.000	0.047 ± 0.000	NA	NA
0.5	0.131 ± 0.000	0.046 ± 0.001	NA	NA
1.0	0.126 ± 0.001	0.047 ± 0.000	NA	NA
1.5	0.126 ± 0.004	0.046 ± 0.000	NA	NA

*Results expressed as means ± standard deviation; Values are means of duplicate from each concentration (n = 2).

3.3. Transparency and viscosity

The transparency of the films was accessed by measuring the absorbance (Abs) and transmittance (T) values using a UV–vis spectrophotometer at 600 nm. The NaAlg film appeared brownish compared to the commercial film. Therefore, the *S. polycystum* film (ABS: 0.09; T = 81.00%) showed a higher absorbance and lower % of transmittance values compared to the commercial sample. Most commonly, the quality of the NaAlg for commercial is the result of several purification processes that removes impurities in the extracts. Even though, the purity of the *S. polycystum* alginate obtained in this study was compromised, nevertheless, the presence of fucoxanthin (yellowish-brown pigment) and other phytochemicals could add value to the edible film as they possess high antioxidant activities (Jeyaraman et al., 2013; Peng et al., 2011).

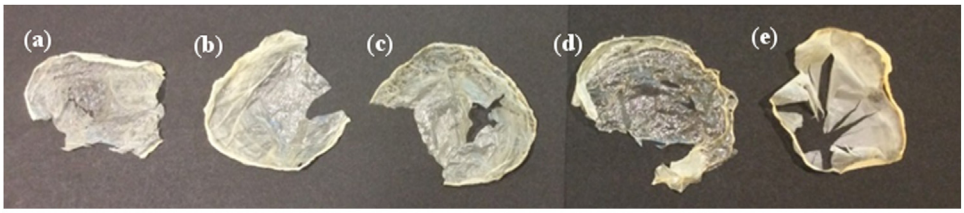
The viscosity of the NaAlg in different concentration of glycerol is shown in Table 1. The viscosity of the commercial samples decreases in the range of 0.149–0.126 Pa s^{-1} with increasing glycerol concentrations. However, the viscosity of the *S. polycystum* alginate with and without glycerol does not deviate significantly at 0.047 and 0.046 Pa^{-1}, respectively (Table 1). Studies have reported that glycerol could reduce the intermolecular forces between the polymers and thus increases the mobility of the polymer chains (Treenate et al., 2015). Generally, *S. polycystum* alginate (0.047 Pa s^{-1}) showed lower intrinsic viscosity compared to the commercial samples (0.149 Pa s^{-1}) with the differences observed could be due to ratio of M/G subunits (Venkatesan et al., 2017). Generally, species alginate has lower viscosity compared to the *Laminaria* species (commercial alginate) (Torres et al., 2007; Fertah et al., 2017). Hence, *S. polycystum* alginate is useful in industries such as pharmaceutical and medical that relies on alginate that is less viscous in their applications (McHugh, 2003).

3.4. Physical appearance of alginate films in $CaCl_2$

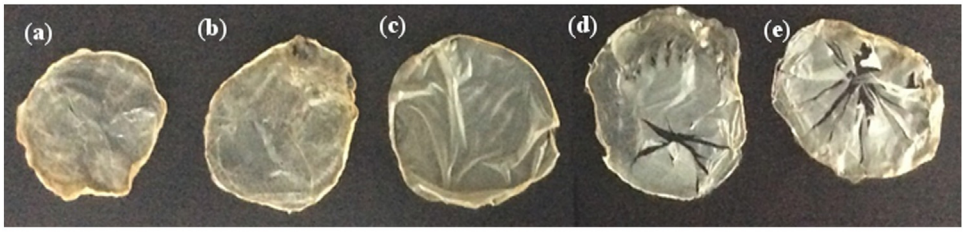
The appearance of the *S. polycystum* films with different glycerol concentrations, cross-linked with $CaCl_2$ are shown in Fig. 1(a-b). In protocol A (Fig. 1a), the *S. polycystum* films produced with different glycerol (0.1–1.5%) concentrations are rigid and brittle. However, the films produced in protocol B (Fig. 1b) maintained its shape and appeared more flexible and less brittle at increasing glycerol concentrations. The opaque films produced, though protocol A and B could be resulted from the cross-linking of alginate with the Ca^{2+}.

3.5. Swelling index and solubility of alginate films

The extent of the simultaneous Ca^{2+} cross-linking and plasticization of the alginate with glycerol was determined by the level of structural organization. The alginate films prepared with different concentrations



(a)



(b)

Fig. 1. (a): *S. polycystum* alginate films (3% $CaCl_2$) prepared following protocol A with different glycerol concentrations; (a) without glycerol, (b) 0.1% glycerol, (c) 0.5% glycerol, (d) 1.0% glycerol and (e) 1.5% glycerol. **(b)**: *S. polycystum* alginate films (3% $CaCl_2$) prepared following protocol B with different glycerol concentrations; (a) without glycerol, (b) 0.1% glycerol, (c) 0.5% glycerol, (d) 1.0% glycerol and (e) 1.5% glycerol.

Table 2
Solubility and swelling index of sodium alginate samples.

		Protocol A		Protocol B	
	$CaCl_2$	Commercial	*S. polycystum*	Commercial	*S. polycystum*
Solubility	**1%**	17.12 ± 2.20	10.77 ± 3.02	7.09 ± 0.85	38.33 ± 4.23
	3%	5.86 ± 0.74	2.40 ± 0.14	3.77 ± 0.45	25.54 ± 2.83
Swelling	**1%**	30.65 ± 1.20	27.35 ± 9.83	41.90 ± 1.84	66.10 ± 18.53
	3%	13.50 ± 4.67	16.25 ± 3.75	25.70 ± 2.26	41.60 ± 29.98

*Results expressed as means ± standard deviation; Values are means of duplicate from each concentration (n = 2).

(a) Protocol A: alginate in 1% $CaCl_2$

(a) Protocol A: after soaking in water

(b) Protocol B: alginate in 1% $CaCl_2$

(b) Protocol B: after soaking in water

Fig. 2. (a,b): The solubility of the commercial alginate films.

of glycerol were treated with $CaCl_2$ using protocol A (external gelation) and protocol B (internal gelation). The alginate that underwent external gelation resulted in a less elastic film with rough surface. However, the film produced by internal gelation method resulted in a more flexible film with a smoother surface (Fig. 1). It was reported that by soaking the alginate film in the bivalent ions, instantaneous cross linking bonds between the polymers results in the rough surface of the film (Pavlath et al., 1999). Alginate composed of homopolymeric regions of

Table 3
Solubility and swelling index of sodium alginate with different glycerol concentrations.

			Protocol A		Protocol B	
	$CaCl_2$	Glycerol	Commercial	*S. polycystum*	Commercial	*S. polycystum*
Solubility	**1%**	0.1	23.48 ± 8.52	11.85 ± 3.44	14.17 ± 1.59	15.05 ± 4.96
		0.5	34.34 ± 3.30	12.98 ± 0.25	37.65 ± 6.58	15.80 ± 0.59
		1.0	52.58 ± 12.95	23.44 ± 2.21	80.06 ± 5.93	27.58 ± 9.09
		1.5	84.37 ± 3.60	66.20 ± 1.22	86.42 ± 0.45	51.14 ± 1.61
	3%	0.1	43.65 ± 1.11	3.31 ± 1.61	15.48 ± 1.68	3.53 ± 2.96
		0.5	46.00 ± 5.66	3.52 ± 2.96	18.24 ± 0.33	3.30 ± 1.61
		1.0	54.69 ± 5.40	7.11 ± 1.73	37.84 ± 7.64	7.11 ± 1.73
		1.5	64.88 ± 4.88	13.10 ± 0.33	44.52 ± 1.68	13.10 ± 0.33
Swelling	**1%**	0.1	35.60 ± 40.16	9.85 ± 1.10	21.85 ± 6.80	39.20 ± 3.19
		0.5	134.20 ± 35.50	11.70 ± 0.42	26.30 ± 3.11	59.3 ± 4.47
		1.0	140.65 ± 16.33	44.65 ± 2.01	39.75 ± 3.32	93.3 ± 1.20
		1.5	178.20 ± 12.87	89.60 ± 7.82	50.05 ± 2.03	97.70 ± 1.70
	3%	0.1	7.30 ± 2.55	8.50 ± 0.85	1.85 ± 0.78	9.95 ± 12.09
		0.5	16.15 ± 2.05	16.95 ± 0.92	3.05 ± 0.35	10.45 ± 9.83
		1.0	36.80 ± 0.99	36.80 ± 0.99	5.70 ± 4.10	95.45 ± 1.14
		1.5	82.70 ± 11.31	69.00 ± 3.69	34.45 ± 2.76	235.20 ± 8.67

*Results expressed as means ± standard deviation; Values are means of duplicate from each concentration (n = 2).

Table 4
Physical characterisation of sodium alginate from *S. Polycystum*.

	Composition fractions		Doublet frequencies				
^{1}H NMR	F_M	F_G	F_{MM}	F_{MG}	F_{GM}	F_{GG}	M/G
S. polycystum	0.423	0.577	0.337	0.086	0.086	0.491	0.733
Commercial	0.260	0.740	0.176	0.084	0.084	0.656	0.351
ATR-FTIR	**Wavenumber (cm^{-1})**		**Functional group**				
S. polycystum	3374.12, 3451.0		stretching vibrations of O–H				
	2944.05, 2923.1, 2839.2		stretching vibrations of C–H				
	1605.30, 1654.2; 1409.70		asymmetrical and symmetrical stretching of carboxylate				
	1090.10, 1030.72, 1091.8, 1025.5		stretching vibrations of pyranose rings				
	945.5		stretching vibration of uronic acid				
	810.67, 840.36		characteristic peak of mannuronic acid				

mannuronic (M) and guluronic (G) residues. Cross-linking of divalent Ca^{2+} ions and GG blocks of two alginate chains, forms an ordered chain and the expulsion of water (Rezende et al., 2007).

In Table 2, the solubility of the *S. polycystum* film in protocol A and B was 10.77% and 38.33% and in 3% $CaCl_2$ the solubility was 2.40% and 25.54%, respectively. In Fig. 2a, the *S. polycystum* alginate soaked in 1% $CaCl_2$ (protocol A) produced a less soluble film with the shape maintained. Meanwhile, the film prepared with the addition of $CaCl_2$ (protocol B) slightly disintegrates during immersion in water (Fig. 2b). Nevertheless, at 3% $CaCl_2$, the integrity of the films produced through both methods were maintained, thus, suggesting that at a higher Ca^{2+} concentration increases the ionic cross-linking of the carboxyl groups and resulting in a less soluble film (Rhim, 2004). Even though protocol A produces films that are less soluble, the appearance of the film was less favourable. In the external gelation, due to the instantaneous cross-linking between alginate and Ca^{2+}, localized gelling areas are produced, affecting the quality of films (Al-Remawi, 2012). Therefore, protocol B which involves slow release of Ca^{2+} deemed as a more suitable method to because a uniform film was produced (Kaletunec et al., 1991). The swelling index of *S. polycystum* films produced by protocol B in 1% and 3% $CaCl_2$ were 27.35% and 16.25%, respectively. In Table 3, the swelling ability improved as glycerol was added. Therefore, increasing the glycerol concentration with the cross-linking solution improved the film volumetric swelling and flexibility and decreased the resistance to tensile stress (Peteiro, 2018).

3.6. Physicochemical characterisation of NaAlg film

The characterizing of both the sequence and distribution of mannuronic (M) and guluronic (G) residues of alginate is shown in Table 4. In Fig. 3, the ^{1}H NMR spectra of NaAlg sample showed specific peaks of

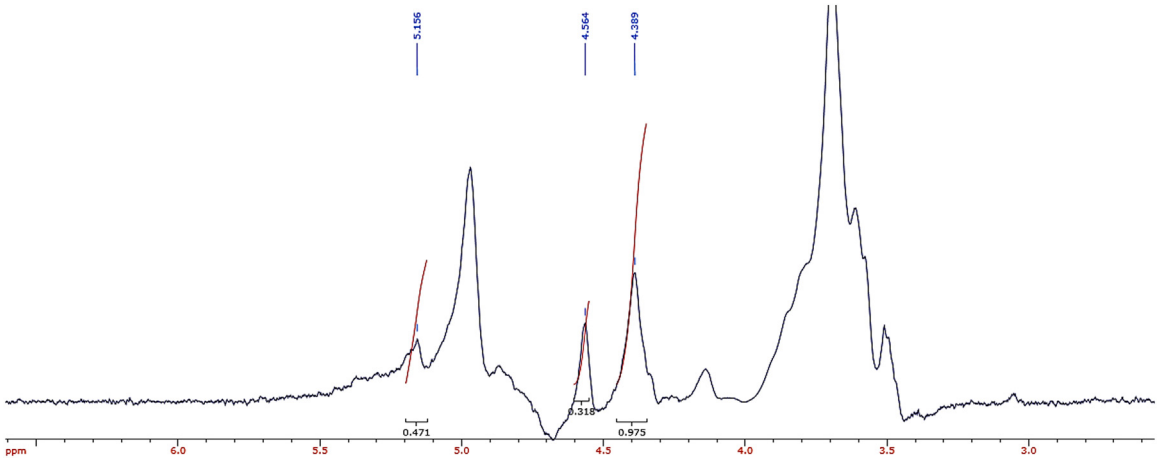


Fig. 3. ^{1}H NMR spectrum of sodium alginate extracted from *Sargassum polycystum*.

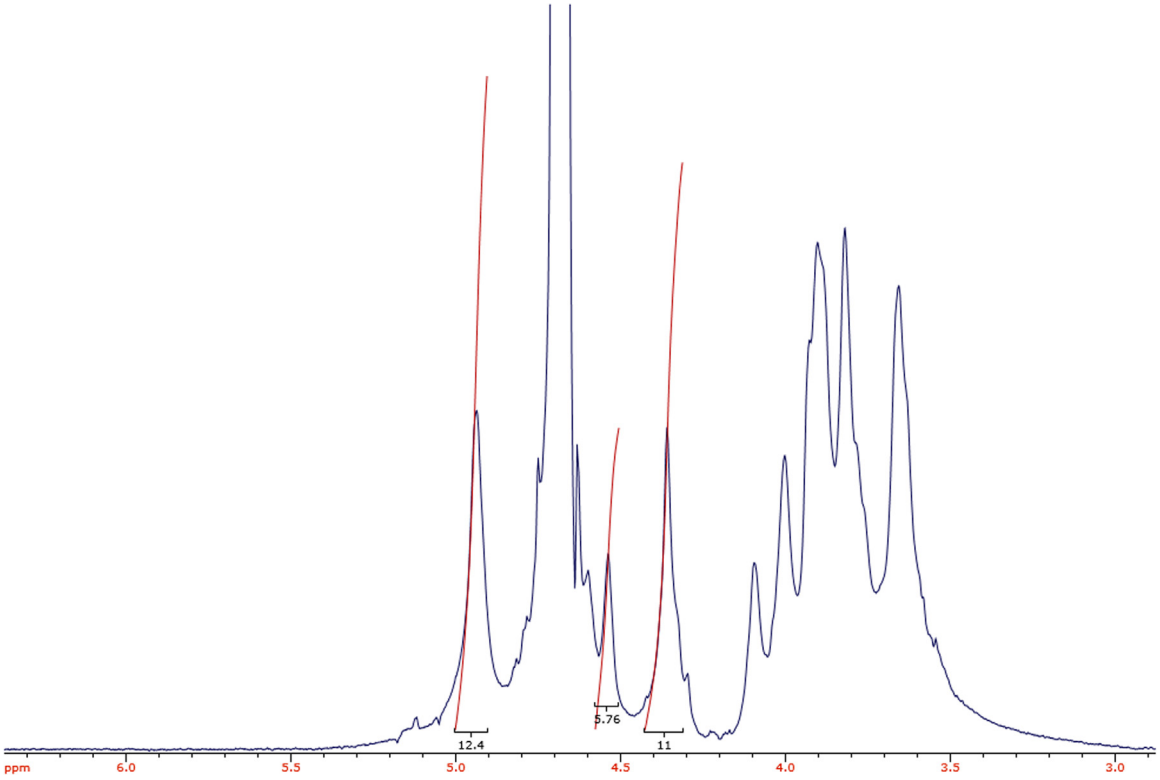


Fig. 4. ^{1}H NMR spectrum of commercial sodium alginate from *Laminarian* species.

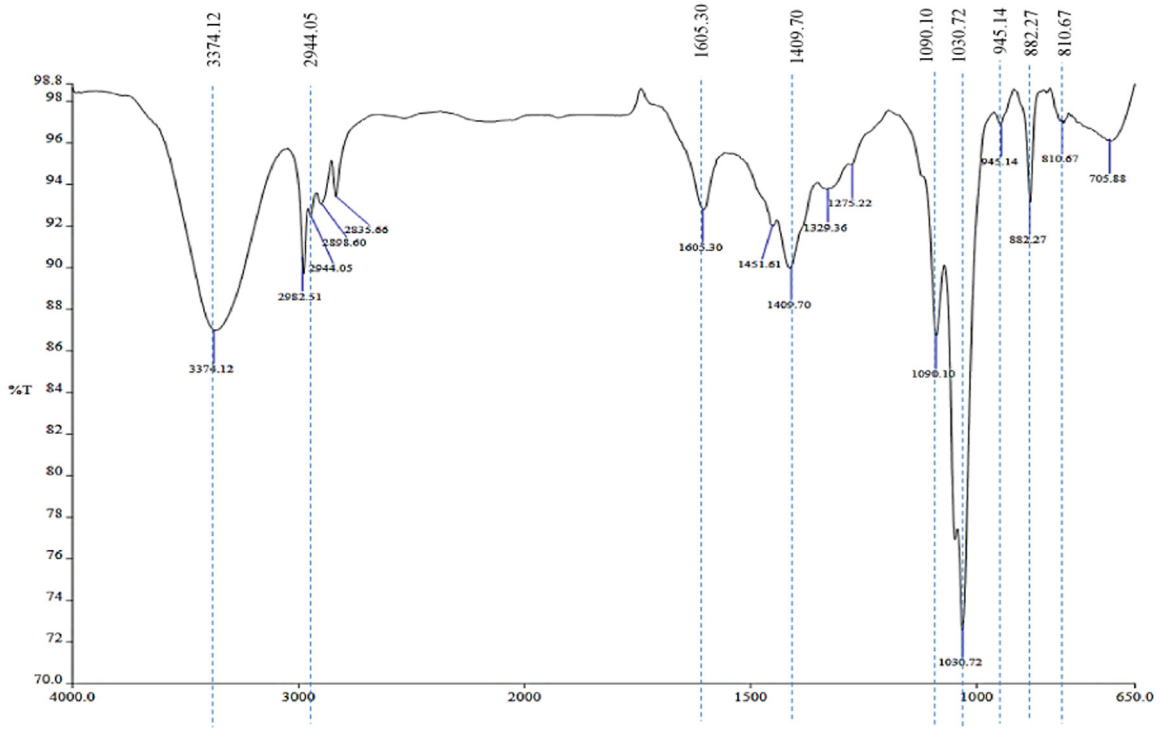


Fig. 5. ATR-FTIR analysis of *S. polycystum* alginate.

5.16, 4.56, and 4.39 ppm assigned to peak A (H1-G), peak B (H1-M + H5-GM) and peak C (H5-GG), respectively. In Fig. 4, the ^{1}H NMR spectra of the NaAlg commercial sample showed peaks at 4.94, 4.54, and 4.36 ppm assigned to peak A, peak B, and peak C, respectively. A study by Yuan and Macquarrie (2015) assigned similar peaks for A, B and C at 4.9, 4.5, and 4.3 ppm, respectively. However, another study by Salomonsen et al. (2008) assigned the limits for peak A (5.18–4.96 ppm), peak B (4.82–4.57 ppm) and peak C (4.55–4.38 ppm) that slightly deviates 0.01–0.03 ppm from the peaks derived from our study. The discrepancies could be contributed by the different procedures employed and the rate of cleavage of the chain by acid hydrolysis (Salomonsen et al., 2008). The composition of the mannuronic and

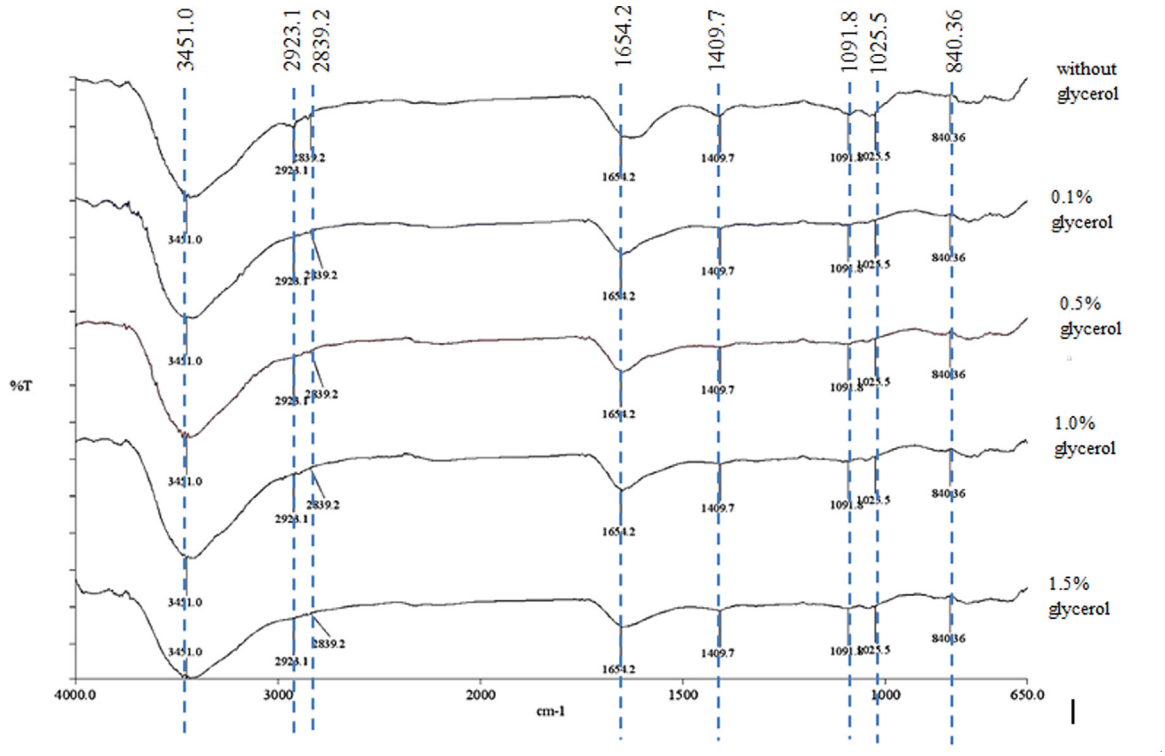


Fig. 6. ATR-FTIR analysis of *S. polycystum* alginate in different glycerol concentrations (0.1–1.5%) immersed in 3% $CaCl_2$.

guluroric acid frequencies in *S. polycystum* showed a high M/G ratio of 0.733. However, the commercial alginate (*Laminaria* species) showed a lower M/G ratio of 0.351. The strength of the gel depends on the content and the length of the guluronic acid whereby alginate with high M/G ratios results in a more elastic gel and low M/G ratios provides brittle gels (Peteiro, 2018).

Fig. 5 shows the ATR-FTIR spectrum of *S. polycystum* NaAlg with functional groups that corresponded to the absorption bands in 4000–650 cm^{-1} range is depicted in Table 4. The absorption bands at 3451.0–33.74.1 cm^{-1}, 2923.1–2839.2 cm^{-1}, 1605.30 cm^{-1} and 1409.70 cm^{-1} corresponded to the stretching vibrations of the O–H group, C–H group, asymmetrical and symmetrical stretching of carboxylate groups, respectively. The absorption bands showed decrease in intensity when the alginate was cross-linked with Ca^{2+}. The differences arise from the participation of hydroxyl and carboxylate groups in the cross-linked structure that resulted in a decrease in its intermolecular hydrogen bonding (Daemi and Barikani, 2012). In Fig. 6, the bonds formed between the alginate and the glycerol has also resulted in the narrowing of the O–H adsorption bands as the glycerol concentration increases from 0.1% to 1.5% which explains the increase in the solubility of the cross-linked films (Table 2).

4. Conclusions

In our study, the characteristics of an edible film are slightly soluble, flexible with high swelling index. On the basis of the results presented here, it can be concluded that the alginate films produced in this study resulted in films with different characteristics. The films were prepared through external and internal gelation methods in 1% and 3% $CaCl_2$ with different glycerol concentrations (0.1–1.5%). The physicochemical properties of the films were affected by the cross-linking with Ca^{2+} and glycerol. Some concluding observations from the investigation are given below.

- The film produced through the internal gelation method produced film with a uniform surface with less creases compared to the external gelation method.
- The alginate film was less soluble in 3% $CaCl_2$ compared to 1% $CaCl_2$ with opaque appearance suggesting cross-linking between the alginate and bivalents. Increasing glycerol has also improved the solubility of the films.
- The Ca^{2+} cross-linked films with increasing glycerol concentrations as plasticizers with 0.5% glycerol as the optimum concentration that maintained the integrity of the film in terms of appearance and flexibility.
- The good antioxidant properties and neutral pH of the film makes them a safe edible material to be consumed.

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Declarations of interest

None.

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