Research Article
Synthesis and Properties of Carboxymethyl Gellan and Application as Edible Film

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Abstract: In order to develop a water solubility material possessing transparent film, Carboxymethyl Gellan (CG) was prepared by the reaction of Gellan (G) with sodium chloroacetate. The products were characterized by Fourier Transform Infrared (FTIR), Nuclear Magnetic Resonance (NMR) spectroscopy and X-Ray Diffraction (XRD). The results showed that the introduction of carboxymethyl groups improved the solubility of G. The CG films were prepared by using the casting solvent evaporation method. The properties of CG film were investigated compared to G films by Thermogravimetric Analysis (TGA), mechanical properties, water barrier properties and transparency and moisture sorption isotherm. CG film had well thermal stability, the lower Tensile Strength (TS) and higher Elongation at Break (EAB), Water Vapor Permeability (WVP) and transparency in contrast to G films. The results suggested that CG would be good use in film application as food package.

Keywords: Carboxymethyl, edible film, gellan, properties

INTRODUCTION

Gellan (G), which produced by the non-pathogenic bacterium *Sphingomonas paucimobilis* ATCC 31461 (Mazen *et al*., 1999), was a linear, anionic polysaccharide based on a tetrasaccharide repeating unit (Jannson *et al*., 1983; Jay *et al*., 1998) composed by the backbone [3)-β-D-Glucose-(1,4)- β-Glucuronic acid-(1,4)- β-D-Glucose-(1,4)- α-L-Rhamnose-(1,]n (Sá-Correia *et al*., 2002; Fialho *et al*., 2008). Due to its excellent physical functional properties and biological activities, gellan was extensively utilized in the food, cosmetics, textile and pharmaceutical industries.

Nowadays, there was a considerable interest in using gellan to prepare edible film (Yang and Paulson, 2000; Sudhamani *et al*., 2003; Xu *et al*., 2007; Lee *et al*., 2004; Pranoto *et al*., 2007; Tapia *et al*., 2007), which could extend the shelf-life of foods. However, aqueous solutions of gellan form three-dimensional network by complexation with cations and hydrogen bonding with water gels in the presence of cations (Morris *et al*., 2012). Therefore, transparent aqueous solution with high mass gellan gum was difficultly obtained even at high temperature. Extensive use of this biopolymer as edible films was hampered by its water solubility. To avoid these problems, chemical modifications of gellan was proposed. Carboxymethyl modification had been studied for gellan (Miyamoto *et al*., 1996; Ahuja *et al*., 2013). It had a number of

sodium carboxymethyl groups (CH$_2$COONa), introduced into the polysaccharides molecule, which promote water solubility.

In this study, carboxymethyl gellan was synthesized and the products were characterized by FTIR, ^{1}H NMR. The film properties including thermogravimetric analysis, moisture sorption isotherm, film transparency, mechanical (tensile strength and elongation at break) and barrier properties of CG were evaluated and compared with G.

MATERIALS AND EXPERIMENTS METHODS

Synthesis of CG: CG was synthesized by reacting G (C P Kelco Inc) with sodium chloroacetate (SC, Sigma). The reaction was carried out in a 250 mL three necked round-bottom flask, equipped with motor-driven stirrer (Shanghai shenshun, China). Sodium hydroxide was added to ethanol in the flask and the mixture was stirred until sodium hydroxide was completely dissolved. G was added to the solution and continued for 30 min at room temperature. Sodium chloroacetate was added to the solution and the temperature was raised to 50°C for 3 h. At the end of the reaction, pH (7.0) was adjusted with 4.0 M HCl solution. The modified CG solution was purified by ethanol for several times to remove the excess of Sodium. Purified CG was obtained and dried in an oven at 60°C for 24 h.

Characterization of CG: The chemistry of the modified materials was analyzed by Fourier transform infrared spectroscopy. The FTIR spectra of G and CG were run as KBr pellets using a 470 Nicolet FTIR spectrometer (Nicolet Instrument Corporation, USA) in the frequency range 400-4000/cm.

The chemical modification to G was assessed by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy. ^{1}H NMR spectra were obtained on an Avance 400 MHz spectrometer (Bruker, Rheinfelden, Germany) in D_2O as a solvent.

Preparation of films: G/CG of 2.4 g was dissolved in 60 mL de-ionized water and heated at 90°C. After complete dissolution, the solution was poured onto leveled glass plates fitted with rims around the edge, followed by drying in an electrical blast drying chest (Shanghai Sanfa Scientific Instruments Co. Ltd., China) at 60°C for 24 h. The resulting films were peeled off from the glass plate and conditioned at 23±1°C and 55±1% RH prior for further testing. All samples were tested in triplicate.

Characterization of films: Thermal stability and degradation of films were analyzed by Thermogravimetric Analyses (TGA), performed with a Mettler TGA-SDTA851^e (Mettler Toledo Instrument Company Limited). Samples were placed in the balance system and heated from 20 to 560°C at a heating rate of 10°C/min.

Moisture sorption isotherm was measured according to Tong *et al.* (2013).

The resulting moisture sorption isotherm data were fitted to the GAB sorption isotherm model Eq. (1); Tong *et al.* (2013) using Origin 8.5 software: where M_0 is the monomolecular layer moisture content and C and K are temperature-dependent parameters related to heats of sorption for monolayer and multilayer water, respectively:

$$M = \frac{M_0 C K a_w}{(1 - K a_w)(1 - K a_w + C K a_w)} \tag{1}$$

Film transparency was measured according to the method of Ubonrat Siripatrawan and by using the UV spectrophotometer (UV-2802H, Unico, Shanghai) and calculated according to Eq. (2). The high values of T indicate lower transparency and higher degree of opacity:

$$T = -\frac{\log T_{600}}{thikness} \tag{2}$$

Tensile Strength (TS) and Elongation at Break (EAB) of the films were determined using a Texture Analyzer (TA-XT2i) according to the ASTM standard method D882-02 (ASTM D882-02, 2002; Tong *et al.*, 2013).

Water vapor permeability (WVP) of the film specimen was measured according to modified ASTM E96 method (ASTM E96, 1993).
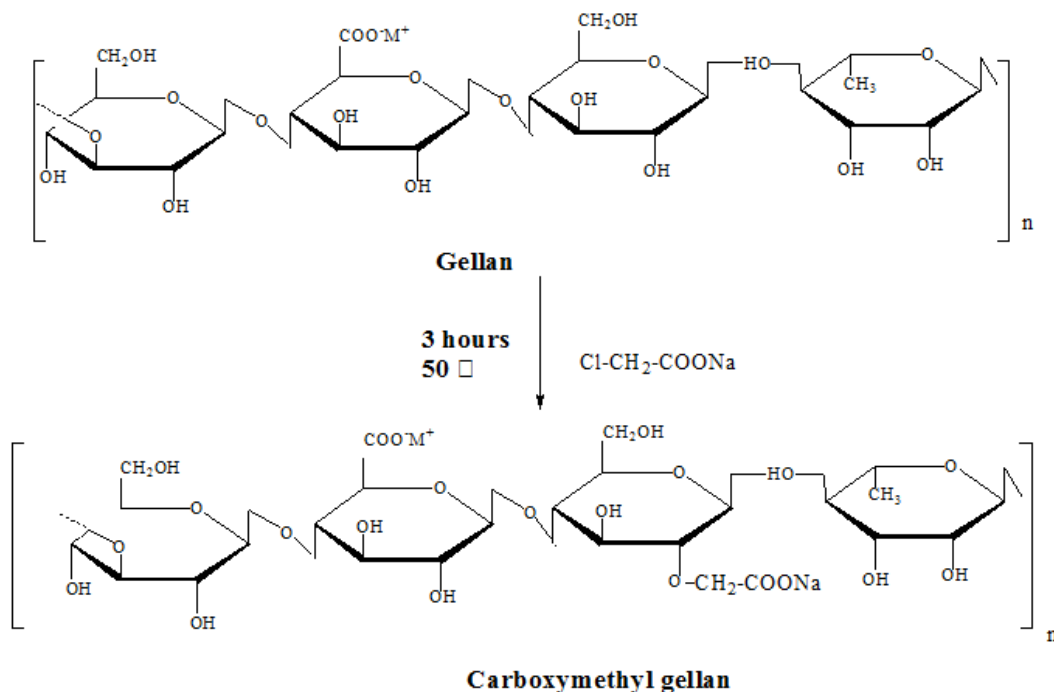

Fig. 1: Synthesis of carboxymethyl gellan

RESULTS AND DISCUSSION

Preparation and Properties analysis of CG: The brief processes and the methods of CG preparation were shown in Fig. 1. CG, which achieved with G (20 g), sodium hydroxide (2.46 g) and Sodium chloroacetate (1.46 g), had good water solubility. The solubility of CG were examined and compared with G. The completely dissolved time in water at 90°C of 1% (w/w) CG and G were 1 min and 30 min, respectively. As expected, the CG displayed better solubility than G, indicating that the introduction of carboxymethyl groups was a convenient and effective method to make G had solubility ability.

Characterization of CG: The FTIR spectra of CG and G are presented in Fig. 2. FTIR spectroscopy showed similarity in CG and G. The band stretch around 3400 cm^{-1} was attributed to hydroxyls on the gellan molecules. The band at 2924 cm^{-1} is attributed to -CH_2 symmetrical stretching vibrations. The absorption peaks at 1410 cm^{-1} and 1610 cm^{-1} could be, respectively, assigned to the symmetry and asymmetry stretch vibration of COO– (Sudhamani *et al*., 2003; Xu *et al*., 2007). In the CG, the COO– band to shift from 1609 to 1613 cm^{-1} and from 1409 to1413 cm^{-1}, respectively. This provided evidence that the symmetry and asymmetry vibrations of COO– bonds were enhanced, due to the carboxymethylation of gellan did take place.

Additionally, the C–O stretching band at 1030 cm^{-1} corresponding to the primary hydroxyl group disappears, verifying a high carboxymethylation of OH the formation of the CG was also confirmed.
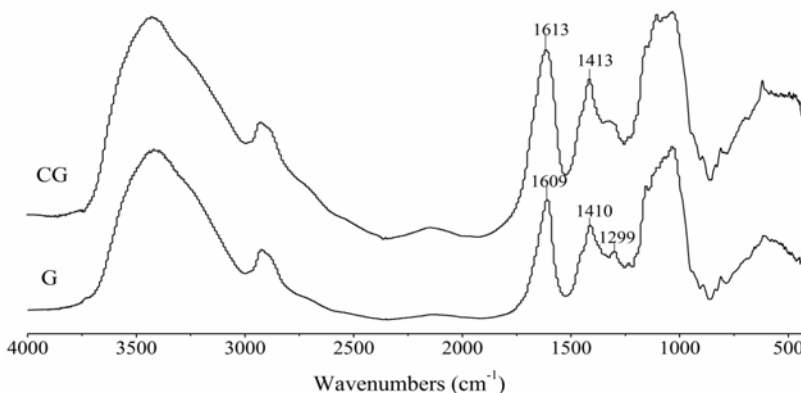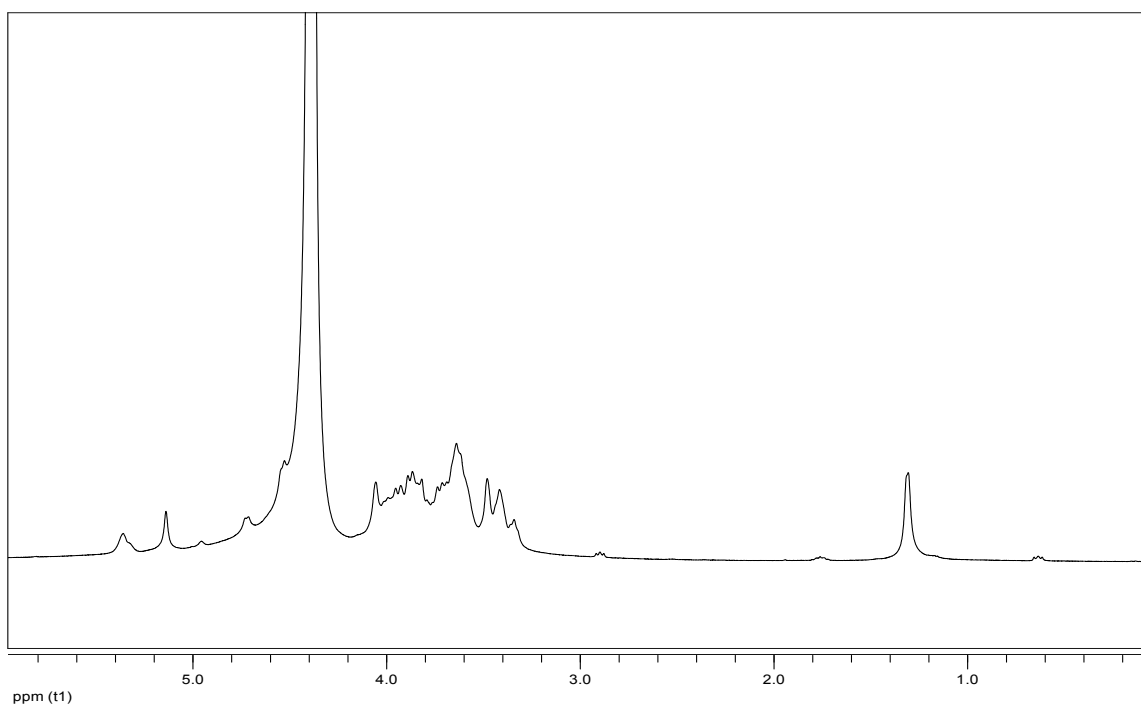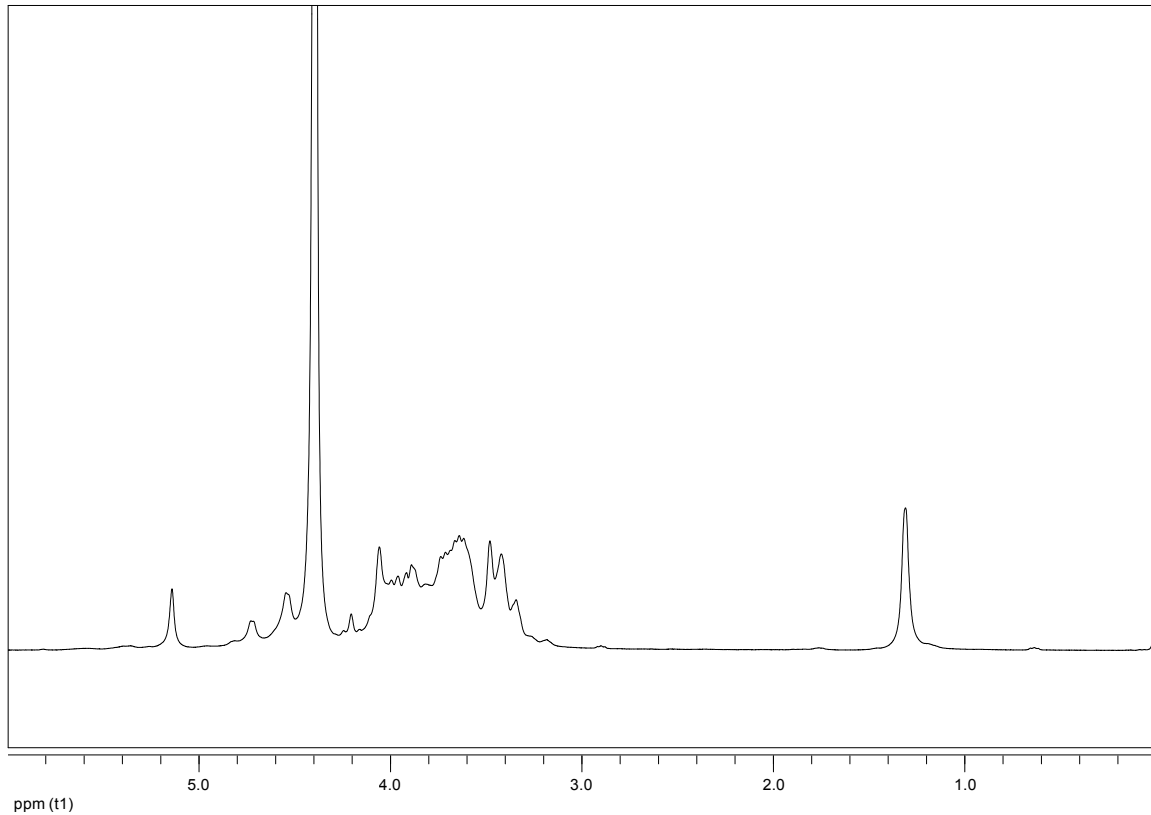

Fig. 2: The FIIR spectra of gellan (G) and CG (CG)

(a)

(b)

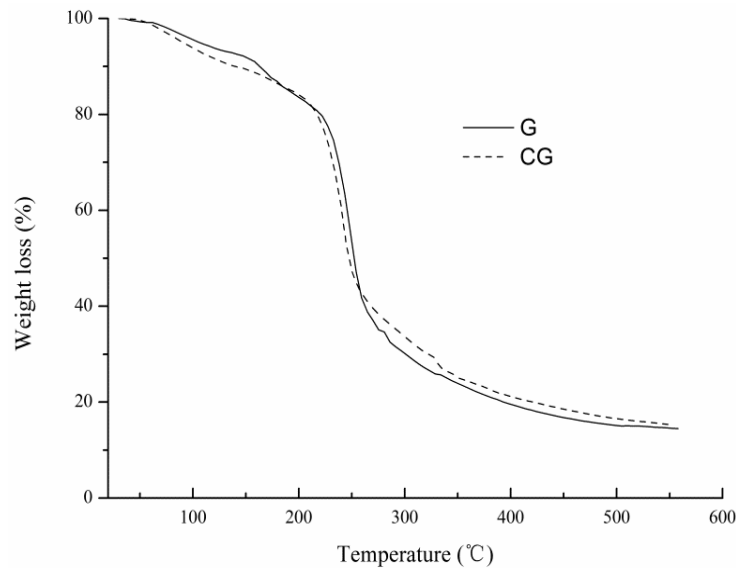Fig. 3: ^{1}H NMR spectra of gellan (a) and carboxymethyl gellan (b)

Fig. 4: TGA thermograms of gellan and carboxymethyl gellan films

The G and CG polymers were analyzed based on the ^{1}H NMR spectra results (Fig. 3). These results showed the presence of characteristic peaks that correspond to CH of rhamnose (δ 5.2-5.6 ppm), CH of glucuronic acid (δ 5.0-5.2 ppm), CH of glucose (ppm) and CH$_3$ of rhamnose (δ 1.3 ppm) (Coutinho *et al.*, 2010; Lee *et al.*, 2012). The spectrum confirms the incorporation of the carboxymethyl group by the presence of methylidyne proton peaks at 4.2. The degree of carboxymethyl substitution can be

conveniently determined by comparing the integrated intensity of the pwohenyl and CH CH peaks of the carboxymethyle group to the integral of the CH_3 protons (δ 1.3 ppm) of gellan. Accordingly, the degree of carboxymethyl substitution was approximately 0.25 (Dong *et al.*, 2005; Lee *et al.*, 2012).

Thermogravimetric Analysis (TGA): TGA was performed to evaluate the stability of the films. TGA curves (Fig. 4) show the thermal events for films. The derivative of weight loss (DTG) curves of films showed at least two significant thermal events (Fig. 5). The first slight weight loss at low temperature could be attributed to the water evaporation. The weight loss of CG film (10.6%) was higher than G film (8.5%), indicating that

the former was more hydrophilic. The greater moisture content for CG can be attributed to the formation of water hydration spheres around the substituted hydrophilic -COONa. The second major weight loss occurring could be attributed to the decomposition of G film (249°C) and CG film (240°C) (Zohuriaan and Shokrolahi, 2004). It was clear from the results that the thermal stability of CG film was nearly equal to G films.

Moisture sorption isotherm: Moisture sorption isotherms of G and CG films are presented in Fig. 6. As shown, the equilibrium moisture content increased slowly with increasing environmental water activities up to about 0.5 and then a steep rise thereafter.
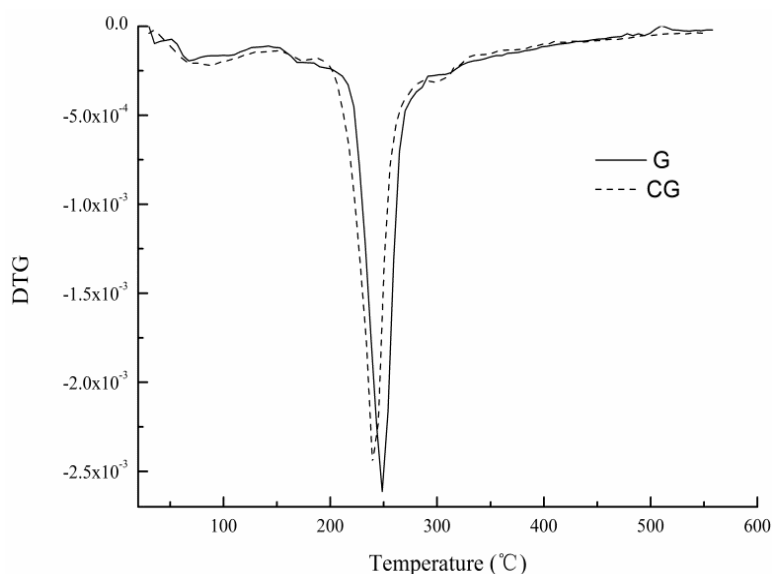

Fig. 5: Derivative thermogravimetric (DTG) curves of gellan and carboxymethyl gellan films
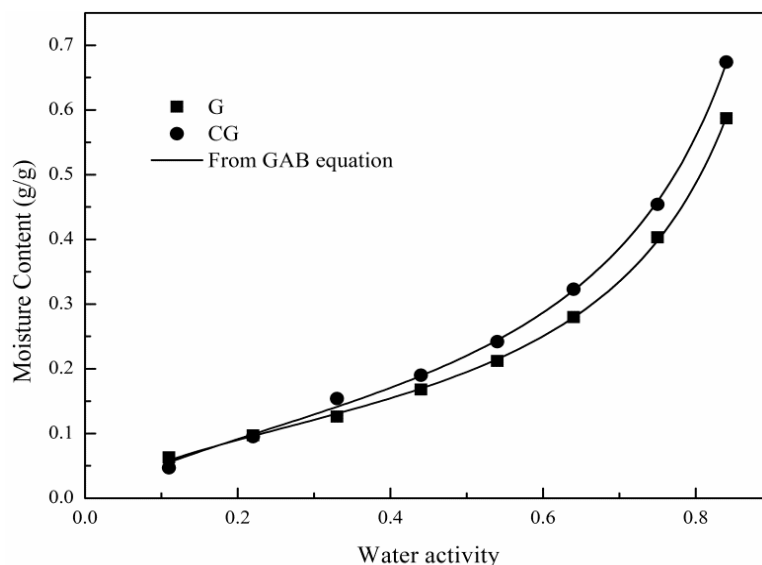

Fig. 6: Moisture sorption isotherms at 25 °C of CG film and G film (solid lines represent the GAB model fit to the data)

Table 1: GAB equation parameters for gellan, CG films at 25°C

Films	M_0	C	K	R^2
Gellan	0.117	6.788	0.961	0.999
CG	0.145	4.478	0.948	0.998

Table 2: The properties of the CG and G films

Film	TS (MPa)	EAB (%)	WVP$\times10^{-7}$ (g m/Pa h m^2)	T
Gellan	17.48	11	2.64	1.23
CG	13.37	23	2.92	0.51

Among the samples tested, CG film exhibited the lower moisture content and G film had the higher. This was consistent with the result of thermo gravimetric analysis.

The model parameters calculated by the GAB equations were summarised in Table 1. Generally, the value of monolayer (M_0) indicated the amount of water that was strongly adsorbed to specific sites of food for optimum stability. The predicted M_0 value of the G film (0.117 g H_2O/g dry basis) was lower than that of the CG film (0.145 g H_2O/g dry basis), implying that the film of CG might have induced a more open film morphology that increased the number of active sites. The C value for the CG films was the lower than G films, suggesting that the monolayer moisture interaction was the weaker for the CG films. The magnitudes of the K values are comparable with GAB fits for biopolymers commonly encountered in foods. It has been reported that the higher the K value, the more pronounced the upswing of the isotherm beyond the intermediate a_w range (Timmermann *et al*., 2001; Xiao *et al*., 2012).

The properties of CG and G films: The transparency of CG and G were summarized in Table 2. As expected, introduction of the carboxymethyl to G led to an increase in the transparency of the films. The transparency of the CG film reached 0.51, which is more than a 50% increment in the transparency of G film.

The tensile strength and elongation at the break of the CG and G films were measured and shown in Table 2. The tensile strength measured film strength and the elongation at the break was an indicator of the film flexibility. TS and EAB values for G films were 17.48 MPa and 11%, respectively. In contrast, CG films had weaker TS and higher EAB, at 13.37 MPa and 23%, respectively. The small amount of cations in commercial G powder could be responsible for cation crosslinks between the G molecular chains. These extensive interchain interactions may contribute to the high mechanical strength and low flexibility of G film (Yang and Paulson, 2000). However, the CG films probably reduced the cation crosslink due to the carboxymethl substitute. As a result, the lower TS and higher extensibility of CG film as compared to G film.

Water vapor permeability is an important criterion for many practical applications of edible films. Low WVP values widen the application of a composite packaging film, especially in a highly humid environment. The WVP data for CG and G films were summarized in Table 2. WVP of CG and G films did not show significant differences. Since CG was more hygroscopic than G, the affinity for water molecules would be higher in these films and therefore resulting in higher water diffusion giving films with higher WVP.

CONCLUSION

CG was successfully synthesized and characterized by FTIR and ^{1}H NMR. It was confirmed that the CG had higher solubility in water. Furthermore, the characteristics of CG film were investigated in comparison with G film. It was demonstrated that CG film exhibited good thermal stability, moisture absorbability, mechanical properties, water barrier properties and transparency, suggesting that CG was good use in film application as food package.

ACKNOWLEDGMENT

REFERENCES

Ahuja, M., S. Singh and A. Kumar, 2013. Evaluation of carboxymethyl gellan gum as a mucoadhesive polymer. Int. J. Biol. Macromol., 53: 114-121.

ASTM D882-02, 2002. Standard Test Method for Tensile Properties of Thin Plastic Sheeting. American Society for Testing and Materials, Philadelphia, PA.

ASTM E96, 1993. Standard Test Methods for Water Vapor Transmission of Materials. American Society for Testing and Materials, Philadelphia, PA.

Coutinho, D.F., S.V. Sant, H. Shin, J.T. Oliveira, M.E. Gomes, N.M. Neves, A. Khademhosseini and R.L. Reis, 2010. Modified Gellan Gum hydrogels with tunable physical and mechanical properties. Biomaterials, 31(29): 7494-7502.

Dong, C.M., X. Wu, J. Caves, S.S. Rele, B.S. Thomas and E.L. Chaikof, 2005. Photomediated crosslinking of C6-cinnamate derivatized type I collagen. Biomaterials, 26(18): 4041-4049.

Fialho, A.M., L.M. Moreira, A.T. Granja, A.O. Popescu, K. Hoffmann and I. Sá-Correia, 2008. Occurrence, production, and applications of gellan: Current state and perspectives. Appl. Microbiol. Biot., 79(6): 889-900.

Jannson, P.E., B. Lindeberg and P.A. Sandford, 1983. Structural studies of gellan gum, an extracellular polysaccharide elaborated by *Pseudomonas elodea.* Carbohyd. Res., 124(1): 135-139.

Jay, A.J., I.J. Colquhoun, M.J. Ridout, G.J. Brownsey, V.J. Morris, A.M. Fialho, J.H. Leitão and I. Sá-Correia, 1998. Analysis of structure and function of gellans with different substitution patterns. Carbohyd. Polym., 35(3-4): 179-188.

Lee, K.Y., J. Shim and H.G. Lee, 2004. Mechanical properties of gellan and gelatin composite films. Carbohyd. Polym., 56(2): 251-254.

Lee, M.W., H.F. Tsai, S.M. Wen and C.H. Huang, 2012. Photocrosslinkable gellan gum film as an anti-adhesion barrier. Carbohyd. Polym., 90(2): 1132-1138.

Mazen, F., M. Milas and M. Rinaudo, 1999. Conformational transition of native and modified gellan. Int. J. Biol. Macromol., 26(2-3): 109-118.

Miyamoto, K., K. Tsuji, T. Nakamura, M. Tokita and T. Komai, 1996. Preparation of carboxymethyl-gellan. Carbohyd. Polym., 30(2-3): 161-164.

Morris, E.R., K. Nishinari and M. Rinaudo, 2012. Gelation of gellan-a review. Food Hydrocolloid., 28(2): 373-411.

Pranoto, Y., C.M. Lee and H.J. Park, 2007. Characterizations of fish gelatin films added with gellan and κ-carrageenan. Lwt-Food Sci. Technol., 40(5): 766-774.

Sá-Correia, I., A.M. Fialho, P. Videira, L.M. Moreira, A.R. Marques and H. Albano, 2002. Gellan gum biosynthesis in *Sphingomonas paucimobilis* ATCC 31461: Genes, enzymes and exopolysaccharide production engineering. J. Ind. Microbiol. Biot., 29(4): 170-176.

Sudhamani, S.R., M.S. Prasad and K. Udaya Sankar, 2003. DSC and FTIR studies on Gellan and Polyvinyl alcohol (PVA) blend films. Food Hydrocolloid., 17(3): 245-250.

Tapia, M.S., M.A. Rojas-Graü, F.J. Rodríguez, J. Ramírez, A. Carmona and O. Martin-Belloso, 2007. Alginate- and gellan-based edible films for probiotic coatings on fresh-cut fruits. J. Food Sci., 72(4): E190-E196.

Timmermann, E.O., J. Chirife and H.A. Iglesias, 2001. Water sorption isotherms of foods and foodstuffs: BET or GAB parameters? J. Food Eng., 48(1): 19-31.

Tong, Q., Q. Xiao and L.T. Lim, 2013. Effects of glycerol, sorbitol, xylitol and fructose plasticisers on mechanical and moisture barrier properties of pullulan-alginate-carboxymethylcellulose blend films. Int. J. Food Sci. Tech., 48(4): 870-878.

Xiao, Q., L.T. Lim and Q.Y. Tong, 2012. Properties of pullulan-based blend films as affected by alginate content and relative humidity. Carbohyd. Polym., 87(1): 227-234.

Xu, X., B. Li, J.F. Kennedy, B.J. Xie and M. Huang, 2007. Characterization of konjac glucomannan-gellan gum blend films and their suitability for release of nisin incorporated therein. Carbohyd. Polym., 70(2): 192-197.

Yang, L. and A.T. Paulson, 2000. Mechanical and water vapour barrier properties of edible gellan films. Food Res. Int., 33(7): 563-570.

Zohuriaan, M.J. and F. Shokrolahi, 2004. Thermal studies on natural and modified gums. Polym. Test., 23(5): 575-579.