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Characterization of physicochemical properties of carboxymethyl agar

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ABSTRACT

A series of carboxymethyl agars (CMAs) with different degree of substitution (DS) were prepared, and their properties were determined and analyzed. The results showed that with the increase of DS, the dissolving temperature, the gelling temperature, the gel melting temperature, the gel strength, the gel hardness, the gel fracturability, and the solution apparent viscosity of CMA all decreased, except that its gel cohesiveness and gel springiness increased. The variation process of agar molecules in solution from coil to helix could be observed by measuring the optical rotation of the solution at such a low concentration, at which even the solution could not form a gel. The gel skeleton microstructures of both agar and CMA were of porous network structure, and the pore size of CMA became smaller and denser with the increase of its DS. After carboxymethylation, the agar hygroscopicity was improved, but its thermal stability was lowered.

1. Introduction

Agar, the gel-forming polysaccharides extracted from *Gelidiaceae* and *Gracilariaceae* species (Freile-Pelegrín & Robledo, 1997), is linear polymers based on a disaccharide repeating structure of 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose units (Labropoulos, Niesz, Danforth, & Kevrekidis, 2002; Lahaye & Rochas, 1991). Agarose and agaropectin are the two components of agar, with the former consisting of neutral polysaccharides with a high gelling ability and the latter consisting of ionic polysaccharides with a low gelling ability (Arnott et al., 1974). Agar is widely used for medical, pharmaceutical, pabular and electronic and experimental purposes due to its combination of renewability, biological activities, biocompatibility, biodegradability and non-toxicity (Cayre, Chang, & Velev, 2007; García-González, Alnaief, & Smirnova, 2011; Scholten & Pierik, 1998; Wijesekara, Pangestuti, & Kim, 2011; Wu, Geng, Chang, Yu, & Ma, 2009).

In recent years, the carboxymethylation of different polysaccharide has gained great interest. In order to obtain the special functional property, there have been a lot of research reports regarding the preparation and properties of diverse carboxymethyl polysaccharides, such as carboxymethyl κ-carrageenan (Tranquilan-Aranilla, Nagasawa, Bayquen, & Dela Rosa, 2012), carboxymethyl starch (Bi, Liu, Wu, & Cui, 2009; Spychaj, Wilpiszewska, & Zdanowicz, 2013; Xie, Zhang, & Liu, 2011), carboxymethyl cellulose (Bhandari, Jones, & Hanna, 2012; Bono et al., 2009), carboxymethyl chitin (Jayakumar et al., 2010; Philippova et al., 2012), carboxymethyl gellan (Ahuja, Singh, & Kumar, 2013; Kaity & Ghosh, 2013) and carboxymethyl inulin (Leong et al., 2011). The carboxymethyl agarose used as ion-exchange gels was firstly patented by Ghctie (1970), but so far, there had been few literature report regarding the preparation and properties of carboxymethyl agar. Herein, a series of carboxymethyl agars with different DS have been prepared, and their properties were subsequently determined and analyzed.

2. Experiment

2.1. Materials

Gracilaria agar (3, 6-anhydro-L-galactose content: 35.5%; sulfate content: 3.2%; gel strength: 1920 g/cm^2) was purchased from Qingdao Agar Manufacturing Company, Shandong Province, China. Chloroacetic acid, sodium hydroxide and hydrochloric acid (all reagent grade) used in this research were purchased from Laiyang Chemical Reagent Plant, Shandong Province, China.

2.2. The preparation of a series of CMA with different DS

Agar (30 g) and alcohol (70 g, 95%) were stirred for 10 min in a 500 ml four-necked flask equipped with a mechanical stirrer, a reflux condenser and a temperature controller. Then a certain amount of sodium hydroxide powder was added in gradually, kept the temperature of the reaction solution at 40 °C to react for 1 h.

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Then a solution of chloroacetic acid in alcohol was added dropwise in 0.5 h. After this, some sodium hydroxide powder was added again, the reaction temperature was raised to 54 °C, and kept at this temperature to react for another 3 h. The reaction solution was then cooled, neutralized, filtered, and washed three times with 60% alcohol. The product was dried in an oven at 60 °C. The DS of CMA was determined by potentiometric titration with potassium hydroxide solution, according to the method described by Rinaudo and Hudry-Clergeon (1967).

2.3. FT-IR spectroscopy

The FT-IR spectra were obtained from samples in KBr pellets using a Nicolet 200 FT-IR spectrophotometer (Nicolet, Madison, WI, USA).

2.4. Determination of the dissolving temperature

In a thermostatic water bath, agar or carboxymethyl agar (1.5 g) and deionized water (98.5 g) were stirred in a 250 ml four-necked flask equipped with a mechanical stirrer, a reflux condenser and a temperature controller. The heating rate was uniform in all cases at 1 °C/min, and the dissolving temperatures were recorded by monitoring the temperature at which the agar was fully dissolved in water.

2.5. Determination of physicochemical properties

The gelling temperatures and the gel melting temperatures were measured as described by Freile-Pelegrín and Robledo (1997). The gel strength was measured using a Nikkansui-type gel tester (Kiya Seisakusho Ltd., Tokyo, Japan). The measurements above were performed on a 1.5 wt% sample solution. Sulfate content was determined based on the method described by Matos, Fortunato, Velizarov, Reis, and Crespo (2008), using a Dionex ion exchange chromatography system (Dionex Corporation, USA). The 3, 6-anhydro-L-galactose content (3, 6-AG) was determined by the colorimetric method of Yaphe and Arsenault (1965) using the resorcinol–acetal reagent and with fructose as standard.

2.6. Gelling time dependence of the gel strength

Gel strength was determined at the time of 3 h, 6 h, 9 h, 12 h, 24 h, and 36 h, respectively, after the completely dissolved hot solution of 1.5% agar or CMA was cooled down to the temperature of 20 °C.

2.7. Determination of rheological properties

Apparent viscosity was measured using a Brookfield Synchrolectric Viscometer (USA). This viscometer could permit deformation to be measured at a constant stress and allow gel samples to be tested at stresses below the rupture point. The gel formation point, T_g, was taken as the temperature at which $\partial\eta/\partial T \rightarrow \infty$, i.e. when a yield stress appears, as described in the study of Plashchina, Muratalieva, Braudo, and Tolstoguzov (1986). No. 1 plunge was used at the speed of 30 rpm. The solution (1.5 wt%) was kept in the viscometer cell for 10 min at 80 °C, the temperature was then lowered at a rate of 0.3 °C/min.

2.8. Analysis of optical rotation

The optical rotation was measured with an ATAGO polarimeter (POL-1/2, Japan) at 589 nm. The measurements were taken in a thermostated silica cell. The optical path length was 1 dm. The measurement was performed on 0.05 wt% and 0.25 wt% solutions, respectively. The solution was kept in the cell for 10 min at 80 °C; temperature was then lowered at a rate of 0.3 °C/min.

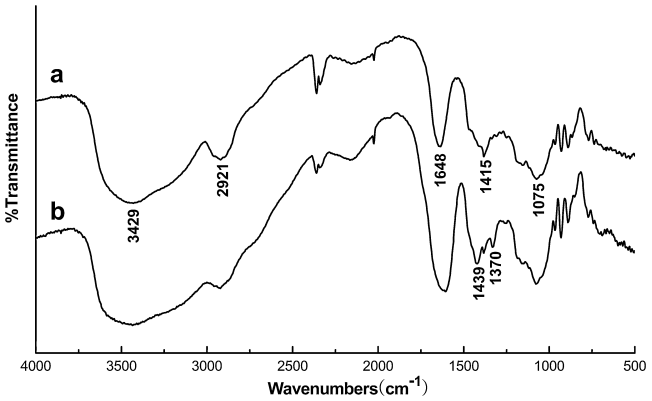

Fig. 1. The FT-IR spectra of raw agar and CMA, (a) raw agar; (b) CMA (DS = 0.351).

2.9. Cryo scanning electron microscopy (Cryo-SEM)

Gel samples (1.5 wt%) were placed into ultra-low temperature freezer for 3 h. The vacuum freeze dryer (FD1, Boyikang Company, Beijing, China) was used to remove water in the gel (about 36 h). The drying samples were cut into small pieces and fixed onto metallic sample holders with conducting silver glue and then sputtered with a layer of gold. The prepared samples were subsequently examined by scanning electron microscopy (JSM-840, Jeol, Japan).

2.10. Textural profile analysis (TPA)

The gels (1.5 wt% samples in water, cooling for 12 h) were subjected to an instrumental texture profile analysis (TPA). The gel specimens were placed between parallel flat plate fixtures fitted to a TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK). The gels were compressed twice at 1 mm/s to 50% of their original height. The results were reported by TPA as the means of duplicate tests. The TPA parameters, namely hardness [peak force on first compression (N)], fracturability [first bite, the force required to produce the first fracture (N)], cohesiveness [ratio of the active work done under the second force-displacement curve to that done under the first compression curve (dimensionless)] and springiness [distance that the sample recovered after the first compression (mm)] were computed.

2.11. Thermogravimetric analysis (TGA)

Thermogravimetric analyses were carried out on a thermal analyzer Perkin-Elmer TG7. The measurements were used the temperature program 30–600 °C at a heating rate of 5 °C/min in a nitrogen atmosphere. All samples were stored in a humidistat for at least 48 h.

3. Results and discussion

3.1. FT-IR spectroscopy

The FT-IR spectra of raw agar and carboxymethyl agar with DS = 0.351 are shown in Fig. 1. Compared with the FT-IR spectrum of raw agar (curve a), the FT-IR spectrum of CMA (curve b) was almost the same as that of raw agar except for the appearance of new characteristic absorption peaks at 1439 cm^{-1} and at 1370 cm^{-1}. The characteristic absorption peaks at 1648 cm^{-1} and 1439 cm^{-1} could be attributed to the asymmetrical and symmetrical stretching

Table 1
Physicochemical properties of CMA with different DS.

DS	0	0.040	0.080	0.119	0.155	0.251	0.351	0.495	0.559
Dissolving temperature (°C)	78	74	70	66	63	54	48	44	–
Gelling temperature (°C)	38	37	36	35	34	31	28	23	–
Gel melting temperature (°C)	79	78	77	76	75	73.5	72	70	–
Gel strength (g/cm^2)	1920	1560	1150	780	480	120	40	20	–
3,6-Anhydro-L-galactose content (%)	35.50	35.36	35.22	35.08	34.96	34.62	34.29	33.82	33.61
Sulfate content (%)	3.200	3.187	3.175	3.162	3.151	3.121	3.091	3.049	3.030

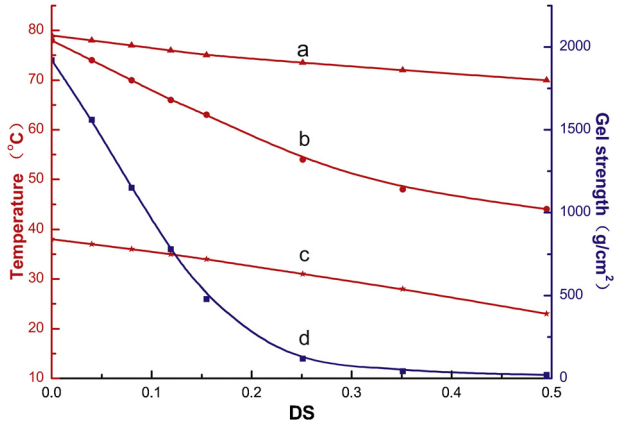

Fig. 2. DS value dependence of physicochemical properties of CMA, (a) gel melting temperature; (b) dissolving temperature; (c) gelling temperature; (d) gel strength.

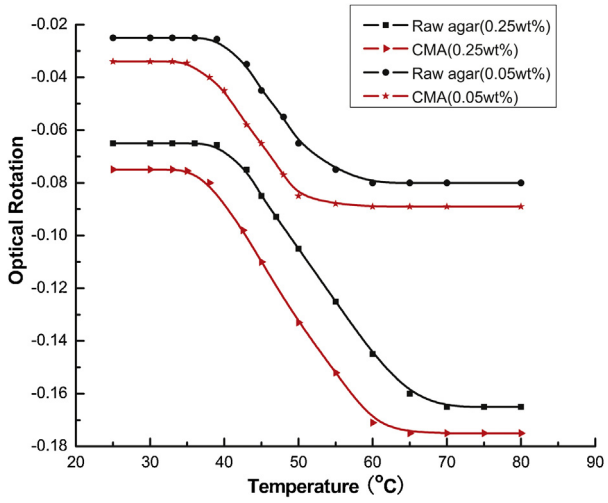

Fig. 3. Temperature dependence of the optical rotation for solutions of raw agar and CMA on cooling.

vibrations of carbonyl group (C=O) of carboxylate anions. Among which, the characteristic absorption peaks at 1648 cm^{-1} of raw agar was obviously enhanced after carboxymethylation. The characteristic absorption peaks at 1370 cm^{-1} could be attributed to scissoring vibration of methylene (—CH_2—) in carboxymethyl group. All these differences between the FT-IR spectra of raw agar and CMA could verify that carboxymethyl group had been successfully introduced into the agar molecule.

3.2. Determination of physicochemical properties

The dissolving temperature, the gelling temperature, the gel melting temperature, the gel strength, the content of 3,6-anhydro-L-galactose and the sulfate content of raw agar and CMAs were tested and are shown in Table 1 and Fig. 2. It could be seen that the CMA could be dissolved in water at room temperature when the DS was equal or above 0.559. This indicated that the introduction of carboxymethyl groups into agar molecules could confer the raw agar better aqueous solubility and make CMA lose its gelation property at room temperature. Both the gel melting temperature and the gelling temperature of CMA decreased in good linear correlation with the increase of DS value from 0 to 0.495, and their linear equations were $Y_a = 78.4 - 18.0X$ (correlation coefficient $R_a = -0.99004$) and $Y_c = 38.4 - 30.3X$ (correlation coefficient $R_c = -0.99841$), respectively. But it was quite different for the dissolving temperature and the gel strength of CMA. With the increase of DS, the dissolving temperature and the gel strength of CMA also decreased in good linear correlation at first. The linear equation of DS value dependence of dissolving temperature of CMA was $Y_b = 77.8 - 95.5X$ (correlation coefficient $R_b = -0.99962$) when the DS value was lower than 0.251, and the linear equation of DS value dependence of gel strength of CMA was $Y_d = 1919.8 - 9413.7X$ (correlation coefficient $R_d = -0.99951$) when the DS value of CMA was lower than 0.155. However, when the DS value was larger than 0.155 for the gel strength, or larger than 0.251 for the dissolving temperature, the decline of the gel strength and the dissolving temperature of CMA became slowly with the increase of its DS, respectively, as shown in Fig. 2.

3.3. Analysis of optical rotation

Studies showed that the mechanism of sol–gel transition for agar solution was due to the double helix structure formed between agar molecules (Arnott et al., 1974; Nordqvist & Vilgis, 2011). When agar aqueous solution was cooled, agar molecules turned into double helix, and when further cooled, the double helix was gathered and generated hard gel. Temperature dependence of the optical rotation for solutions of raw agar and CMA (DS = 0.155) on cooling is shown in Fig. 3. It could be seen that the optical rotation values of both raw agar and CMA were negative, and almost constant at high temperature (more than 70 °C) and low temperature (less than 30 °C), and their variation trend on cooling were approximately the same. The variation process of agar molecules in solution from coil to helix could be observed not only in a higher concentration solution (0.25 wt%) at which the solution could form a gel, but also in a lower concentration solution (0.05 wt%) at which even the solution could not form a gel. The temperature of the transition beginning point (from coil to helix) of the agar molecules was higher in a higher concentration solution (0.25 wt%) than that in a lower concentration solution (0.05 wt%), but its temperature of the transition end point (from coil to helix) was almost the same in both higher and lower concentration solution. As for this, it might be attributed to that the probability of collision between agar molecules was greater in the higher concentration solution than that in the lower concentration solution. Meanwhile, it could be also found that the temperature of the transition end point of CMA was obviously lower than that of raw agar. This meant that the introduction of carboxymethyl groups into the agar molecule could hinder the agar molecules to form double helix structure in water solution.

The results of the effect of gelling time on gel strength of CMAs were determined. The results showed that the gel strength values of raw agar and CMAs went up with the increase of gelling time, and the CMAs gel took more gelling time to reach the biggest gel strength with the increase of DS. When the gelling time were above 6 h, 9 h, 12 h and 24 h, respectively, the gel strength values of raw agar, CMA (DS = 0.119), CMA (DS = 0.251) and CMA (DS = 0.495) became constant successively. This indicated that the introduction of carboxymethyl groups into the agar molecule could hinder the formed double helix structure of CMA molecule to gather and generate the hard gel.

3.4. Rheological properties

Apparent viscosity values of CMA and raw agar at different temperature are shown in Fig. 4. It could be seen that the apparent viscosity values of all samples increased gradually as the temperature decreased, and the falling amplitude of apparent viscosity for all samples was small at the temperature above 50 °C. The solution apparent viscosity values of all CMAs were lower than that of raw agar under the same condition, and the CMA with the higher DS would have the lower apparent viscosity. The solution apparent viscosity of all samples increased sharply at the temperature range of 30–40 °C, which meant that all solutions of raw agar and CMA were gelling in this range of temperature. The gel formation point

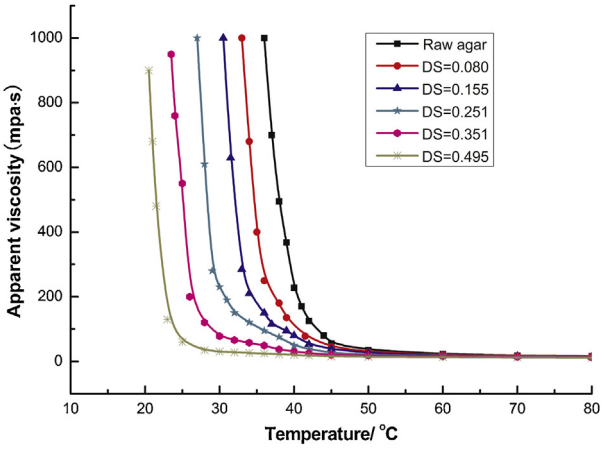

Fig. 4. Temperature dependence of the solution apparent viscosity of CMA with different DS.

temperatures (T_g) of raw agar and CMAs could be calculated from Fig. 4, which were 38 °C (raw agar), 36 °C (CMA, DS = 0.080), 34 °C (CMA, DS = 0.155), 30 °C (CMA, DS = 0.251), 25 °C (CMA, DS = 0.351), 20 °C (CMA, DS = 0495), respectively. These data were in almost perfect agreement with corresponding values of gelling temperature

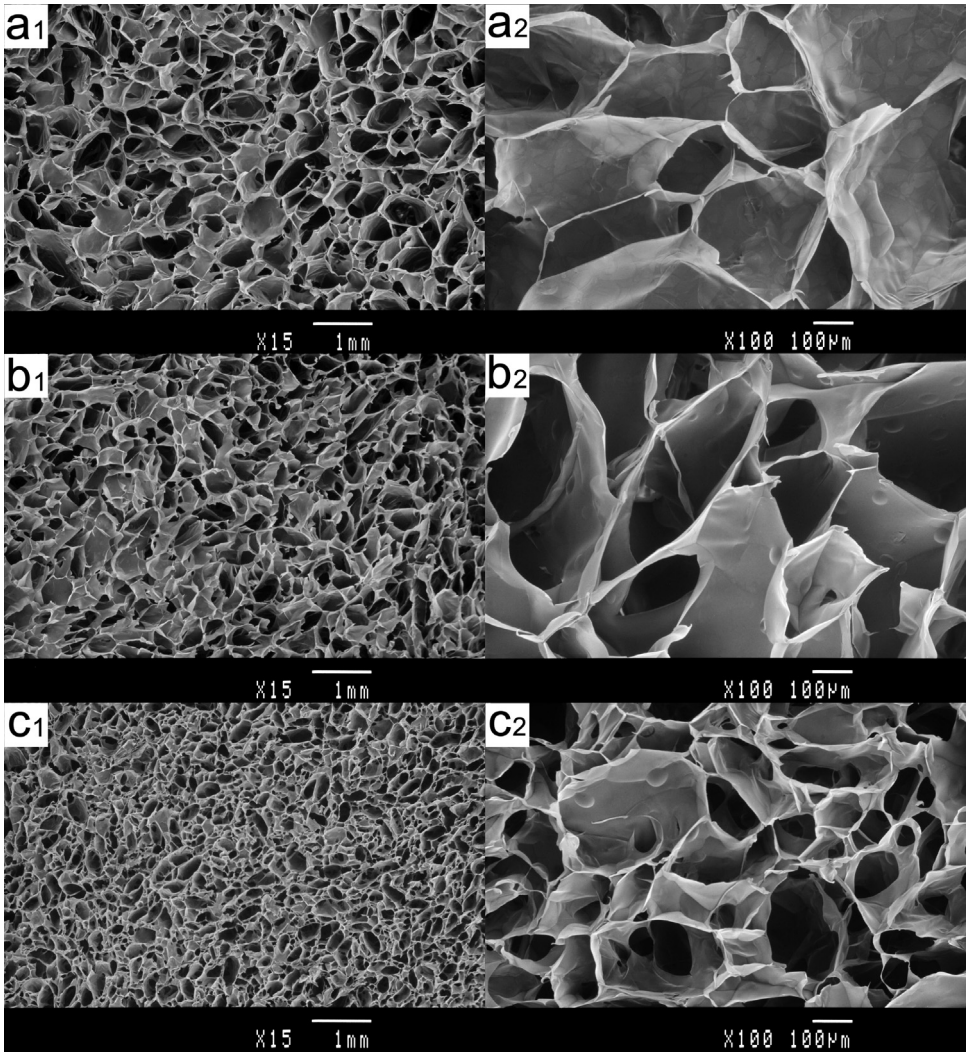

Fig. 5. Cryo scanning electron microscopy images of the gels of raw agar and CMA, (a_1, a_2): raw agar; (b_1, b_2): CMA (DS = 0.119); (c_1, c_2): CMA (DS = 0.495).

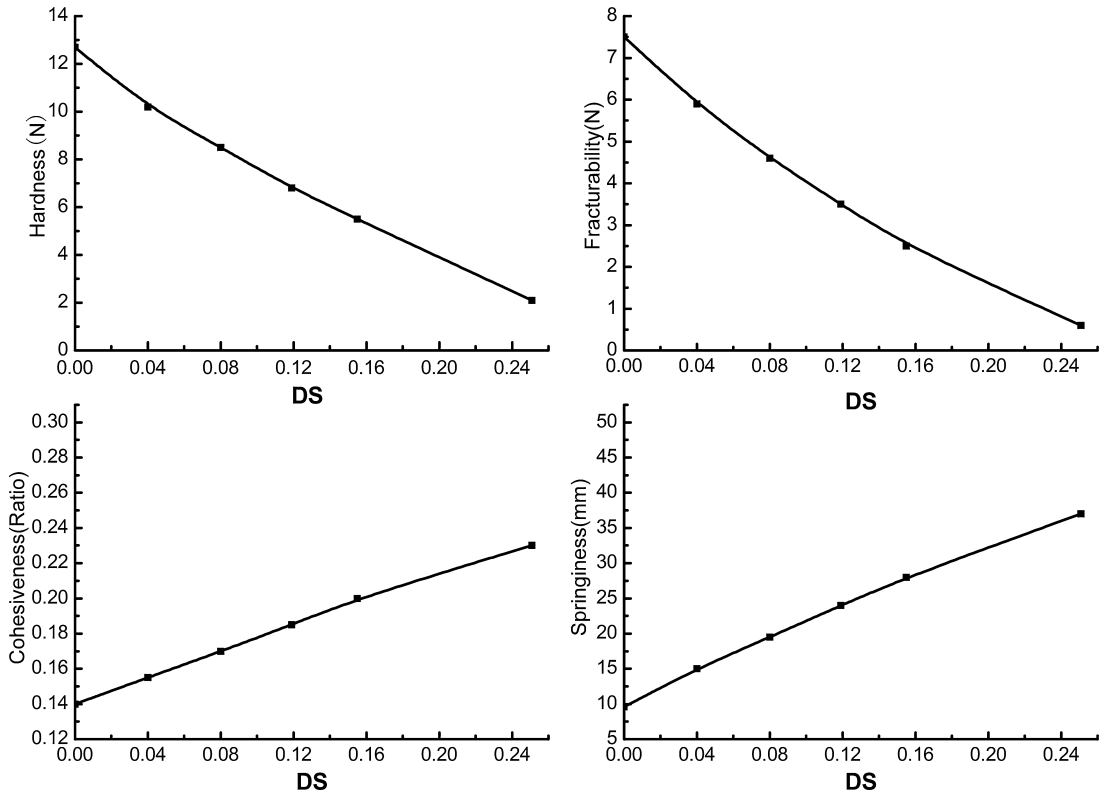

Fig. 6. DS dependence of the hardness, the fracturability, the cohesiveness and the springiness of CMA gel.

in Table 1, and further indicated that the gelling temperature of CMA was decreased with the increase of its DS.

3.5. Cryo-SEM

The gel microstructures of agar and CMA were characterized by Cyro-SEM, and the results are shown in Fig. 5. It could be seen that the gel skeleton structures of raw agar and CMAs were similar and all of the porous network microstructures. Notably, these photographs were similar to the images captured by Sousa, Borges, Silva, and Gonçalves (2013) for agar gel and by Tuvikene et al. (2008) for agarose gel, respectively. The pores in the network microstructure came from the sublimation of ice crystals in the frozen gel, and the ones of agar were more regular and well-defined than those of CMA. The pores of gel skeleton structure of CMA tended to be smaller and denser than those of the raw agar, and became even smaller and denser with the increase of its DS, that is, the size of the water droplet trapped in gel skeleton structure of CMA was smaller than that of the raw agar, and became even smaller with the increase of its DS. This meant that the gel skeleton structure of CMA would have much higher capillary force to hold water in its gel than that of raw agar according to the capillary effect, that is, the CMA would have the higher gel water holding capacity than raw agar, and the higher the DS of the CMA, the higher the gel water holding capacity. However, the pore wall of CMA became thinner than that of raw agar, and with the further increase of DS, the pores of CMA became irregular and broken. This was in line with the result that the CMA would lose its gelation property with the increase of its DS.

3.6. Textural properties

The gel textural properties of agar and CMAs were determined by the texture analyzer and shown in Fig. 6. It could be seen that with the increase of DS, the gel hardness and gel fracturability of the CMA decreased, whereas its gel cohesiveness and gel springiness increased. It was worthwhile to note that the relationships between each of TPA parameters (the gel hardness, the gel cohesiveness and the gel springiness) of CMA and its DS value in the range from 0 to 0.251 was in good linear correlation, and their linear equations were $Y_h = 12.1 - 41.3X$ (correlation coefficient $R_h = -0.99398$), $Y_c = 0.14 + 0.36X$ (correlation coefficient $R_c = 0.99868$) and $Y_s = 10.5 + 108.8X$ (correlation coefficient $R_s = 0.99759$), respectively. The relationship between the gel fracturability of the CMA and its DS value in the range from 0 to 0.251 was near to linear correlation, its linear equation was $Y_f = 7.0 - 27.3X$ (correlation coefficient $R_f = -0.98954$). The gel strength and the gel hardness of the CMA had the same variation trend with the increase of its DS. This result was in agreement with the result obtained by Lau, Tang, and Paulson (2000). The results that the gel hardness and the gel fracturability reduced with the increase of the DS indicated that the introduction of carboxymethyl groups into the agar molecule could reduce the linkage force between aggregated helices of CMA, thus making the gel strength of the CMA become weaker and easier to be broken. And the results that the gel springiness and the gel cohesiveness rose with the increase of the DS indicated that the gel of CMA was hard to be broken into many smaller pieces. For this result, it could be supported in Fig. 5, which showed that the pores of porous skeleton structures of CMA gel tended to be smaller and denser with the increase of its DS.

3.7. Thermogravimetric analysis (TG)

TG curves of agar and CMA (DS = 0.495) are shown in Fig. 7. It could be seen from the TG curves in Fig. 7 that a weight loss of raw agar around 19.63% was recorded in the range of temperature from 30 °C to 260 °C. This weight loss could be explained as the

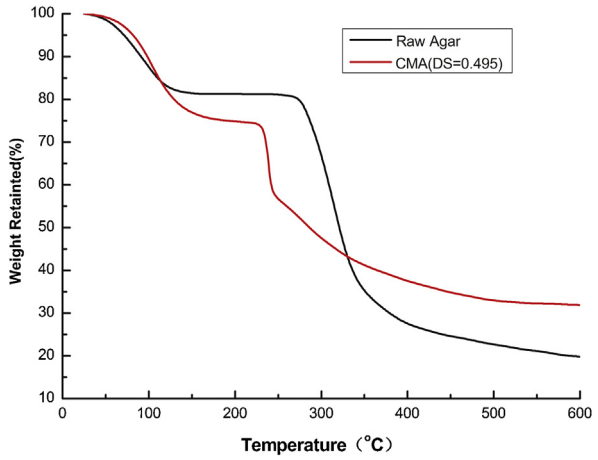

Fig. 7. The thermogravimetric analysis of raw agar and CMA (DS = 0.495).

loss of moisture of raw agar sample. After that, the raw agar sample showed a much accentuated mass loss of 55% in the region of the temperature from 260 °C to 400 °C, which marked the decomposition of the sample. As the temperature continued slowly to be increased up to 600 °C, the remaining residue was 20% in mass of the starting material. In the case of the CMA sample, the loss mass behavior as the function of the temperature was quite different. The moisture of CMA sample which made up around 25.34% was removed with the increase of the temperature from 30 °C to 200 °C, which indicated that the hygroscopicity of CMA was bigger than that of the raw agar. The CMA sample degradation with about 41% weight loss started at 200 °C and ended at 350 °C, and the remaining residue at the temperature of 600 °C was 31% in mass of the starting material. The lowering of the degradation temperature of CMA compared to that of the raw agar was probably due to the presence of carboxymethyl group, which had adverse effect on the thermal stability of agar molecule. Similar to starch, the thermal stability of agar was also decreased after carboxymethylation (Li et al., 2010; Wang, Pan, Hu, Miao, & Xu, 2010).

4. Conclusions

A series of carboxymethyl agars with different degree of substitution (DS) were prepared, and their properties were determined and analyzed. The results showed that with the increase of DS, the dissolving temperature, the gelling temperature, the gel melting temperature, the gel strength, the gel hardness, the gel fracturability and the solution apparent viscosity of CMA all decreased except that its gel cohesiveness and gel springiness increased. The introduction of carboxymethyl group into agar molecule could confer the raw agar better aqueous solubility and make CMA lose its gelation property at room temperature when the DS was equal or above 0.559. The variation process of agar molecules in solution from coil to helix could be observed through the solution optical rotation measurement, which could be carried out not only in a higher concentration (0.25 wt%) at which the solution could form a gel, but also in a lower concentration solution (0.05 wt%) at which even the solution could not form a gel. The temperature of the transition beginning point (from coil to helix) of the agar molecule was higher in higher concentration (0.25 wt%) than that in lower concentration (0.05 wt%), but its temperature of the transition end point (from coil to helix) was almost the same in both higher and lower concentration. The cyro-SEM showed that the gel skeleton microstructures of both agar and CMA were of porous network structure, the size of the water droplet trapped in gel skeleton structure of CMA gel were smaller than that of raw agar gel, and become even smaller with the increase of its DS. Following carboxymethylation, the agar hygroscopicity was improved, but its thermal stability was lowered.

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