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The physicochemical property characterization of agar acetate

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ABSTRACT

A series of agar acetates with different degree of substitution (DS) were prepared, and their properties were determined and analyzed. The results showed that the gelling temperature, the gel melting temperature, the gel strength, the gel hardness, the gel fracturability, the gel springiness and the solution apparent viscosity of agar acetates all decreased except that their gel cohesiveness increased with the increase of DS. The variation process of agar molecules in solution from coil to helix could be also observed by measuring solution optical rotation in a lower concentration at which even the solution could not form a gel. The gel skeleton structures of agar acetates were of porous network structures, and the pores became smaller and denser with the increase of DS. After acetylation, the water holding capacity of the agar 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 repeat structure of 1,3-linked β-D-galactopyranose and 1,4-linked 3,6 anhydro-α-L-galactopyranose units (Arnott et al., 1974; Labropoulos, Niesz, Danforth, & Kevrekidis, 2002). Agarose and agaropectin are the two components of agar, 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; Yaphe, 1984). Agar is widely used for medical, pharmaceutical, food and electronic industrial and laboratory experimental purposes due to its combination of renewability, biological activities, biocompatibility, biodegradability and nontoxicity (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 esterification of different polysaccharides has gained great interest. As described by Sweedman, Tizzotti, Schäfer, and Gilbert (2012), starches modified with octenyl succinic anhydride had been used in a range of industrial applications, particularly as a food additive. Superabsorbent hydrogels were prepared from native celluloses by esterification crosslinking with 1,2,3,4-butanetetracarboxylic dianhydride (Kono & Fujita, 2012). Poplar wood fibers were chemically modified by esterification (acetate, propionate, benzoate), and the properties were shown to be more thermally stable and hydrophobic than unmodified fibers (Wei, McDonald, Freitag, & Morrell, 2013). However, the literature and reports on agar esterification were very few. Acetylation of agar was firstly patented by Kenneth and Guiseley (1976), but no more properties of the agar acetates were described except the gelling temperature. In this research, a series of agar acetates with different DS were prepared, and their properties were determined and analyzed.

2. Experiment

2.1. Materials

Gracilaria agar (3,6-anhydro-L-galactose content: 35.5%; sulfate content: 3.2%; gel strength: 1930 g/cm^2) was purchased from Qingdao Agar Manufacturing Company, Shandong Province, China. All reagent used in this research were purchased from Laiyang Chemical Reagent Plant, Shandong province, China.

2.2. The preparation of agar acetates with different DS

Agar acetates were prepared by the method of Kenneth and Guiseley (1976) described in US3956273. Degree of substitution (DS) was determined by titration according to the method of Tupa, Maldonado, Vázquez, and Foresti (2013).

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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. Physicochemical properties

The gelling temperature and the gel melting temperature 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.

2.5. Test of apparent viscosity

Apparent viscosity was measured by using a Brookfield Synchrolectric Viscometer (USA). This viscometer could permit deformation to be measured at a constant stress and allowed gel samples to be tested at stress below the rupture point. The gel formation point, Tg, 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). The solution (1.5 wt%) was kept in the viscometer cell for 10 min at 80 °C, and then the temperature was lowered at a rate of 0.3 °C/min.

2.6. Test of optical rotation

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

2.7. Texture profile analysis of gels

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.

2.8. Thermogravimetric analyses

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. The samples with equal actual moisture content were prepared by the method of Zeleznak and Hoseney (1987).

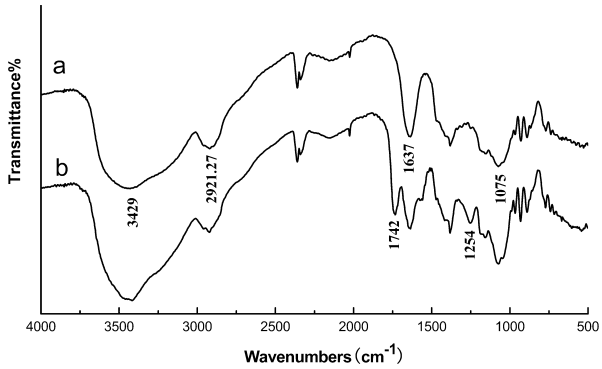

Fig. 1. The FT-IR spectra of raw agar and agar acetate, (a) raw agar; (b) agar acetate (DS = 0.365).

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 ready samples were examined by scanning electron microscopy (JSM-840, Jeol, Japan).

3. Results and discussion

3.1. FT-IR spectroscopy

The FT-IR spectra of raw agar and agar acetate with DS = 0.365 are shown in Fig. 1. Compared with the FT-IR spectrum of raw agar (curve a), the FT-IR spectrum of agar acetate (curve b) was almost the same as the one of raw agar except for the appearance of new absorption peaks at 1742 cm^{-1} and at 1254 cm^{-1}. The absorption peak at 1742 cm^{-1} indicated the presence of carbonyl group, corresponding to C=O of acetic ester. The absorption peak at 1254 cm^{-1} in the fingerprint region could be attributed to the C—O—C (acetic ester group) bond stretching vibration.

3.2. Physicochemical properties

Physicochemical properties of the prepared agar acetates were tested and shown in Table 1. It could be seen from Table 1 that the gelling temperature, the gel melting temperature and the gel strength of agar acetates were all decreased with the increase of DS. Among which, the variation trend of the gelling temperatures with the increase of DS was in agreement with the data reported in the patent by Kenneth and Guiseley (1976). It was worthwhile to note that the relationship between gel strength and DS values (in the range from 0 to 0.365) was in good linear correlation (correlation coefficient $R = -0.99834$) as it could be seen in Fig. 2.

3.3. Optical rotation analysis

Studies had shown that the mechanism of agar solution from sol to gel was due to the double helix structure formed between molecules (Arnott et al., 1974; Nordqvist & Vilgis, 2011). When agar water solution cooled, agar molecules turned into double helix, further cooled, the double helix was gathered and generated the hard gel. Temperature dependence of the optical rotation for solutions of raw agar and agar acetate (DS = 0.365) on cooling was shown in Fig. 3.

Table 1
Physicochemical properties of the prepared agar acetates.

DS of agar acetate	0	0.149	0.210	0.268	0.315	0.365
Gelling temperature (°C)	39	37.5	36.5	35	33	31
Melting temperature (°C)	79	77	75.5	74	72	70
Gel strength (g/cm^2)	1930	1290	1050	850	700	530
Acetyl content (%)	0	2.05	2.87	3.63	4.24	4.88
3,6-AG content (%)	35.50	34.79	34.51	34.24	34.03	33.80
Sulfate content (%)	3.20	3.14	3.11	3.08	3.06	3.04

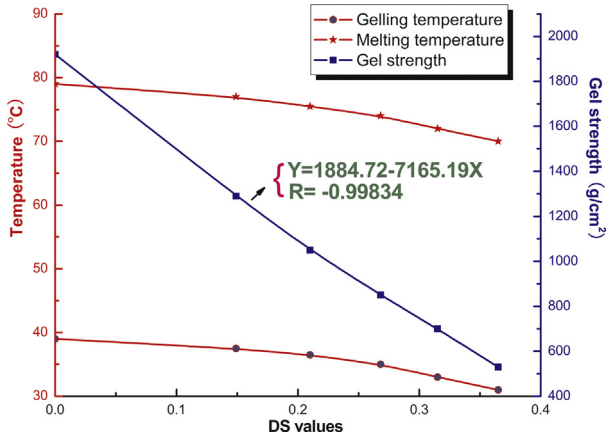

Fig. 2. DS values dependence of gelling temperature, melting temperature and gel strength.

It could be seen from Fig. 3 that the solution optical rotation values of both raw agar and agar acetate were almost constant at high temperature (higher than 70 °C) and low temperature (lower than 30 °C), and their variation trend on cooling were approximately the same. This meant that the molecules of both raw agar and agar acetate had not changed from single coil to double helix, and were evenly dispersed in solution at high temperature (higher than 70 °C), but had turned into double helix and gathered completely at low temperature (lower than 30 °C). As the agar acetate was prepared only by using acetyl group to replace the hydrogen atom of hydroxyl group of agar molecule, the molecule of agar acetate still owned the characteristic of chirality like agar molecule. This may be the reason why agar acetate and raw agar had the similar variation trend of solution optical rotation on cooling. However, the introduction of acetyl group could lower the solution optical rotation value of raw agar. 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 agar acetate was obviously lower than that of raw agar.

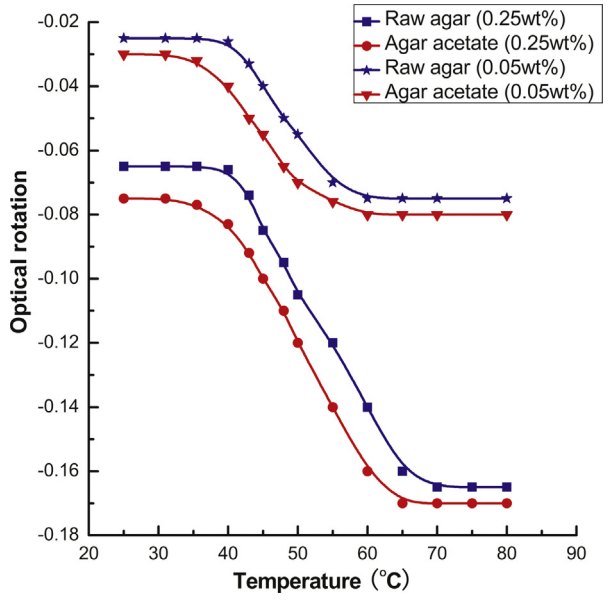

Fig. 3. Temperature dependence of the optical rotation for solutions of raw agar and agar acetate on cooling.

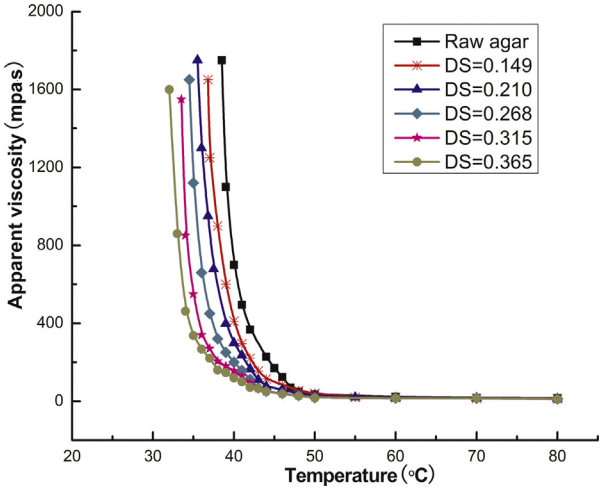

Fig. 4. Temperature dependence of the solution apparent viscosity of raw agar and agar acetates.

3.4. Rheological studies

The solution apparent viscosity of raw agar and its acetates at different temperature was shown in Fig. 4.

It could be seen from Fig. 4 that the solution apparent viscosity values of raw agar and agar acetates increased gradually as the temperature decreased, and the increase amplitude of solution apparent viscosity for all samples was small at the temperature above 50 °C. All solution apparent viscosity values of agar acetates were lower than the one of raw agar under the same conditions, and the solution of the agar acetates with bigger DS had lower viscosity. It could be found from Fig. 3 above that the molecules of both raw agar and agar acetate began to change from single coil to double helix when the temperature was lower than 70 °C.

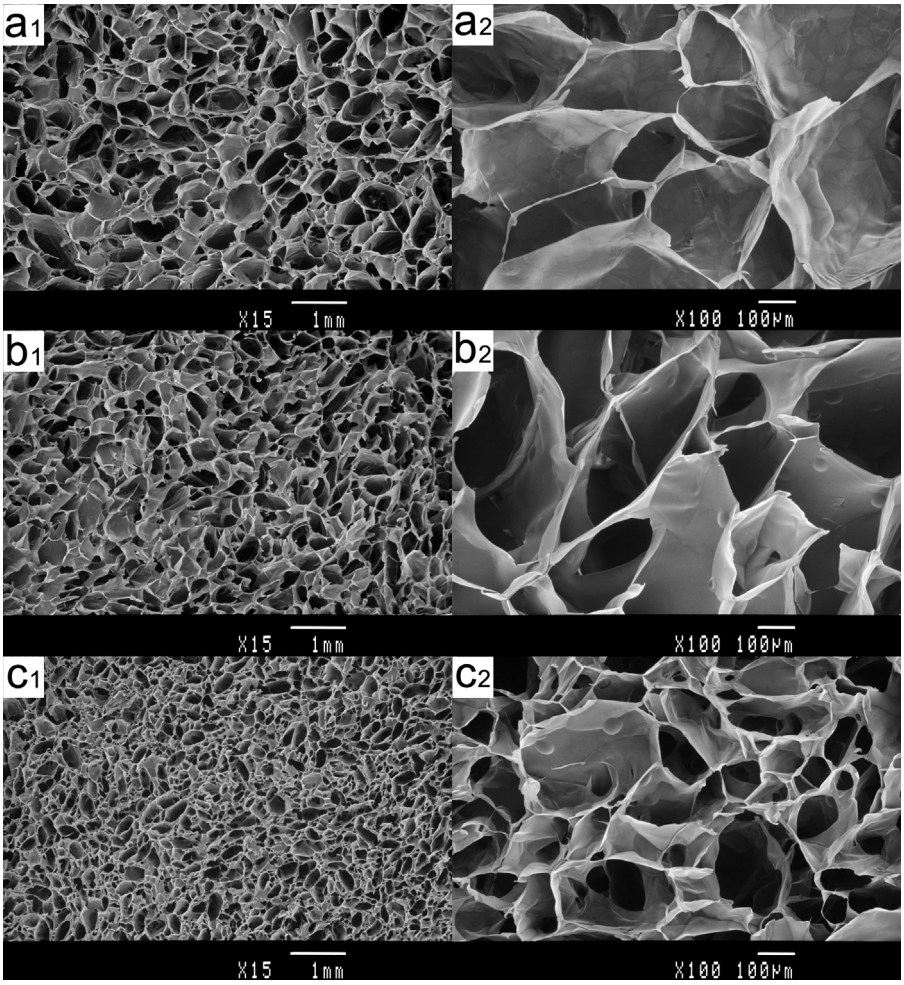

Fig. 5. Cryo scanning electron microscopy images of the gels of raw agar and agar acetates (a_1, a_2) raw agar, (b_1, b_2) agar acetate (DS = 0.149); (c_1, c_2) agar acetate (DS = 0.365).

This meant that the molecules of both raw agar and agar acetate existed in the mixture form of single coil and double helix, and were still evenly dispersed in solution when the solution temperature was in the range 50–70 °C. The change of the molecules of both raw agar and agar acetate in solution from single coil to double helix had no obviously effect on their solution apparent viscosity, which indicated that the formed double helix had not begun to gather to gel at the temperature higher than 50 °C. As the apparent viscosity of the raw agar solution was mainly due to the intermolecular hydrogen bonding interaction between its molecules, and the introduction of acetyl group could weak its intermolecular hydrogen bonding interaction, this may be the reason why the solution apparent viscosity values of agar acetates were lower than the one of raw agar under the same conditions. The solution apparent viscosity of all samples increased sharply at the range of temperature 30–45 °C, which meant that the formed double helix began to gather together, and all solutions of raw agar and its acetates were gelling in this range of temperature. When the temperature was lower than 30 °C, the hard gel was formed completely. The temperatures of gel formation point (Tg) of agar and agar acetates could be calculated from Fig. 4, which were 39 °C (raw agar), 37 °C (agar acetate, DS = 0.149), 36 °C (agar acetate, DS = 0.210), 34 °C (agar acetate, DS = 0.268), 33 °C (agar acetate, DS = 0.315), 32 °C (agar acetate, DS = 0.365) respectively, which were in almost perfect agreement with corresponding values of gelling temperature in Table 1, and further indicated that the gelling temperature of agar acetate was decreased with the increase of DS.

3.5. Cryo-SEM

The gel skeleton structures of agar and agar acetates were characterized by Cyro-SEM, and the results are shown in Fig. 5.

It could be seen from Fig. 5 that the gel skeleton structures of raw agar and agar acetate were similar and all of the porous network microstructures. These photographs were similar to the images captured by Sousa, Borges, Silva, and Gonçalves (2013) and Tuvikene et al. (2008) for agar and agarose, respectively. The pores in the network microstructures came from the sublimation of ice crystals in the frozen gels. The pores of gel skeleton structures of agar acetate tended to be smaller and denser than the one of the raw agar, and became even smaller and denser with the increase of DS, i.e., the size of the water droplet trapped in gel skeleton structures of agar acetate was smaller than that of raw agar, and become even smaller with the DS increase of agar acetate. This meant that the gel skeleton structure of agar acetate would have much higher capillary force to hold the water in gels than that of raw agar gel according to the capillary effect, i.e., the agar acetate would have the higher gel water holding capacity than raw agar, and the higher the DS of the agar acetate, the higher the gel water holding capacity.

3.6. Texture profile analysis

The gel textural properties of agar and agar acetates were determined by the texture analyzer and shown in Fig. 6.

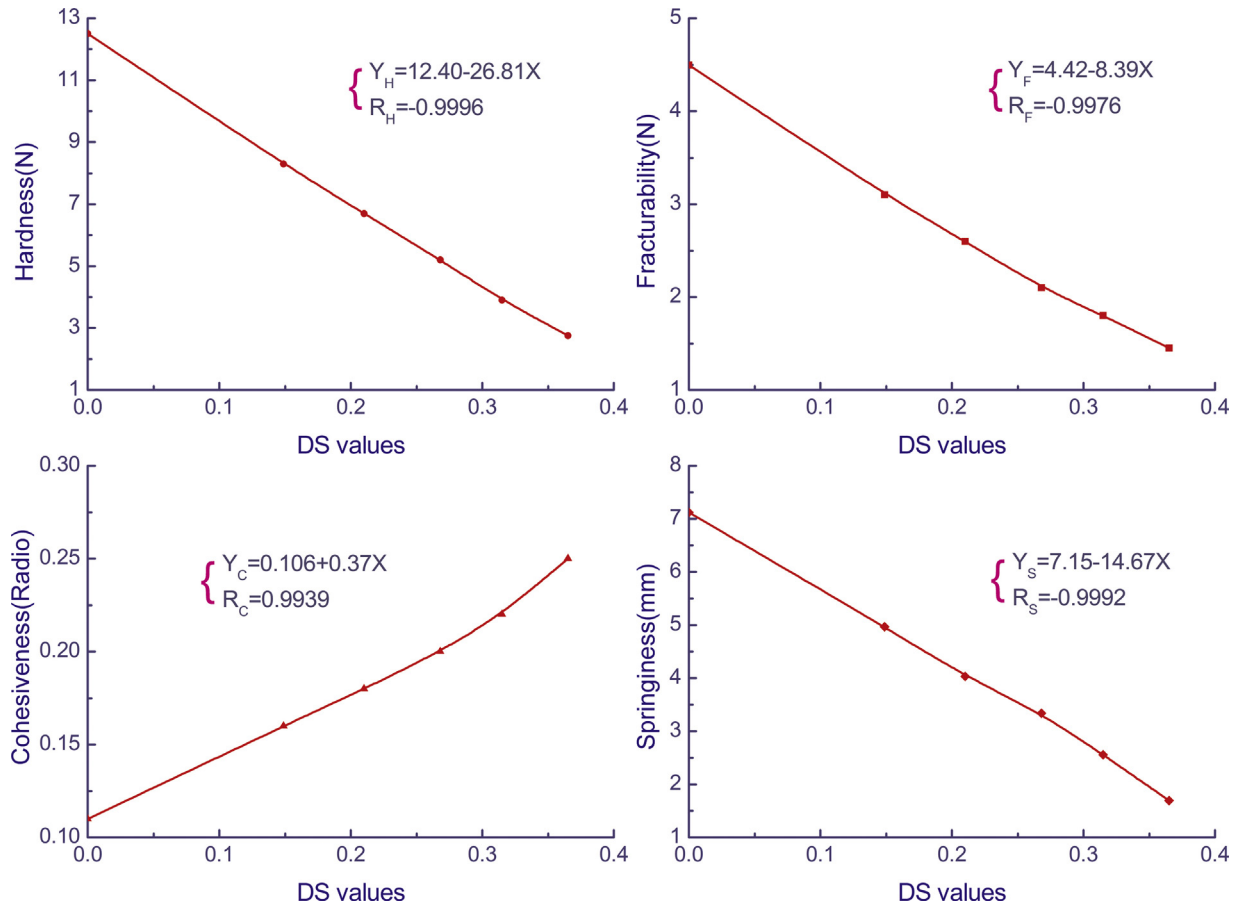

Fig. 6. DS values dependence of hardness, fracturability, cohesiveness and springiness of agar acetate gels.

It was worthwhile to note that the relationships between each of TPA parameters (gel hardness, gel fracturability, gel cohesiveness and gel springiness) of agar acetate and its DS values (in the range from 0 to 0.365) were in good linear correlation (the correlation coefficients were $R_H = -0.9996$, $R_F = -0.9976$, $R_C = 0.9939$ and $R_S = -0.9992$, respectively) as they were shown in Fig. 6, and the gel hardness, the gel fracturability and the gel springiness of agar acetate all decreased except that its gel cohesiveness increased with the increase of DS. The gel strength and the gel hardness of the agar acetate had the same variation trend with the increase of DS. This result was in agreement with the result obtained by Lau, Tang, and Paulson (2000). It could be attributed to that the meshes of porous skeleton structures of agar acetate gels tended to be smaller and denser with the DS increase of agar acetate (see Fig. 5) that both of the result that the gel springiness of the agar acetate decreased and the result that the gel cohesiveness of the agar acetate increased with the DS increase of agar acetate. These results were also in good agreement with the results obtained by Marshall and Vaisey (1972) and Wolf, LaVelle, and Clark (1989), respectively.

3.7. Thermogravimetric analysis (TG)

TG curves of agar and agar acetate (DS = 0.365) were shown in Fig. 7.

It could be seen From the TG curves shown in Fig. 7 that an equal weight loss of raw agar and agar acetate around 19% was recorded in the range of temperature from 30 °C to 180 °C. This weight loss could be explained as the loss of free water presented in the samples. Because of the same moisture content of the two samples, water holding capacity could be characterized through the water loss rates. The water loss rate of raw agar was obviously larger than that of agar acetate in moisture drying region, which indicated that water holding capacity of agar acetate was better than that of raw agar. In the region of the temperature from 260 °C to 400 °C, the raw agar sample showed a much accentuated mass loss of 55%, which marked the decomposition of the sample, and slowly continued as the temperature was increased up to 600 °C. The remaining residue was 20% in mass of the starting material. In the case of the agar acetate sample, the loss mass behavior as a function of the temperature was quite different. The sample degradation with 40% weight loss started at 200 °C and ended at 300 °C. Remaining residue at

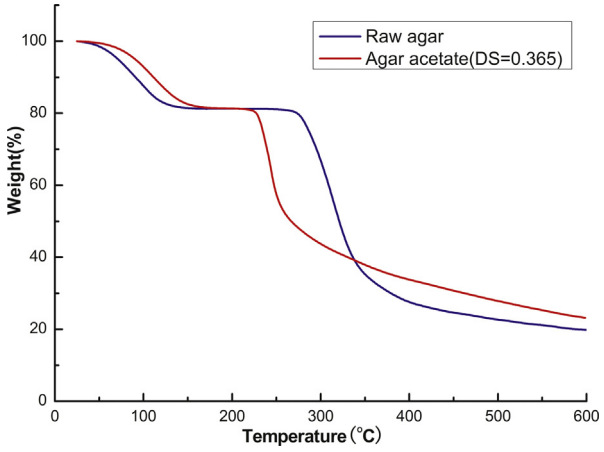

Fig. 7. Thermogravimetric (TG) curves of raw agar and agar acetate (DS = 0.365).

the temperature of 600 °C was 25% in mass of the starting material. This lowering of the degradation temperature of agar acetate compared to raw agar was probably due to the presence of acetyl ester group, which has adverse effect on the thermal stability of agar molecule. This result was different from other polysaccharides such as starch and xylan which thermal stability were increased after acetylation (Fundador, Enomoto-Rogers, Takemura, & Iwata, 2012; Xia, Wenyuan, Qianqian, Luqi, & Changxiao, 2011; Zhang, Xie, Zhao, Liu, & Gao, 2009).

4. Conclusions

A series of agar acetates with different DS were prepared, and their properties were determined and analyzed contrastively with the raw agar. The results showed that the gelling temperature, gel melting temperature, gel strength and apparent viscosity of agar acetates were all decreased with the increase of DS. The variation process of agar molecules in solution from coil to helix could be observed through the optical rotation measurement, which could be carried out not only in a higher concentration (0.25 wt%) at which it could form a gel, but also in a lower concentration (0.05 wt%) at which even it 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. According to the texture profile analysis (TPA), the hardness, the fracturability and the springiness of agar acetate gels all decreased except that the cohesiveness of agar acetate gels increased with the increase of DS. The Cyro-SEM showed that the gel skeleton microstructures of both agar acetate gel and raw agar gel were of porous network structure, the size of the water droplet trapped in gel skeleton structure of agar acetate gels were smaller than the one of raw agar gel, and become even smaller with the DS increase of agar acetate. After acetylation, the water holding capacity of the agar was improved, but its thermal stability was lowered.

References

Arnott, S., Fulmer, A., Scott, W. E., Dea, I. C. M., Moorhouse, R., & Rees, D. A. (1974). The agarose double helix and its function in agarose gel structure. *Journal of Molecular Biology*, *90*, 269–284.

Cayre, O. J., Chang, S. T., & Velev, O. D. (2007). Polyelectrolyte diode: Nonlinear current response of a junction between aqueous ionic gels. *Journal of the American Chemical Society*, *129*, 10801–10806.

Freile-Pelegrín, Y., & Robledo, D. (1997). Influence of alkali treatment on agar from Gracilaria cornea from Yucatán, México. *Journal of Applied Phycology*, *9*, 533–539.

Fundador, N. G. V., Enomoto-Rogers, Y., Takemura, A., & Iwata, T. (2012). Acetylation and characterization of xylan from hardwood kraft pulp. *Carbohydrate Polymers*, *87*(1), 170–176.

García-González, C. A., Alnaief, M., & Smirnova, I. (2011). Polysaccharide-based aerogels—promising biodegradable carriers for drug delivery systems. *Carbohydrate Polymers*, *86*, 1425–1438.

Kenneth, B., & Guiseley (1976). Modified agarose and agar and method of making same. US3956273.

Kono, H., & Fujita, S. (2012). Biodegradable superabsorbent hydrogels derived from cellulose by esterification crosslinking with 1,2,3,4-butanetetracarboxylic dianhydride. *Carbohydrate Polymers*, *87*, 2582–2588.

Labropoulos, K. C., Niesz, D. E., Danforth, S. C., & Kevrekidis, P. G. (2002). Dynamic rheology of agar gels: Theory and experiments. Part I. Development of a rheological model. *Carbohydrate Polymers*, *50*, 393–406.

Lau, M. H., Tang, J., & Paulson, A. T. (2000). Texture profile and turbidity of gellan/gelatin mixed gels. *Food Research International*, *33*, 665–671.

Marshall, S. G., & Vaisey, M. (1972). Sweetness perception in relation to some textural characteristics of hydrocolloid gels. *Journal of Texture Studies*, *3*, 173–185.

Matos, C. T., Fortunato, R., Velizarov, S., Reis, M. A., & Crespo, J. G. (2008). Removal of mono-valent oxyanions from water in an ion exchange membrane bioreactor: Influence of membrane permselectivity. *Water Research*, *42*(6), 1785–1795.

Nordqvist, D., & Vilgis, T. A. (2011). Rheological study of the gelation process of agarose-based solutions. *Food Biophysics*, *6*, 450–460.

Plashchina, I. G., Muratalieva, I. R., Braudo, E. E., & Tolstoguzov, V. B. (1986). Studies of the gel formation of κ-carrageenan above the coil–helix transition temperature range. *Carbohydrate Polymers*, *6*, 15–34.

Scholten, H. J., & Pierik, R. L. M. (1998). Agar as a gelling agent: Differential biological effects in vitro. *Scientia Horticulturae*, *77*, 109–116.

Sousa, A. M., Borges, J., Silva, F., & Gonçalves, M. P. (2013). Influence of the extraction process on the rheological and structural properties of agars. *Carbohydrate polymers*, *96*, 163–171.

Sweedman, M. C., Tizzotti, M. J., Schäfer, C., & Gilbert, R. G. (2012). Structure and physicochemical properties of octenyl succinic anhydride modified starches: A review. *Carbohydrate Polymers*, *92*, 905–920.

Tupa, M., Maldonado, L., Vázquez, A., & Foresti, M. L. (2013). Simple organocatalytic route for the synthesis of starch esters. *Carbohydrate Polymers*, *98*, 349–357.

Tuvikene, R., Truus, K., Kollist, A., Volobujeva, O., Mellikov, E., & Pehk, T. (2008). Gel-forming structures and stages of red algal galactans of different sulfation levels. *Journal of Applied Phycology*, *20*(5), 527–535.

Wei, L., McDonald, A. G., Freitag, C., & Morrell, J. J. (2013). Effects of wood fiber esterification on properties, weatherability and biodurability of wood plastic composites. *Polymer Degradation and Stability*, *98*, 1348–1361.

Wijesekara, I., Pangestuti, R., & Kim, S. K. (2011). Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. *Carbohydrate Polymers*, *84*, 14–21.

Wolf, C. L., LaVelle, W. M., & Clark, R. C. (1989). Gellan gum/gelatin blends. US4876105.

Wu, Y., Geng, F., Chang, P. R., Yu, J., & Ma, X. (2009). Effect of agar on the microstructure and performance of potato starch film. *Carbohydrate Polymers*, *76*, 299–304.

Xia, L., Wenyuan, G., Qianqian, J., Luqi, H., & Changxiao, L. (2011). Study on the morphology, crystalline structure, and thermal properties of Fritillaria ussuriensis Maxim. starch acetates with different degrees of substitution. *Starch-Stärke*, *63*(1), 24–31.

Yaphe, W. (1984). Properties of Gracilaria agars. *Hydrobiologia*, *116*, 171–186.

Yaphe, W., & Arsenault, G. P. (1965). Improved resorcinol reagent for the determination of fructose, and of 3,6-anhydrogalactose in polysaccharides. *Analytical Biochemistry*, *13*(1), 143–148.

Zeleznak, K. J., & Hoseney, R. C. (1987). The glass transition in starch. *Cereal Chemistry*, *64*, 121–124.

Zhang, L., Xie, W., Zhao, X., Liu, Y., & Gao, W. (2009). Study on the morphology, crystalline structure and thermal properties of yellow ginger starch acetates with different degrees of substitution. *Thermochimica Acta*, *495*(1), 57–62.