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Characterizations of fish gelatin films added with gellan and κ-carrageenan

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Abstract

Fish gelatin is known to be inferior to mammalian gelatins. Gellan and κ-carrageenan were added to improve properties of the fish gelatin films. Initially, polysaccharides were added to make fish gelatin gels, and tested for the melting point. Mechanical, barrier, color and microstructure properties, as well as Fourier transform infrared (FTIR) and thermal analysis (DSC) of the modified fish gelatin films were evaluated. The addition of gellan and κ-carrageenan increased the melting point of fish gelatin gels, gellan being more effective. Polysaccharides modified fish gelatin films by increasing tensile strength and barrier against water vapor, but made films slightly darker. Scanning electron microscopy (SEM) microstructure analysis revealed that gellan eliminated cracks present in the film matrix resulting in a more uniform structure. FTIR and DSC analyses showed that both polysaccharides effectively interacted with fish gelatin, and moreover, gellan being more effective. Overall, addition of gellan up to 2 g/100 g of gelatin performed better in enhancing fish gelatin films properties.

Keywords: Fish gelatin; Film; Gellan; Carrageenan; Properties

1. Introduction

Gelatin, an extracted protein from animal collagen, has several functions for foods, pharmaceutical, medical, cosmetic and photographic industries. The majority of gelatin in the world is produced from pigskin and bovine hide. However, Moslem and Jewish do not accept any pig-related food products, while Hindu does not consume cow-based foods. In addition, bovine spongiform encephalopathy (BSE) becomes an issue in consuming products from cow (Baziwane & He, 2003; Gudmundsson, 2002). Therefore, finding an alternative to the mammalian gelatin acceptable to these religious groups and overcoming food safety issues are in urgent need. Gelatin from fish is the potential alternative to mammalian gelatins. In recent years, fish gelatin has been actively studied (Cho, Gu, & Kim, 2005; Gómez-Guillén & Montero, 2001; Gómez-Guillén et al., 2002; Gudmundsson, 2002; Montero & Gómez-Guillén, 2000; Muyonga, Cole, & Duodu, 2004a). The studies covered extraction and the characterization of gelatin properties from several fish skins like megrim, cod, tuna, tilapia, yellowfin tuna and Nile perch. Several fish gelatin products are now commercially available and have been used for several applications in place of mammalian gelatins.

Fish gelatin, however cannot replace the role of mammalian gelatin totally due to its inherent limitations. The principal limitations of fish gelatin are low melting point and gel strength but relatively high viscosity

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compared to mammalian gelatin. Similarly, the gels formed tend to be less stable and have worse rheological properties, thereby limiting their field of applications (Fernández-Díaz, Montero, & Gómez-Guillén, 2001; Gómez-Guillén et al., 2002; Montero & Gómez-Guillén, 2000). Soft gel formation is due to its lower amounts of proline and hydroxyproline content which are important during the hydrogen bonding of gelatin in water solutions. In addition, the properties of fish gelatin also vary with species. The gelatin from warm water fish has higher gelling and melting temperature than those from cold water fish (Baziwane & He, 2003; Kołodziejska, Kaczorowski, Piotrowska, & Sadowska, 2004; Muyonga et al., 2004a). Some efforts have been made to modify fish gelatin properties to meet several requirements. The modifications were carried out by chemical and enzymatic treatments. Chemical cross-linking agents, including salts and enzyme transglutaminase have been used to improve fish gelatin gel strength, rheological properties and to increase melting temperature (Fernández-Díaz et al., 2001; Kołodziejska et al., 2004; Sarabia, Gómez-Guillén, & Montero, 2000). Chemical agents such as formaldehyde and glutaraldehyde are very effective to improve gelatin properties. However, they are considered toxic for human consumption (de Carvalho & Grosso, 2004). Fish gelatin properties can also be modified by adding some polysaccharides such as gellan and carrageenan. Gellan is a polysaccharide produced by fermentation of Sphingomonas paucimobilis and consists of tetrasaccharides repeat units of β-D-glucose, β-D-glucuronic acid and α-L-rhamnosa in the molar ratios of 2:1:1. On the other hand, carrageenan is a sulphated galactant extracted from red algae. The κ-carrageenan backbone consists of repeating disaccharides unit of (-4) 3,6-anhydro-α-D-galactose(1-3) β-D-galactose-4-sulfate (1-). The addition of low acyl gellan into gelatin gels has been found to increase the gelation rate constant. Gellan may form coupled networks with the gelatin molecule wherein the anionic domains of the gellan forms new heterolytic junction zones with cationic domain of the gelatin molecules leading to increases in gelation temperature, gelation rate and gel strength (Fonkwe, Narsimhan, & Cha, 2003). In order to overcome the inferiority of fish gelatin, Haug, Draget, and Smidsrød (2004) mixed fish gelatin with κ-carrageenan and resulted in solutions and gels with varying degree of turbidity, and stabilized by electrostatic interaction. An improved composite film property from κ-carrageenan and chitosan was obtained by Park, Lee, Jung, and Park (2001) by utilizing their opposite ionic charges.

Characterization of a sheet form of gelatin films could be useful assessment of hard and soft pharmaceutical capsules. The film formed as uniform sheet is easier to use in assessing the properties (Baziwane & He, 2003). Reported were studies on the mammalian gelatin-based films (Lim, Mine, & Tung, 1999; Sobral, Menegalli, Hubinger, & Roques, 2001; Vanin, Sobral, Menegalli, Carvalho, & Habitante, 2005), and attempts to modify properties by using tranglutaminase, glyoxal and formaldehyde (de Carvalho & Grosso, 2004). In addition, Lee, Shim, and Lee (2004) mixed pigskin gelatin with gellan to obtain composite film for packing or coating materials. However, to date there has been limited information and comprehensive studies on fish gelatin films as well as the attempts to enhance the properties. Particularly, employing polysaccharides onto formation of fish gelatin films has not been reported yet. Thus, this study aimed to improve the characteristics of fish gelatin films by adding polysaccharides of gellan and κ-carrageenan. Mechanical, physical, color, microstructure (SEM) as well as thermal property (DSC) and infrared (FTIR) analyses were methodically carried out to evaluate the fish gelatin films.

2. Materials and methods

2.1. Materials

The fish gelatin used was gelatin granule extracted from tilapia fish skin (Bloom = 200 g, moisture content <12 g/100 g, Ash <2 g/100 g and pH$\sim$6) (Vyse Gelatin Company Inc., IL, USA). Bloom value indicates the gel strength of the gelatin, defined as the weight in gram necessary to apply to the surface of gelatin gel by means of a piston 12.7 mm in diameter, to produce a 4 mm depth depression. Gellan (gelrite gellan gum) was purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Kappa-carrageenan was donated by MSC Co. Ltd. (Seoul, Republic of Korea). Fish gelatin gels and films were prepared with and without gellan and κ-carrageenan at 1 and 2 g/100 g of gelatin. These small amounts of polysaccharides are expected to be optimum level to enhance fish gelatin film properties as similarly has been reported earlier (Fonkwe et al., 2003).

2.2. Determination of melting point of fish gelatin gels

The melting point was determined following the method of Muyonga et al. (2004a) with slight modification. Fish gelatin solutions (6.67 g/100 ml of distilled water) with and without polysaccharides were filled in screw cap test tubes (15 mm × 125 mm) with some headspace. The sample in test tubes closed with screw cap were held in a refrigerator 5 °C for 16–18 h, and transferred into a 10 °C water bath in inverted position, so the headspace was at the bottom. The water bath was warmed gradually at a rate of 1 °C/min, and the gel melting temperature was recorded as gas moving up of the headspace.

2.3. Film preparation

Fish gelatin films were prepared according to casting method involving dehydration of the film forming solutions. The gelatin film forming solutions were made by dissolving granule of fish gelatin into distilled water to obtain concentration of 5 g/100 ml. Gellan and κ-carrageenan were added into gelatin solution to final concentration

of 1 and 2 g/100 g gelatin granule, whereas a control was gelatin without polysaccharides. To enhance solubility, the hydrated gelatins were heated in the water bath of 50 °C and mechanical agitation was given for 30 min. Afterwards, the gelatin film solutions were conveniently casted onto teflon-coated glass plates followed by air drying at room temperature for 24 h.

2.4. Tensile strength and elongation at break

Tensile strength (TS) and elongation at break (E) were measured following the ASTM (1995a) method using a universal testing machine (Instron 4465, LabX, Canada). Five films specimen 10 × 2.5 cm strips were pre-conditioned at 25 °C and 50% RH for 48 h before testing. The samples were mounted between the grips with initial separation of 50 mm and then pulled apart at cross-head speed of 50 mm/min. TS was calculated by dividing the maximum force at break by cross-sectional area of film, and expressed in MPa. E was calculated based on the length extended and original length of the films. Measurements were made in five replications.

2.5. Water vapor permeability (WVP)

WVP of gelatin films was measured according to the ASTM E96-95 (1995b) with slight modification using a cup made from polymethylacrylate with 45.5 mm internal diameter and 20 mm depth. Distilled water was added into the cup with 10 mm headspace and the film was tightly covered over the cup mouth. The cup was kept in a controlled chamber of 25 °C and 50% RH. The weight was measured every 2 h until 12 h under this controlled environment. The weight losses of the cups with the time were measured and the slope of moisture losses over time was determined using a linear regression analysis. The water vapor transmission rate (WVTR) value was calculated by dividing the slope by the open area of the cups. WVP was calculated using the following equation:

$$\mathrm{WVP} = (\mathrm{WVTR} \times \mathrm{L})/\Delta p, \quad (1)$$

where L is the average thickness of the sample film, and Δp is the difference of the partial vapor pressure (Pa) across the film. The WVP value was expressed in g mm/m^2 h kPa and obtained from quadruplicate measurements.

2.6. Color measurement

The surface colors of fish gelatin films were measured using a Hunter Lab color meter (model Color QUEST II, Hunter Associates Laboratory, Inc., VA, USA). The parameters measured were L, a and b in quadruplicate. These values are L black (−) to white (+), a green (−) to red (+), and b blue (−) to yellow (+).

2.7. Microstructure observation

Cross-section of fish gelatin films were viewed at × 25,000 magnification employing scanning electron microscopy (SEM) (Hitachi S-4300SE, Hitachi Science System Ltd., Japan). The samples were prepared by fracturing the films in liquid nitrogen, mounted on alumunium stubs, and sputter coated with platinum.

2.8. Thermal property

The melting point (T_m) and melting enthalpy (ΔH_m) of fish gelatin films were measured employing differential scanning calorimetry (DSC) (EXSTAR 6000 DSC, SII Seiko Instruments Inc., Tokyo, Japan). The exact amount of samples were weighed and placed into aluminum pans, and heated at the rate of 5 °C/min, from 5 to 150 °C, in inert atmosphere (100 ml/min of N_2) along with an empty pan as a reference. The moisture content of the same sample was determined by oven drying at 105 °C. The maximum peak of temperature of the endothermic was taken as T_m, and the area under the endothermic peak as ΔH_m.

2.9. Fourier transform infrared (FTIR) analysis

The spectra of fish gelatin films (control and modified ones, with gellan and κ-carrageenan) were recorded by a FTIR-430 (Jasco Corporation, Tokyo, Japan). Light source of transmittance was in the middle range infrared 400–4000 cm^{-1}. The detector used was tri-glycine-sulfate (TGS) with resolution 4 cm^{-1}. The spectra obtained were used to determine the interactions of functional groups between fish gelatin and gellan or κ-carrageenan.

2.10. Statistical analysis

A one-way ANOVA was used to determine the difference between samples using Statistical Package for Social Science software (SPSS Inc.). When there were any significant differences between samples, Duncan's multiple range test was used to determine the significance of the average ($P < 0.05$).

3. Results and discussion

3.1. Melting temperature of fish gelatin gels

Melting point of gelatin gel is one of the important properties of gelatin beside gel strength, visco-elastic and gelling temperature. The gelation of this biopolymer, normally occurs by physical cross-linking leading to the formation of junction zone and ultimately a three-dimensional branched network with the present water entrapped (Gilsenan & Ross-Murphy, 2000), and thereby, the solid or rigid form is obtained. The melting temperature of the fish gelatin gels, native and modified ones are shown in Table 1.

Table 1
Effect of the addition of gellan and κ-carrageenan on the melting temperature of fish gelatin gels and on the tensile strength, elongation at break and water vapor permeability of the fish gelatin films

Gelatin gels and films	Melting temperature (°C)	Tensile strength (MPa)	Elongation at break (%)	Water vapor permeability (g mm/m^2 h kPa)
Control	24.55 ± 0.13[a]	101.23 ± 1.56[a]	5.08 ± 0.37[a]	2.40 ± 0.12[a]
Gellan 1 g/100 g	33.53 ± 0.17[b]	109.76 ± 1.11[b]	5.37 ± 0.41[a]	1.79 ± 0.12[b]
Gellan 2 g/100 g	39.20 ± 0.14[c]	104.39 ± 0.58[c]	6.24 ± 0.42[b]	1.75 ± 0.03[b]
Carrageenan 1 g/100 g	27.50 ± 0.18[d]	103.63 ± 2.71[c]	5.04 ± 0.39[a]	2.28 ± 0.31[a]
Carrageenan 2 g/100 g	28.55 ± 0.21[d]	104.48 ± 1.68[c]	6.81 ± 1.10[b]	2.17 ± 0.16[a]

[a–d]Mean ± standard deviation. Means in the same column with different superscript letters are significantly different ($P < 0.05$).

The native fish gelatin extracted from tilapia had melting temperature of 24.55 °C. It is a slightly higher than that of red tilapia (22.45 °C) and lower than that of black tilapia (28.90 °C) reported by Jamilah and Harvinder (2002), and close to those of gelatins extracted from fish skins of adult Nile perch and yellowfin tuna, which revealed melting points of 26.3 °C (Muyonga et al., 2004a) and 24.3 °C (Cho et al., 2005), respectively. The same study on fish tilapia gelatin having Bloom value 200 (same as used in this experiment) at concentration of 8 g/100 ml resulted in a melting point of 26 °C (Choi & Regenstein, 2000). In addition, the melting point was found to be dependent on the concentration of gelatin, maturation time, maturation temperature, pH and the influence of NaCl and sucrose. It has been known that the melting point of fish gelatin is lower than mammalian gelatin due to the amino acid composition and molecular weight distributions. The mammalian gelatins of bovine and porcine are shown to have melting point of 33.8 and 36.5 °C, respectively (Cho et al., 2005). Addition of gellan and κ-carrageenan up to 2 g/100 g significantly ($P < 0.05$) increased melting temperature. Gellan was significantly ($P < 0.05$) more effective in increasing melting temperature of fish gelatin gel than κ-carrageenan.

3.2. Mechanical properties of fish gelatin films

Mechanical properties evaluated in our study were tensile strength (TS) and elongation at break (E). TS is a measure of film strength, whereas E is a measure of film stretchability prior to breakage. Both properties are important in evaluation of packaging materials (Krochta & Johnston, 1997). Film prepared from fish gelatin resulted in tensile strength of 101.23 MPa (Table 1) which is close to that (106.7 MPa) of blended gelatin–pectin films (Jo, Kang, Lee, Kwon, & Byun, 2005). Our TS is higher than those of gelatin films reported by Xiao, Liu, Lu, and Zhang (2001), and much higher than that of the bovine gelatin film reported by de Carvalho and Grosso (2004), which revealed TS of 73 and 15.12 MPa, respectively. This remarkable difference is due to varying gelatin source and the process to prepare the films. As an example, de Carvalho and Grosso (2004) put glycerol as a plasticizer, while Xiao et al. (2001) did not mention the source of the gelatin used to make a blended film. The addition of polysaccharides as expectedly, caused a significantly ($P < 0.05$) increased TS. It showed that the addition of gellan 1 g/100 g resulted in the highest increased TS of the fish gelatin film (109.76 MPa). On the other hand, the addition of κ-carrageenan at both 1 and 2 g/100 g resulted in TS which are not significantly different ($P > 0.05$) from that of gelatin with gellan 2 g/100 g. Gellan could have formed networks with the gelatin molecules between anionic domains of gellan and cationic domain of gelatin, thereby strengthening the film structure (Fonkwe et al., 2003). In the preparation of composite films from porcine skin gelatin and gellan, Lee et al. (2004) found that TS was improved by increasing the gellan to gelatin ratio, with the highest TS at the film made from gellan. In our gelatin film system, gelatin is the major and dominant phase, 2 g/100 g appeared to be beyond the optimum level. A similar phenomenon was also observed by Fonkwe et al. (2003) in investigating gelling system of gelatin and gellan, wherein higher gellan decreased gelation rate. κ-carrageenan also improved significantly ($P < 0.05$) TS of gelatin film, although the effect was less than that of gellan. κ-carrageenan forms polyelectrolyte complex with positive charges of gelatin resulting in stronger films (Haug et al., 2004).

Elongation at break (E) of fish gelatin films, native and polysaccharides added ones is shown in Table 1. Native fish gelatin film had E value of 5.08%. This E value is much lower than that of E of bovine gelatin film (39.24%) investigated by de Carvalho and Grosso (2004). Addition of polysaccharides led to increase slightly E value. A significant increased E value was shown by the addition of gellan and κ-carrageenan at 2 g/100 g which resulted in 6.24% and 6.81%, respectively. In general, increased TS is followed by the decrease of E, but it seems not to be shown here. As these polysaccharides are kinds of macromolecules having relatively length chains, therefore, beside the existence of cross-linking reaction with gelatin leading to increased TS, it might extend macromolecular relaxations leading to increased E.

3.3. WVP of fish gelatin films

WVP is a measure of ease of the moisture to pass through a material, like in such biopolymer films (Pranoto,

Rakshit, & Salokhe, 2005). Table 1 shows the effect of the addition of gellan and carrageenan on WVP of fish gelatin films. Native fish gelatin film had WVP of 2.40 g mm/m^2 h kPa. It is not easy to compare the WVP with other similar works due to variability in the unit of quantity and measurement method. There are two basic methods to determine water vapor transmission, namely dessicant method (dry cup) and the water method (wet cup). The latter was used in this study. The wet method tends to give higher values (McHugh & Krochta, 1994). Glycerol-plasticized bovine gelatin film had WVP of 0.198 g mm/m^2 h kPa measured by dry method (de Carvalho & Grosso, 2004), whereas the report on WVP of fish gelatin is not available. In our study, the addition of gellan significantly ($P < 0.05$) reduced WVP of gelatin film with no significant difference between 1 g/100 g and 2 g/100 g. The hydrophilicity and the compactness of the polymeric matrix of the films are the major factors that affect WVP. The ionic interaction between gelatin and gellan may have formed a denser polymeric matrix, thus hindering passing of water molecule through. Similarly, κ-carrageenan was also able to form ionic complex with gelatin, but the compact matrix appeared to be weaker than that of gellan. As a result, it did not significantly ($P > 0.05$) reduce WVP of gelatin films. These results are in agreement with the previous evaluations (melting point and tensile strength) in which gellan influenced the fish gelatin characteristics to a greater extent than κ-carrageenan.

3.4. Color of films

In visual observation, all fish gelatin films performed transparent appearance. After addition of polysaccharides up to 2 g/100 g gelatin, films remained clear and transparent. L value significantly ($P < 0.05$) decreased by the addition of gellan and κ-carrageenan at 2 g/100 g (Table 2), indicated that the films were getting a bit darker in comparison with control of native fish gelatin film. Meanwhile, a (green to red) and b (blue to yellow) values did not differ significantly ($P < 0.05$) due to the addition of both polysaccharides in the concentrations studied.

3.5. Microstructure characteristic (SEM)

Fig. 1 presents the micrographs of the cross-sectional morphology of the native and polysaccharides-added fish gelatin films at ×25,000 magnification by SEM. Native fish gelatin film (Fig. 1A) shows the internal structure of polymeric film with the presence of discontinuous zone characterized by horizontal direction cracks randomly distributed along the networks. These discontinuous zones were attributed to the formation of preferential channel during drying process of filmogenic solution (Bigi, Bracci, Gojazzi, Panzavolta, & Roveri, 1998). Addition of gellan was noted to modify internal structure of fish gelatin film, in which gellan eliminated the present cracks, resulting in compact and dense appearance. Gellan might initiate some linkages and or resided between the fibrillar zones of fish gelatin through polyelectrolytes association with the gelatin. Different from that of added with gellan, addition of κ-carrageenan showed a light modified internal structure of fish gelatin films. However both levels resulted in the film matrices with the remaining cracks present. Even though interaction between κ-carrageenan and fish gelatin might occur, but it was not as effective as gellan. The microstructure observations of these results also answer the improvements of tensile strength and WVP properties of modified fish gelatin films, in which gellan was found markedly to modify the film matrix than that of κ-carrageenan.

Table 2
Color performance of fish gelatin films; native and added with polysaccharides

Film samples	Color parameter		
	L	a	b
Control	$93.22 \pm 0.03^{a,c}$	$-1.39 \pm 0.04^{a,b}$	$1.37 \pm 0.16^{a,b}$
Gellan 1 g/100 g	$93.16 \pm 0.02^{a,b}$	$-1.39 \pm 0.01^{a,b}$	$1.34 \pm 0.04^{a,b}$
Gellan 2 g/100 g	93.12 ± 0.02^{b}	$-1.41 \pm 0.01^{a,b}$	$1.43 \pm 0.04^{a,b}$
Carrageenan 1 g/100 g	93.23 ± 0.01^{c}	-1.38 ± 0.01^{b}	1.29 ± 0.02^{a}
Carrageenan 2 g/100 g	93.12 ± 0.08^{b}	-1.42 ± 0.02^{a}	1.46 ± 0.09^{b}

[a–c]Mean ± standard deviation of the color measurements. Means in the same column with different superscript letters are significantly different ($P < 0.05$).

3.6. Thermal property

The DSC thermograms of the fish gelatin films, as well as the films added with gellan and carrageenan are shown in Fig. 2. Fish gelatin films made from our experiment were known to have moisture content of approximately 14 g/100 g gelatin film, which is a slightly higher than that of moisture content of fish gelatin granule (12 g/100 g). The moisture content was appropriately used to calculate in determination of melting enthalpy (ΔH_m). Native fish gelatin film had T_m of 76.5 °C and ΔH_m of 21.78 J/g, which are higher than those films made from bovine skin gelatin (de Carvalho & Grosso, 2004), who found T_m and ΔH_m of 65.06 °C and 20.49 J/g, respectively. On the other hand, the dry film made from pigskin gelatin had T_m of 91 °C and ΔH_m of 26 J/g (Bigi, Cojazzi, Panzavolta, Rubini, & Roveri, 2001), while Vanin et al. (2005) reported different T_m and ΔH_m, which were 121.1 °C and 18 J/g, respectively, when the pigskin gelatin film was plasticized with glycerol 10 g/100 g. This difference of the thermal property is reasonable due to the different specification of gelatin source used and the effect of plasticizer added, although both films were prepared from the same material source. The addition of both polysaccharides gellan and κ-carrageenan modified T_m and ΔH_m of the fish gelatin films, with increased T_m and decreased ΔH_m values. The addition of gellan 1 g/100 g raised T_m from 76.5 to 79.4 °C,

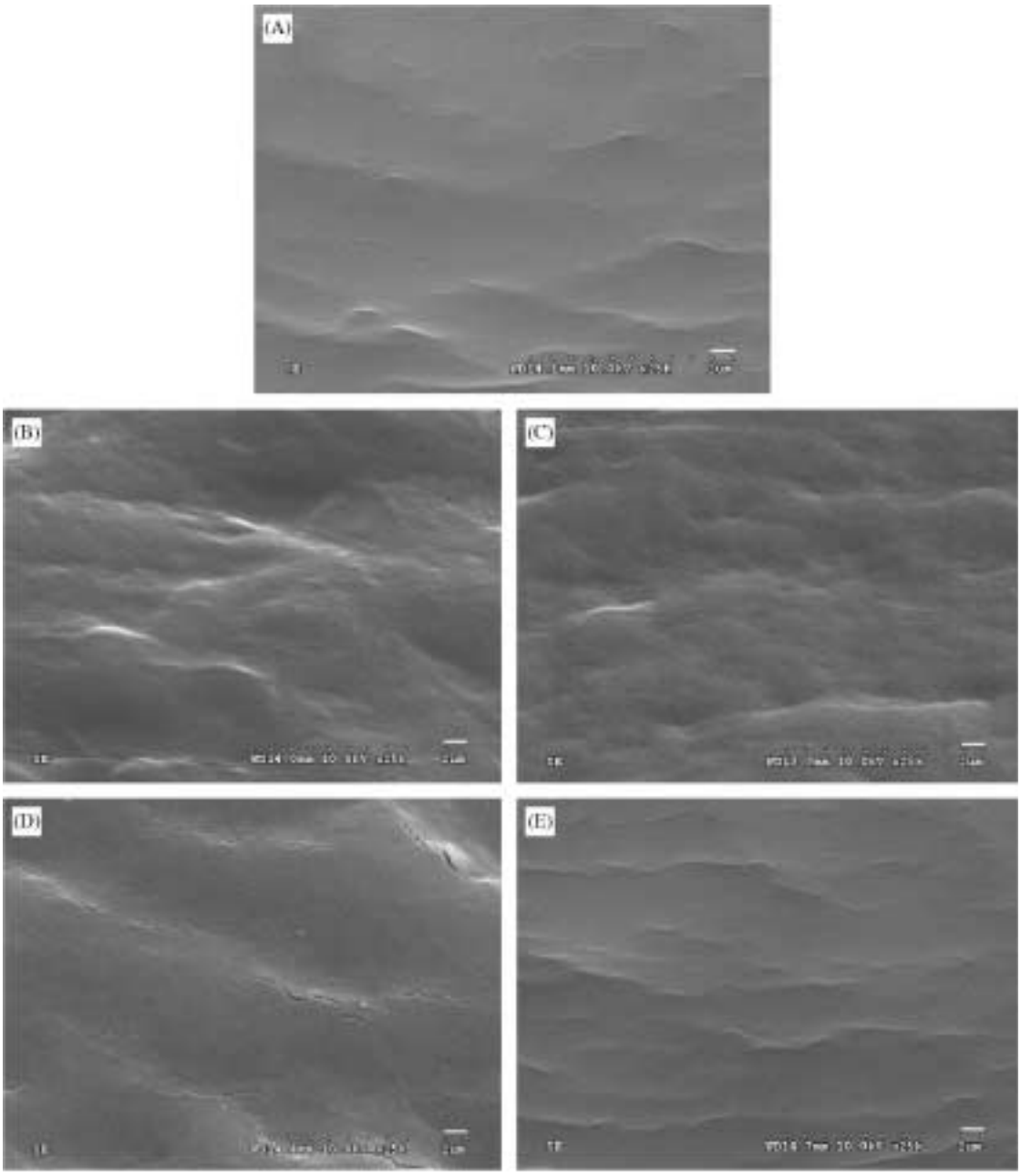

Fig. 1. Micrographs (SEM) of the cross-sectional view of internal microstructure of fish gelatin films: (A) native fish gelatin film; (B) fish gelatin film added with gellan 1 g/100 g; (C) fish gelatin film added with gellan 2 g/100 g; (D) fish gelatin film added with κ-carrageenan 1 g/100 g; and (E) fish gelatin film added with κ-carrageenan 2 g/100 g of gelatin. White bar = 2 μm.

but lowered ΔH_m from 21.78 to 10.63 J/g. Increasing the level of gellan to 2 g/100 g caused to shift T_m to 85.1 °C and reduce ΔH_m to 5.26 J/g. Kappa-carrageenan at 1 g/100 g was also found to shift the T_m to 80.4 °C and ΔH_m to 14.58 J/g. The increased T_m is slightly higher than that of gelatin added with gellan at the same concentration. However, increasing the level of carrageenan to 2 g/100 g resulted in a less increase in T_m and a little decrease in ΔH_m. Results showed that gellan >1 g/100 g still showed considerable effect on the T_m and ΔH_m, not with κ-carrageenan. In the study of cross-linked gelatin films using enzyme (transglutaminase) and chemical agents (formaldehyde and glyoxal), de Carvalho and Grosso (2004) confirmed that the degree of cross-linking reflected an increase in T_m accompanied a decrease in ΔH_m. Similar results were previously observed by Bigi et al. (2001) in investigation of thermal properties of gelatin films cross-linked with glutaraldehyde. Cross-linking increases the thermal stability of gelatin films, as shown by the shift of T_m to a higher value. Addition of gellan and κ-carrageenan showed similar results with those added with cross-linking agents reported earlier, in which cross-linking reaction

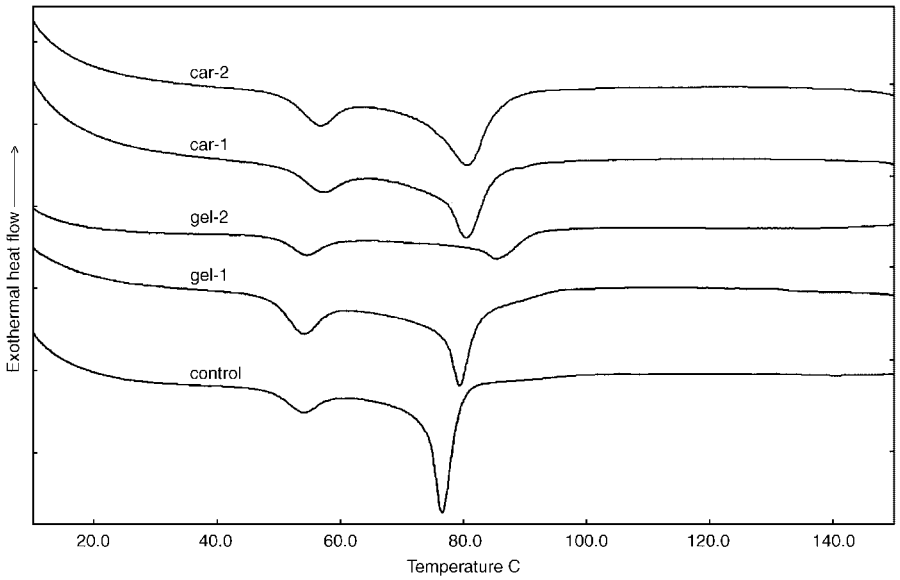

Fig. 2. DSC thermograms of fish gelatin films; native fish gelatin film (control), fish gelatin film added with gellan 1 g/100 g (gel-1), fish gelatin film added with gellan 2 g/100 g (gel-2), fish gelatin film added with κ-carrageenan 1 g/100 g (car-1) and fish gelatin film added with κ-carrageenan 2 g/100 g of gelatin (car-2).

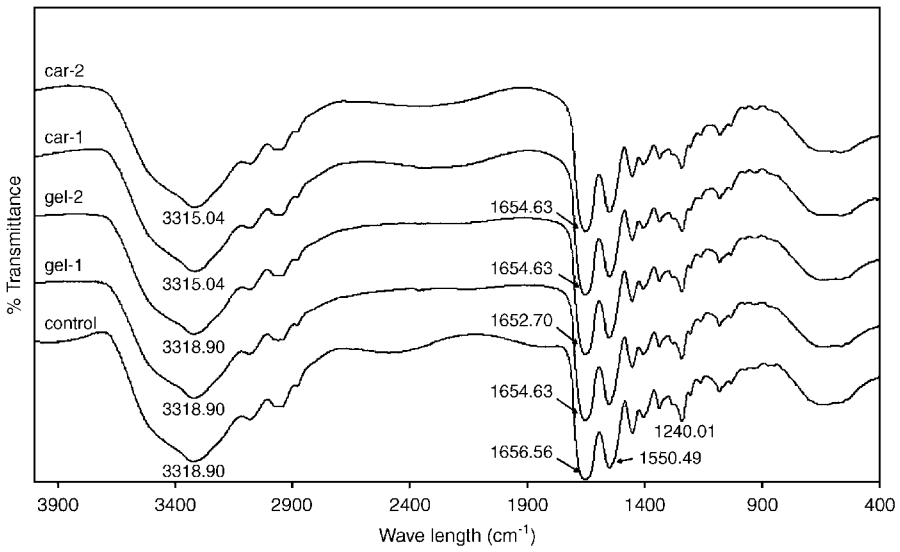

Fig. 3. FTIR spectra of fish gelatin films; native fish gelatin film (control), fish gelatin film added with gellan 1 g/100 g (gel-1), fish gelatin film added with gellan 2 g/100 g (gel-2), fish gelatin film added with κ-carrageenan 1 g/100 g (car-1) and fish gelatin film added with κ-carrageenan 2 g/100 g of gelatin (car-2).

might have occurred due to the associations of positively charged gelatin with negative charges present in gellan and κ-carrageenan.

3.7. FTIR spectroscopy

The infrared spectra of fish gelatin films, native and added with polysaccharides are depicted in Fig. 3. The spectrum of all the films showed a similar pattern, which indicates that there were no major changes in the functional groups of fish gelatins due to interaction between gelatin and polysaccharides. Fish gelatin film (control) revealed absorption bands at 3318.90 cm^{-1} (NH-stretching), 1656.56 cm^{-1} (amide I, CO and CN stretching), 1550.49 cm^{-1}(amide II) and 1240.01 cm^{-1} (amide III). Among these absorption bands, the amide I band between 1600 and 1700 cm^{-1} is the most useful peak for infrared analysis of the secondary structure of protein like gelatin (Muyonga, Cole, & Duodu, 2004b). The addition of gellan and carrageenan to the fish gelatin caused a slight shift of the peak of amide I to lower number. The distinctively shifted peak was shown by the addition of gellan at 2 g/100 g gelatin, which shifted the peak to 1652.70 cm^{-1}, whereas the others shifted the peaks to 1654.63 cm^{-1}. Such

shift of peak by the addition of gellan occurred due to coupling COO^- of gellan with the groups of amide I. In κ-carrageenan, the negatively charged sulfate ester groups might associate with positively charged gelatin represented in amide peaks. The addition of gellan, however, did not result in shift of peak 3318.90 cm^{-1}, which corresponds to NH stretching group. Carrageenan at 1 g/100 g and 2 g/100 g shifted this peak to lower number of 3315.04 cm^{-1}. The similar shift of this peak caused by interrupting intramolecular hydrogen bonding was also observed by Xiao et al. (2001) in the blended film prepared from sodium alginate and gelatin. FTIR analysis supports mechanical and thermal analyses in which the level of interaction of gellan with gelatin depended on the concentration.

4. Conclusions

The addition of gellan and κ-carrageenan modified fish gelatin properties. Gellan being more effective in increasing the melting temperature of the fish gelatin gels and both gellan and κ-carrageenan are effective in increasing mechanical and barrier properties of the films. These polysaccharides, however, made films slightly darker. Observed by SEM, gellan eliminated the discontinuous zone present in the biopolymer matrix of fish gelatin film, and thereby markedly modified the films properties than κ-carrageenan. DSC and FTIR analyses support the results of gel melting temperature, mechanical and permeability properties of fish gelatin films. Gellan appeared to be more effective in cross-linking action with gelatin. In general, the addition of these negatively charged polysaccharides could improve the properties of films made from fish gelatin by increasing T_m and mechanical properties and decreasing permeability. Hence, it is possible to replace mammalian gelatin in some applications.

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